PERCHLORATE AND HUMAN HEALTH IN 2008: THE IMPLICATIONS OF NEW SCIENCE FOR THE EPA PRELIMINARY DETERMINATION

COMMENTS IN RESPONSE TO THE EPA NOTICE *DRINKING WATER: PRELIMINARY REGULATORY DETERMINATION ON PERCHLORATE*
[EPA-HQ-OW-2008-0068; FRL-8727-6]
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FINAL

Prepared for:

PERCHLORATE STUDY GROUP

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Intertox, Inc.
600 Stewart St.
Suite 1101
Seattle, WA 98101

206.443.2115 phone
206.443.2117 facsimile

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I. INTRODUCTION

The Environmental Protection Agency (EPA) has released its preliminary regulatory determination on perchlorate for public comment. The EPA is required by the Safe Drinking Water Act (SDWA) to publish Maximum Contaminant Level Goals (MCLG) and national primary drinking water regulations (NPDWR) **IF** (emphasis added),

> (a) the contaminant may have an adverse effect on the health of persons;
> (b) the contaminant is known to occur in public water systems with a frequency and at levels of public health concern; and
> (c) in the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems (73 FR 60264).

Based on these criteria, the EPA has presented their rationale in the determination not to set an NPDWR for perchlorate as it would not present "a meaningful opportunity for health risk reduction for persons served by public water systems." This decision was made based on the derivation of a Health Reference Level (HRL) for perchlorate and was calculated to be 15 ppb based on a relative source contribution (RSC) of 62% (73 FR 60276). .

Even a cursory review of the 50 years of perchlorate research shows that the weight of scientific evidence clearly supports EPA's decision.

The purpose of this document is to review the key scientific studies and how these studies support the EPA's findings in the proposed determination. We present a brief review of the National Research Council of the National Academy of Sciences (NRC) assessment and update key studies that have been added to the scientific literature.

Between 1997 and 2002, at least 13 toxicological studies of perchlorate, all using EPA protocols, were conducted in animals; these include pharmacokinetic studies, subchronic studies, developmental studies, immunotoxicology studies, and a multigenerational reproductive study. All studies were conducted over a range of doses and evaluated independently by EPA. Of the many results obtained from this work, the most sensitive target organ was the thyroid gland and its influence on the body. Studies of potential mutagenic effects were conducted but no such effects were found (U.S. EPA, 2002). During the same time period, there were several clinical exposure studies in humans and a number of epidemiologic and ecological studies, some in occupational settings and some in populations exposed to perchlorate via the community drinking-water supply.

The EPA's National Center for Environmental Assessment (NCEA) prepared draft risk assessments for perchlorate in 1998 and 2002. The proposed reference doses (RfD) were 0.0009 mg/kg-day in 1998 and 0.00003 mg/kg-day in 2002. Using EPA's conventional (or default) conversion factors, these reference doses translated to drinking water values of 32 and 1 ppb, respectively. Both reference doses proposed by EPA/NCEA were based on the results of animal studies. In 2002, EPA/NCEA interpreted these studies as showing adverse effects in

The body of science supports the Agency's decision not to establish a national primary drinking water standard as there is no meaningful opportunity for health risk reduction.

the pups of rats exposed during pregnancy to a perchlorate dose as low as 0.01 mg/kg-day (U.S. EPA, 2002).
Public comments on the 2002 draft risk assessment raised serious concerns about the validity of using these animal studies for human health risk assessment (U.S. EPA, 2003). The EPA, NRCA, and the Departments of Energy and Defense jointly sponsored a review of the underlying science by the NRC.

On basing an RfD on a no effect level for a non adverse effect, the NRC states: "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."

For this current regulatory determination, EPA has the benefit of a rigorous and independent peer review of the available science. These include the study by the NRC and more recently the Agency for Toxic Substances and Disease Registry, an agency of the Department of Human Health Services (ATSDR, 2008).

In 2005, the NRC perchlorate panel reached a consensus and recommended an RfD of 0.0007 mg/kg per day. The NRC panel comprised 15 leading scientists and physicians with wide-ranging expertise necessary to evaluate all aspects of the available science related to perchlorate. The NRC process occurred over a 15-month time period, providing ample time for the panel to review studies and consider oral testimony and written comments prior to issuing its conclusions and recommendations. As part of this process, the NRC panel performed an exhaustive review of the wide body of available animal and human studies as well as other scientific data relevant to understanding the health effects of perchlorate. The NRC panel noted that "emphasis was given to studies with the soundest scientific methods to draw conclusions regarding the effects of perchlorate exposure" (NRC, 2005).

The charge to the Committee was to "critically evaluate the scientific literature, including both human and animal data, and…assess the key studies underlying EPA's 2002 Draft Toxicological Review and Risk Characterization for Perchlorate in terms of quality, reliability, and relevance to draw conclusions about the health implications of exposure to low levels of perchlorate in drinking water." As stated in the NRC Committee Report, "EPA has been criticized that it did not appropriately consider all the relevant data for its assessments and that it based its conclusions on flawed scientific studies" (NRC, 2005).

The NRC independently evaluated the science which was published in the report *Health Implications of Perchlorate Ingestion* (2005). The NRC report recommended an RfD of 0.7 μg/kg-d based on a No Observed Effect Level (NOEL), or the dose at which no effects occur, adverse or otherwise (NRC, 2005). One of the critical studies that served as the basis for this RfD was the study by Greer et al. (2002) which demonstrated that there was no inhibition of iodide uptake by the thyroid at a dose of 7 μg/kg-d. Importantly, the NRC did not examine Greer in isolation. According to the NRC report, the findings in Greer are supported by other clinical studies, occupational and environmental epidemiologic studies, and studies of long-term perchlorate administration to patients with hyperthyroidism.

Further, the NRC states with emphasis, "Inhibition of iodide uptake by the thyroid clearly is not an adverse effect; however if it does not occur, there is no progression to adverse health effects." The committee views its recommendation to use [IUI] by the thyroid as the basis of

the perchlorate risk assessment to be the most health-protective and scientifically valid approach (NRC, 2005).

After review all scientific data, including studies since the release of the NRC perchlorate review, ATSDR has adopted the EPA's chronic RfD recommended by the EPA (2005) for the chronic MRL"

In using a NOEL, the NRC committee was conservative as this dose is already lower than any dose in which adverse effects occur; a safety factor of 10 is further applied to the already conservative NOEL to account for the most sensitive individuals in a population, in this case, hypothyroid or iodine-deficient pregnant women and their developing fetuses. The NRC panel stated that using a NOEL as the point of departure is a more conservative and health-protective approach than EPA's customary approach of using the adverse effect (NRC, 2005). Thus, for example, the use of IUI as a point of departure is a more cautious health protective approach than using changes in thyroid hormones (a precursor to possible adverse effects) or to some adverse effect such as hypothyroidism.

The panel also took time to differentiate between a NOAEL and a NOEL, finding that there was confusion between the two and stating that the NOAEL is based upon an adverse effect, whereas the NOEL is based upon a nonadverse effect (NRC, 2005). With the incorporation of an uncertainty factor of 10, the RfD recommended by the NRC was many times lower than the point at which adverse effects occur. It should be noted that the NRCNRC report concluded that no other uncertainty factors were necessary. Specifically on the issue of adequacy of database, the NRC concluded that the database contained sufficient studies from which to determine an RfD, finding that the database contained both human and animal data from which to evaluate IUI. The NRC also concluded that no LOAEL to NOAEL conversion factor was required, since the point of departure was the NOEL, again, a more conservative approach. Some have also argued that the Greer study was of short duration and have argued for a subchronic to chronic extrapolation. On that issue, the NRC concluded that since the point of departure is based upon IUI—a short-term event—any chronic effects "would have no greater effect" than any short term effects that may occur. (NRC, 2005).

The EPA has based its RfD for perchlorate and Integrated Risk Information System (IRIS) summary on the NRC report.

This year, the ATSDR released its *Toxicological Profile for Perchlorates* (2008) as new data have become available since the last profile of perchlorates in 2005. As a part of their review, ATSDR derives minimum risk levels (MRLs) using available peer reviewed information. MRLs "…are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels" (ATSDR, 2008). Considering the NRC report along with new studies published since the NRC report, "ATSDR has adopted the EPA's chronic RfD recommended by the NRC (2005) for the chronic MRL" (ATSDR, 2008).

ATSDR based their MRL on the same most sensitive population, pregnant women and their fetuses, although they also discussed other sensitive populations, preterm and nursing infants. They also state that their decision "…was made after a careful evaluation of the NRC report and of studies that have been published after the NRC (2005) report. The results from newer studies do not change the bottom-line recommendation" (ATSDR, 2008).

The ATSDR Toxicological Profile for Perchlorates was peer reviewed and made available for public comments. "The expert peer reviewers on April 18, 2007 concluded that the MRL should still be based on the RfD as recommended by the NRC Panel Report (2005) given the research data available at the time of the 2007 peer review" (ATSDR, 2008).

Based on the traditional and foundational approach used in science, the weight of evidence—or the number of well conducted studies in humans and animals with appropriate dose-response information—supports EPA's decision not to establish a NPDWR. As shown in the assessment by the NRC in 2005 and the subsequent literature published since then, including the ATSDR Toxicological Profile, the weight of evidence shows that,

- inhibition of iodide uptake in the thyroid gland is a key biochemical event,
- the inhibition of iodide uptake is not an adverse effect,
- there are doses of perchlorate below which inhibit iodide uptake in healthy adults,
- the dose of perchlorate that might be sufficient to cause an adverse effect is as *sustained exposure* greater than 0.4 mg/kg-d (NRC, 2005), and
- there are natural occurring agents, such as thiocyanate and nitrate, that also effect iodide uptake in the thyroid gland.

The EPA has determined that setting a NPDWR will not present a meaningful opportunity for health risk reduction based on assessments done by multiple authoritative scientific bodies, numerous well conducted human and animal experiments using a range of doses of perchlorate, and the occurrence and concentration of perchlorate in drinking water systems in the U.S. One of the rationales for this decision was the calculation of the HRL which was based on the RfD. The RfD combined this previous knowledge to determine a dose which is well above the threshold in healthy adults that does not cause inhibition of iodide uptake and dividing that dose by 10 to account for sensitive populations.

It should be noted that the RfD aims to protect the most sensitive individuals within a population (i.e., pregnant women and their fetuses). While some have argued that the infant or child is the more sensitive individual in the population; the experts of the Perchlorate NRC committee, EPA, ATSDR, OEHHA, and others, disagree. In addition, no reliable scientific data at relevant environmental exposures for this change in sensitive population has been put forward. One of the central considerations in these arguments is the disregard of dose and exposure. Any chemical at a sufficient dose will cause an adverse effect.

However, dose is a foundational tenant of the standard medical sciences (e.g., toxicology,

Studies published since the NRC report in 2005 add to an already well-developed database, adding more studies that are consistent to previous analyses and reducing uncertainty. Thus, the science supports EPA's decision that the current RfD for perchlorate is both conservative and health protective to even the most sensitive individuals.

endocrinology, pharmacology, etc.). Dose consideration is an absolute foundation of the risk assessment process by all recognized authoritative bodies.

The weight of evidence shows that the fetal brain is more sensitive to hypothyroidism than the neonate or infant brain (Boelaert and Franklyn, 2005). Breast milk concentrations of perchlorate have been measured at levels greater than the HRL (Kirk et al., 2005; Pearce et al., 2007b). However, the RfD assumes that if a person consumes 15 ppb of perchlorate not just once, but every day for a lifetime, they will be consuming a concentration of perchlorate that is not anticipated to cause an adverse effect. Furthrermore, a HRL, MRL, PHG[1], RfD, etc. are not bright lines above which health effects would be expected to occur. As noted with perchlorate, an RfD based on a no effect level for a non adverse effect in humans with an additional 10-fold uncertainty ("safety") factor provides a health protective approach. The development of a value lower than the RfD will only increase the level of caution. Therefore, if an infant consumes one dose of perchlorate that is greater than the RfD, it is not expected to be any more harmful than a lesser dose. Lastly, a solely breast fed infant will not have exposures through other media.

[1] A Public Health Goal, or PHG, is the State of California's acceptable level of a chemical in drinking water that is not expected to cause an adverse effect if consumed every day for a lifetime.

II. KEY SCIENTIFIC STUDIES PUBLISHED SINCE 2005

Since the publication of the NRC report in 2005, several studies have been published that support the conservative nature of the current EPA RfD. Overall, these studies demonstrate that perchlorate is a ubiquitous chemical in the environment, food, and the human body. In a recent study by Blount et al. (2007), all samples of urine measured had low, but detectable concentrations of perchlorate. The sources of perchlorate appear to be both natural and anthropogenic. There is an increasing body of literature demonstrating that much of the environmental perchlorate may be natural or due to non-point sources (DasGupta et al., 2005; SERDP, 2005; DasGupta et al., 2006; Rajagopalan et al., 2006).

The weight-of-evidence of human and animal studies that include sufficient dose-response data, show that possible adverse health effects are several orders of magnitudes above environmentally-relevant levels of perchlorate and that the current RfD is a conservative toxicity guideline value such that contaminants present in drinking water at or below the HRL of 15 ppb would pose no significant health risk to the most sensitive individuals who consume the water on a daily basis over a lifetime.

Despite the widespread low levels of perchlorate in the environment, studies have not demonstrated an adverse effect due to perchlorate exposure. The NRC report states,

> "The committee emphasizes that inhibition of iodide uptake by the thyroid has been the only consistently documented effect of perchlorate exposure in humans. The continuum of possible effects of iodide-uptake inhibition caused by perchlorate exposure is only proposed and has not been demonstrated in humans exposed to perchlorate (with the exception that in patients with hyperthyroidism doses of 200 mg daily or higher may reduce thyroid secretion). More important, the outcomes at the end of the continuum are not inevitable consequences of perchlorate exposure." (*NRC, 2005*) [2]

The new studies contribute to an already well developed database, adding more studies that are consistent with previous analyses. Furthermore, additional studies that are consistent with the literature also reduces uncertainty (reducing "what we do not know"), thereby reinforcing that the current RfD for perchlorate is both conservative and health protective. Thus, perchlorate concentrations equal to or above the EPA-recommended HRL of 15 ppb in water would also be conservative and health protective for sensitive populations.

A. Environmental Studies

EPA states: "If adequate human studies (confirmed for validity and applicability) exist, these studies are given first priority in the dose-response assessment, and animal toxicity studies are used as supportive evidence." and "If adequate human data are available, this information is used as the basis of the RfD (EPA, 1989). The scientific studies regarding perchlorate and

[2] The noted dose of 200 mg/d is a conservative therapeutic value. When use of perchlorate to treat hyperthyroidism was common, doses of 400 mg/d were commonly prescribed, but were found to be slow to control thyrotoxicity and doses needed to be repeated 4 to 5 times/d, due to the rapid excretion of the drug. Although it still took an average of 9.4 weeks, doses of up to 2000 mg/d were given to reduce hyperthyroidism to a remission state (Wolff, 1998).

health are predominately based upon human data, with a range of parameters and dose-response data. The types of human studies are clinical, occupational, or ecological. To date, there is only one epidemiological study which measures perchlorate exposures and outcomes in pregnant women and neonates (Téllez et al., 2005). In contrast to ecological studies measuring collective exposures and outcomes, an epidemiological study measures individual exposure and outcome. There are also some well designed animal studies that have contributed to the health effects database. Many of these studies investigate the relationship between perchlorate exposure and adverse outcomes among especially vulnerable groups. In addition to the clinical studies reviewed by NRC, epidemiological studies are valuable in exploring the health effects due to perchlorate in a chronically exposed genetically-diverse population that includes the population of concern (pregnant women and their fetuses).

In the EPA notice, several of these association studies were taken together, leading EPA to conclude that the results of "…studies of the effects of perchlorate exposure on hormone levels have been mixed" (p. 60266), citing Amitai et al. (2007) showing no effects and Blount et al. (2006) identifying hormonal changes (although none were outside normal ranges). However, when the strength of the study design is taken into consideration, the weight-of-evidence demonstrates that the current RfD is a conservative toxicity guideline value such that contaminants present in drinking water at or below the HRL of 15 ppb would pose no significant health risk to the most sensitive individuals who consume the water on a daily basis over a lifetime.

i. 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 neonates. Perchlorate is found naturally at high levels in Chilean soils and water supplies. Some have questioned the relevance of this study to the U.S. population as historically, Chile has had high levels of dietary iodide supplementations. However, this supplementation has been decreased to levels that are similar to U.S. levels. In this study, the maternal excretion (and therefore ingestion) of iodine was at levels that were between those reported in NHANES I and NHANES III. The authors measured maternal and neonatal TSH, Tg, and free T4. They also measured neonatal 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 the World Health Organization (WHO) recommendations. This study strengthens the evidence that the RfD and HRL are conservative health based values.

ii. 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 within 36 to 48 hours after birth, but there was no comment on whether the infants were breast fed or formula fed during the postnatal period. They found that there were no differences between neonatal T4 levels among the groups.

This study provides evidence that the current RfD and values greater are conservative and health protective to the most sensitive individuals in the population.

iii. Murray et al. (2008)
The Food and Drug Administration (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 intake doses. 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 cities 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). This is due to the high intake of dairy products coupled with low body weight. 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.[3] 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 at 40, 78, and 24.4 μg perchlorate/kg wet weight of food, respectively.

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. The study does not aim to evaluate overall body steady-state serum concentrations of perchlorate, only dose. Children age 2 have a higher renal excretion rate than adults or infants do, which would reduce their steady-state serum concentration despite the higher intake.[4]

iv. Blount et al. (2006; cited at Blount et al., 2006b in the EPA Notification)
Using the NHANES 2001-2002 data set and a cross-sectional study design, this study reports measurement of urinary perchlorate, urinary iodide, serum TSH, and serum total T4 levels in men and women over the age of 12. The 2001-2002 NHANES data provide the largest group of subjects to date from which sampling data can be derived. The authors report 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 report 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 the WHO statement that a median urinary iodine concentration for the entire population, based on spot samples of less than 100 μg/L is indicative of overall iodine deficiency for that 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

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

[4] Perchlorate is a non-metabolized pharmaceutical agent with nearly complete urinary excretion. Data on adult and child clearances is available for other pharmaceuticals making it possible to extrapolate the rate of a child's clearance of perchlorate compared to an adult's. Based on the clearance of gabapentin, gentamicin, isepamicin, ticarcillin, and vancomycin, the clearance in a child is 1.6 times that of an adult (unpublished data).

which cause any measurable inhibition of iodide uptake. However, there are a number of considerations that should be noted that to better understand the significance of the data reported.

First, as stated above, ATSDR (2008) reviewed this study in their assessment and did not feel that it merited any special consideration over other studies in the well developed perchlorate literature database. They state that their decision "…was made after a careful evaluation of the NRC report and of studies that have been published after the NRC (2005) report. The results from newer studies do not change the bottom-line recommendation" (ATSDR, 2008).

Second, it is important to understand what the methods inherent to scientific research are. For example, knowing what is meant by "statistical significance and "clinical significance." A "statistically significant" difference simply means it is unlikely that there is a difference between two variables (e.g., numbers) that is due to chance. With a well designed experimental study, statistical significance can be extremely important. However, statistical significance is also a function of sample size, variability in the variables measured, etc. Statistical significance does not necessarily mean the difference is large, important, or biologically significant. However, for biological data, we are keenly interested if something is statistically significant is it also clinically significant, that is is there change in the variable or parameter that is large enough to affect a clinical or medical condition (e.g., development of hypothyroidism). Blount et al., 2006 report statistically significant variables, but do not report any variables with clinical significance.

This type of study conducted by Blount and his colleagues cannot determine causation, only *association* between the variables studied (Wartenberg and Buckler, 2001). 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.

Furthermore, perchlorate did not actually lower (or was even associated with) thyroid hormones outside the normal range of values. Even if it had, the NRC 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).

The study was considered by ATSDR; however, ATSDR states, "limitations of the study acknowledged by the investigators include those common to cross-sectional analyses, the assumption that urinary perchlorate correlate with levels in the thyroid stroma and tissue, and the measurement of total T4 rather than free T4" (ATSDR, 2008).

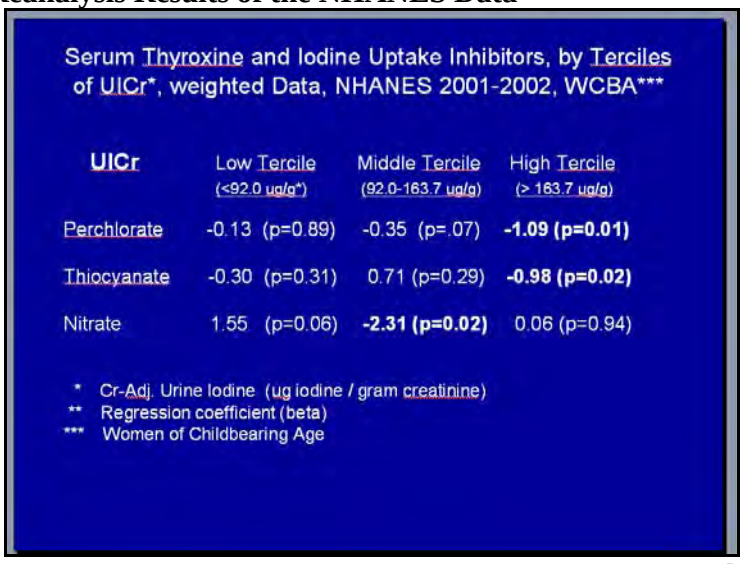
Third, any study must have careful consideration for the parameters and variables that are measured or available. The purpose of NHANES is designed to collect information about the health and diet of people in the United States. It is a survey for a broad range of possible diseases related to diet and health. While it obtains parameters for thyroid function, those parameters fall far short of the parameters needed to clearly understand thyroid health. For example, there were only two

measures of thyroid function (total T4 and TSH) in the NHANES dataset used in this study; NHANES currently uses spot urine samples for iodide status which are neither the preferred, nor most reliable, measure of urinary iodide (Barr et al. 2005); there was lack of normalization for the dilution of individual urine samples using urinary creatinine levels; and due to restraints of the NHANES database, the authors were not able to note the incidence of autoimmune thyroiditis, the most common thyroid disorder in the U.S., which causes changes in serum T4, T3, and TSH concentrations due to errant immune mechanisms attacking the thyroid gland (NRC, 2005). A complete assessment produced by Intertox is attached as Appendix A. In the appendix, there is a more thorough discussion regarding the use of spot urine testing, confounders, and use of the available NHANES variables to drawn conclusions.

This study did 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 (Furnee et al. 1994).

At a recent symposium on perchlorate held in conjunction with the annual Society of Toxicology (SOT) 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. Lamm et al. 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 92 µg/g (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 1. Urinary Reanalysis Results of the NHANES Data



Lamm et al., 2008

In a peer-reviewed letter to the editor of *Thyroid*, Gibbs and Van Landingham (2008) reviewed data from their previously published study (Téllez et al., 2005), 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. (2008), both presented at the Seattle SOT meeting.

The American Thyroid Association (ATA) issued a public health statement cautioning against the paper's use in making decisions on regulating perchlorate. The ATA noted that "serum thyroxine was measured as total thyroxine rather than as free thyroxine, the most frequently used clinical measurement and the physiologically available form of thyroxine. Thyroid autoantibodies, an important confounder in thyroid physiology, have not yet been measured. These have an especially high prevalence in women and may have contributed to the reported correlations. The presence of potentially confounding pharmaceutical and medical factors, such as estrogen use or autoimmune thyroid disease, was not used as a basis for exclusion from the analysis. Inclusion of laboratory results from multiple laboratories may need to be more carefully considered. The reason that perchlorate, but no other measured goitrogen studied, influenced thyroid function at low urinary levels of iodine is not explained. The development of further laboratory information is necessary before the implications of the findings can be understood. The issues raised are important and additional study to resolve them should be pursued" (ATA, 2006).

Fourth, 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 (NRC, 2005; Greer et al., 2002).

Finally, taken from the premise that science is incremental and knowledge builds collectively over time, concerns raised by scientists related to the Blount et al. study should be considered an opportunity for further study. According to Dr. Benjamin Blount, a new study will be conducted using a new data set, NHANES 2007, and will include 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).[5] 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. We believe more research should be conducted to better understand the results of this study.

Steinmaus et al. (2007) used the same dataset as Blount et al. 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. Many of the same methodological issues reported for Blount et al. apply

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

to this study. As in Blount et al., 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.

Both Blount et al. and Steinmaus et al. (2007) show an association between exposures to low, environmentally-relevant levels of perchlorate and non-adverse reductions in thyroid hormones that remain within the normal ranges. However, these studies contradict the wide body of direct studies showing no effect even at low levels. These studies are valuable in that they test a large population; however, neither study shows that exposure to perchlorate at environmentally relevant doses causes an adverse health effect, even in a sensitive population.

v. 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 RfD and below the level of inhibition of iodide uptake. The NRC states that "…inhibition of iodide uptake by the thyroid clearly is not an adverse effect; however if it does not occur, there is not progression to adverse health effects" (NRC, 2005).

B. Occupational or Clinical Studies

i. Braverman et al. (2005)

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 NRC 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 Greer et al. (2002).

ii. 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, respectively, 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." (emphasis added)

This study was similar to Greer et al. (2002), 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 (Crawford-Brown et al., 2006).[6] 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 Greer et al. (2002), and relied upon by the NRC as the basis for its recommended RfD.

C. Breast Milk Studies

For many infants, breast milk is the sole source of food and therefore represents the greatest potential exposure to perchlorate. Although the population theoretically considered the most susceptible to the effects of high levels of perchlorate is developing fetuses, the effects of doses of perchlorate that are sufficiently high to block iodide uptake by the thyroid may alter normal growth and development in infants and children. Taken together, the following studies show that there is no association between environmentally relevant levels of perchlorate in drinking water and perchlorate or iodide in breast milk. Furthermore, no effect, adverse or otherwise, has been found due to environmentally relevant doses of perchlorate.

i. 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." 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 perchlorate 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.

[6] "If one assumes that the individuals in the study by Greer et al. (2002) were exposed to the same background levels of perchlorate as the rest of the U.S. population (there is nothing in their diets or in the study design to preclude this), then no further RSC adjustment is needed to reflect total exposures via all routes because the risk coefficient from the study already reflects the incremental risk from ingestion of perchlorate in water above and beyond the contributions to perchlorate exposure via the other routes" (Crawford-Brown et al., 2006).

ii. 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. 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.[7] 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.

iii. Dasgupta et al. (2008)

In a very recently released study, Dasgupta et al. report the results of their study. "The objective of this present study was to study the excretion of perchlorate, thiocyanate, and iodine in milk and urine and relate the observed pattern within the broad framework of parallel / competitive transport by the NIS." Using breast milk and urine samples from 13 lactating women and using EPA default values for infant body weights and milk intakes, the authors mathematically modeled infant intakes and doses of iodide, perchlorate, and thiocyanate. They calculated the fraction of iodide, perchlorate, and thiocyanate in breast milk compared to the total that is excreted in both breast milk and urine. They used a ratio of these fractions in milk to determine the selectivity of either perchlorate or thiocyanate over iodide. They report "that 12 of 13 infants did not have an adequate intake of iodine…and 9 out of 13 infants were likely ingesting perchlorate at a level exceeding the reference dose…" They also concluded that the selectivity of perchlorate over iodide was 3.14 ± 1.2.

There are a number of concerns related to the experimental design of this paper. The population is small and there is no information on the selection process of the participants (e.g., they are not a random sample). There are only three biological variables that were measured from these women. The rest of the variables, including the variables used to derive the conclusions, are calculated from these these three in addition to

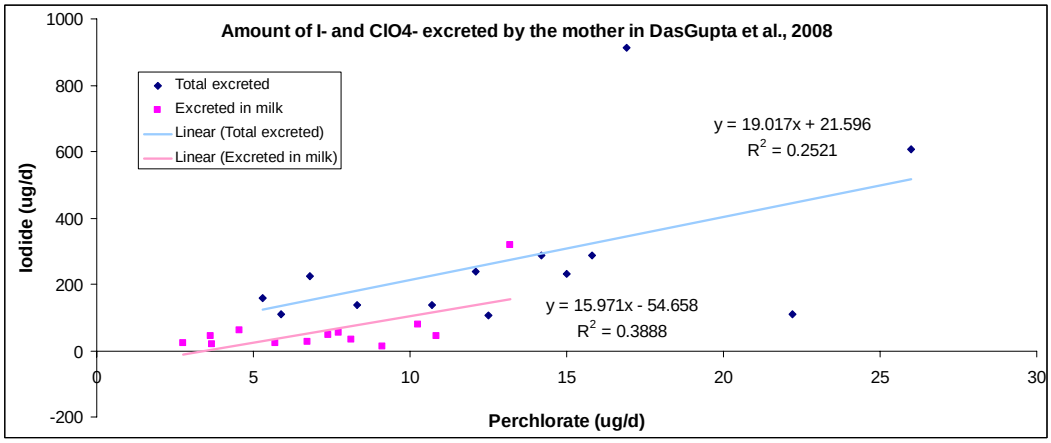
Taken together, studies on perchlorate in breast milk show that there is no association between environmentally relevant levels of perchlorate in drinking water and perchlorate or iodide in breast milk. Furthermore, no effect, adverse or otherwise, has been found due to environmentally relevant doses of perchlorate.

[7] 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."

using default values for infant intake and infant weights. The three measurements were the concentrations of of perchlorate, iodine, and thiocyanate in both urine and breast milk. Where other research has measured more biologically relevant information, such as serum concentrations of analytes which would be most reflective of relative concentrations at the NIS (Tonacchara et al., 2004; Pearce et al., 2007) thus, this work is limited. The estimated doses, the key variable for understanding possible NIS effects, are based on average body weight and intakes for an infant of that age with the concentration in breast milk. For such a simple and critical variable, it is suprising that actual body weights were not measured.

In addition to study design, the data presented in this paper appears to contradict the interpretation by the authors of the paper. Based on estimated weights, the authors state that 9 out of 13 infants are consuming daily doses in excess of the RfD. However, from examining Table 1 from Dasgupta et al., there are 8 infants who exceed the RfD of 0.7 μg/kg-d (subjects 1, 2, 8, 11, 13, 15, 16, 20). Furthermore, if one reviews these estimates to measured variables, the infants with estimated perchlorate doses greater than the RfD are also estimated to have the greatest iodine intake. By plotting the values for total iodide excretion (column 4), total perchlorate excretion (column 7), estimated maternal breast milk iodide excretion (equivalent to estimated infant iodide intake; column 5), and estimated maternal breast milk perchlorate excretion (product of total perchlorate excreted and percent of perchlorate in milk; column 7 x column 9) found in Table 1 of the paper, the results demonstrate that perchlorate and iodide are positively correlated (See Figure 3). If perchlorate was inhibiting the transport of iodide into milk, the association would be negative.

Figure 2. Plotting of data reported by Dasgupta et al., 2008 to illustrate the association between concentrations of iodine and perchlorate in urine and milk in lactating women.



In addition to the concerns regarding interpretation of the data presented in this paper, there are concerns about the interpretation of their previous work (Kirk et al., 2005) and how that study impacts this recent study. For example, Dasgupta et al. state in this current paper that "in real mothers perchlorate does inhibit the transport of iodine into milk and because of competitive inhibition both analytes cannot be high at the same time." However, this statement is based on a previous study in which the

researchers defined their cut off values[8] above which milk iodide or perchlorate were considered "high." They report that no milk samples had both high perchlorate and high iodide (Kirk et al., 2005). In the present study, they report the same trend although they do not define a cut off value. However, if the previous cut off values are applied to the current study, there are many samples that had simultaneously high perchlorate and iodide (Figure 3). Based on the previous conclusions about competitive inhibition of analytes (Kirk et al., 2005), this study does not demonstrate that perchlorate competitively inhibits iodide transport into milk at the concentrations experienced by these women.

iv. Dohán et al. (2007)

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 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).

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.[9] The perchlorate doses administered in this study are high enough such that they should effectively block,

[8] The cutpoints from Kirk et al. (2005) were defined as follows: High iodide in breast milk was greater than 60 μg/L and high perchlorate in breast milk was greater than 20 μg/L.

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

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.

III. IODINE SUFFICIENCY IN THE UNITED STATES

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 healthy adults; Greer et al. 2002), perchlorate exposure would not exacerbate iodine deficiency. As stated above, this dose was adjusted by an uncertainty ("safety") factor of 10. Thus, the approach used by EPA in deriving their HRL based on the RfD of 0.7 μg/kg-d, remains a conservative and health protective approach that accounts for substantial uncertainty. The NRC definitively states

> "…a dose that does not inhibit thyroid iodide uptake will not affect thyroid function, even in subjects with an abnormal thyroid gland or a very low iodide intake."

There is concern that there are low iodine levels in the U.S. population. The concern is that low iodine levels in a pregnant woman for prolonged periods of time can cause a change in thyroid hormones that may affect the neurodevelopment of the fetus.

First and foremost, it is important to note that no study has demonstrated that exposure to environmentally-relevant levels of perchlorate causes or is associated with iodine deficiency. Taken at face value, Blount et al. (2006) show an association between thyroid disruptions and perchlorate; however, even if this association represented an actual biological effect at doses below the inhibition of iodine uptake, this hypothetical effect does not raise or lower the measured thyroid hormones outside of the normal ranges and do not show any adverse effect due to the exposure. An epidemiological study of chronically exposed populations in Chile, including children who were exposed throughout gestation via maternal exposure and in infancy through breastmilk and later dairy, show no adverse effects with exposure much higher than the RfD even with iodine intakes similar to that in the U.S. (Téllez et al., 2005).

Second, the TDS and NHANES both show that population intake of iodide exceed the recommended adequate intake for iodine (Murray et al., 2008; Blount et al., 2006). Although there have been questions regarding iodine sufficiency in the U.S., Borak (2005) points out that there has not been consistency regarding the application of WHO definitions of iodine insufficiency and interpreting the great variability in daily iodine levels (Borak, 2005). For instance, for a population to be considered iodine deficient by the WHO definition, the median urinary iodine concentration must be below 100 μg/L and at least 20% of the population must be below 50 μg/L. Borak notes that much of the confusion in the interpretation of NHANES data comes from this seemingly high number of people that may be below 50 μg/L urinary iodine. He explains "…the reason for such seemingly inconsistent criteria is the need to anticipate potentially large variability when iodine levels are measured in spot urine samples" (Borak, 2005).

Third, despite the presence of perchlorate in breast milk, studies have shown that this level of perchlorate does not cause adverse effects in children (Téllez et al., 2005; Amitai et al., 2007). Moreover, these studies have all shown doses of perchlorate that are below the NOEL from Greer et al. (2002), and are therefore insufficient to inhibit iodide uptake by the NIS. If a person, or a population, is iodine insufficient, reducing perchlorate, at environmental

relevant concentrations, will not improve iodine insufficiency. Supplemental iodine will (e.g., iodized salt) reduce or negate iodine insufficiency.

Fourth, the NRC perchlorate panel concluded that iodide deficiency if it even exists in the U.S., is mild and "…perchlorate exposure most likely would not exacerbate it" (NRC, 2005). Finally, the RfD is a conservative health protective value that is based on a NOEL with the addition of a safety factor to account for the most sensitive individuals. The HRL, which is even more conservative than the RfD, was calculated based on pregnant women and was verified using PBPK modeling to consider whether the HRL would be protective of other sensitive subpopulations, including infants, by factoring in body weight, water intake, and food intake.

IV. CONCLUSIONS

The results of this assessment demonstrate that the RfD continues to be a conservative and health-protective toxicity guideline value. As such, perchlorate concentrations in drinking water at or below the HRL proposed by EPA pose no significant health risk to the most sensitive individuals consuming water on a daily basis over a lifetime. Since the release of the RfD, additional studies have been conducted and continue to add to the scientific weight of evidence supporting the RfD (and HRL) as conservative estimates. The RfD and HRL include uncertainty spanning perhaps an order of magnitude, and estimate a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The EPA has further determined that perchlorate is not found in public water supplies at a frequency and a level that would cause public health concern. Based on this, the EPA has determined that there is not "a meaningful opportunity for health risk reduction for persons served by public water systems" by determining a NPDWR.

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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.[10]

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

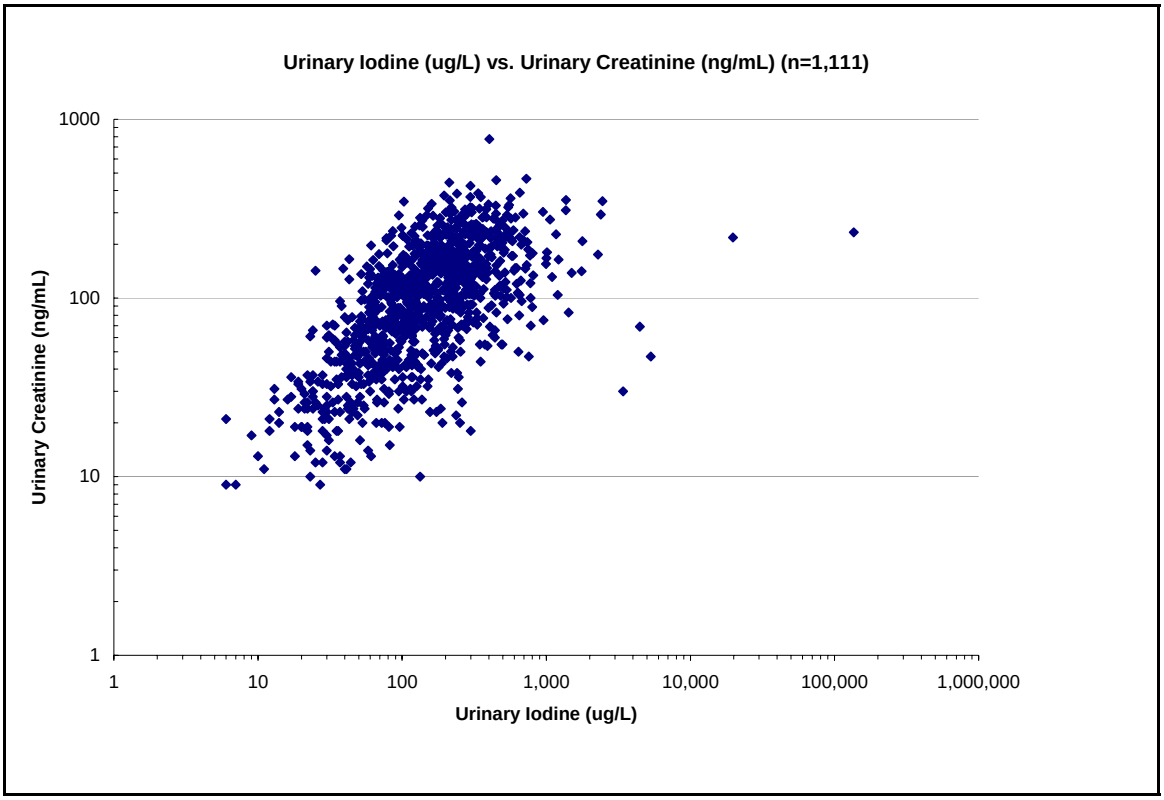
- Urinary iodine concentrations in the study group ranged from 6 to 136,161 µg/L. The geometric mean was 127 µg/L (SE = 5.4) and the 5^{th} and 95^{th} percentiles were 22 and 568 µg/L, respectively. Our findings for this group are consistent with the summary of urinary iodine measurements in NHANES 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—the WHO criteria are not meant to diagnose iodine deficiency in individuals

[10] 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.

(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)



1. 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).

2. 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.

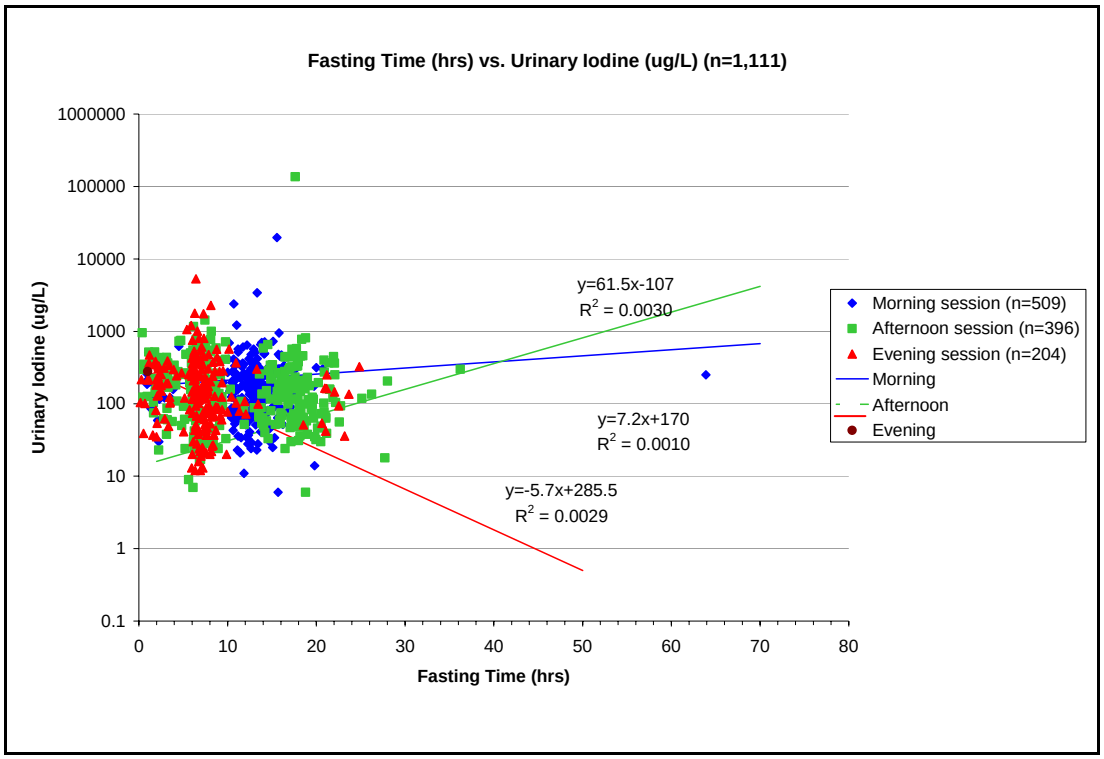
- About 2% (22/1111) admitted taking a dietary supplement during the pre-blood and urine collection fasting period. *There is evidence that dietary supplements can affect thyroid function. For example, short-term dietary supplementation with kelp (high in iodine) significantly increases both basal and 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



3. 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.

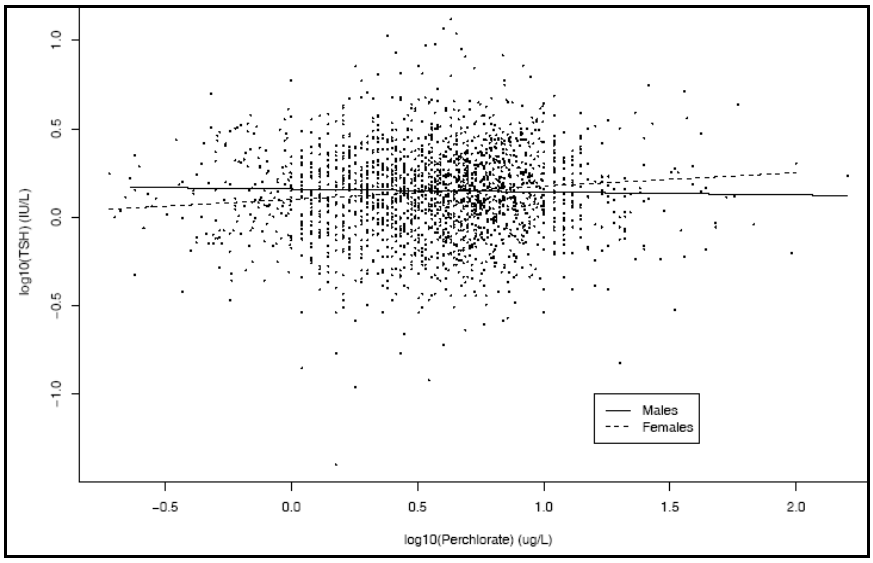
    - Thyroid autoantibodies are not measured in NHANES 2001-2002. *Women are known to be about 2.7 times more likely to acquire an autoimmune disease than men* (Jacobson et al., 1997), *and to have a greater incidence of thyroid autoimmunity* (Chiovato et al., 1993). *Thyroid autoantibody levels have been 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).

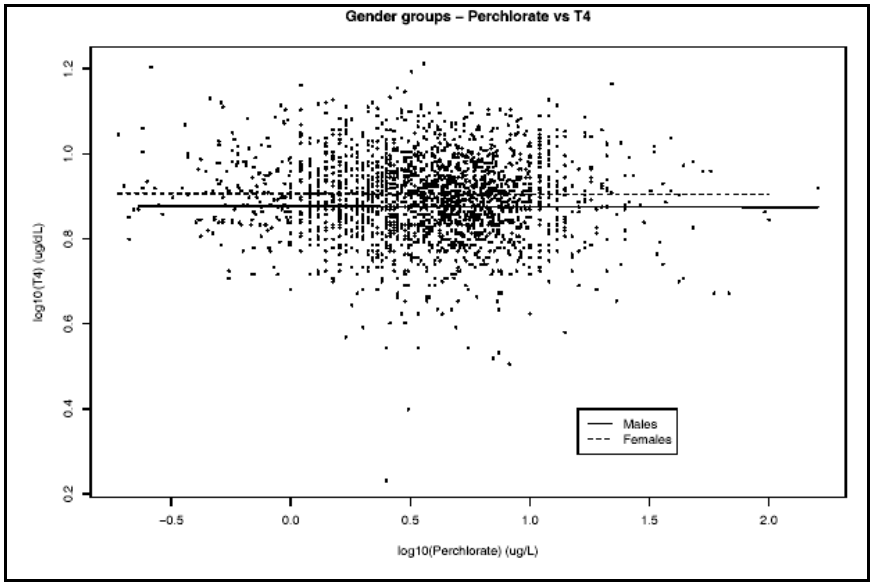
- Figures 3A and 4A depict the variability in the NHANES data set and the nominal changes noted in thyroid hormones.

Figure 3A. Variability of Perchlorate in NHANES Data Set



Charnley, 2008

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



Charnley, *personal communication*

4. 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.