



**SCIENTIFIC COMMENTS ON THE PUBLIC HEALTH GOAL FOR PERCHLORATE IN
DRINKING WATER, CALIFORNIA OEHHA, 2011**

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EXECUTIVE SUMMARY

On January 7, 2011, California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) published a risk assessment for perchlorate (the draft document) that proposes a reduction in the perchlorate PHG from its current value of 6 parts per billion (ppb) established in 2004 to 1 ppb. Perchlorate is both a naturally occurring and human made chemical. Centers for Disease Control (CDC) researchers have demonstrated that in a subset of National Health and Nutrition Examination Survey (NHANES) subjects, all participants had detectable levels of perchlorate in their urine with a median concentration of approximately 4 ppb. Exposure to this compound is not solely from human made sources. At the levels found in urine, the 95th percentile of the distribution of estimated daily perchlorate doses in the adult population is 0.234 mg/kg-day. As a result, reduction of the PHG from 6 ppb to 1 ppb in drinking water is unlikely to have any public health benefit.

The scientific support offered in the draft document is inconsistent with the weight of scientific evidence on perchlorate and is disproportionately restrictive compared with OEHHA's assessment of other environmental agents. By proposing a PHG of 1 ppb, the draft document suggests that perchlorate essentially reflects a comparable public health concern to other noncarcinogenic agents such as inorganic mercury (PHG = 1.2 ppb), beryllium (PHG = 1 ppb), and simazine (PHG = 4 ppb), and greater concern than nickel (PHG = 12 ppb). The proposed PHG for perchlorate is also similar to those for carcinogenic agents such as pentachlorophenol (PHG = 0.3 ppb).

The draft document presents several justifications for the proposed decrease in the perchlorate PHG from 6 ppb to 1 ppb. The weight of evidence does not support the draft document's approach. First, the draft PHG for perchlorate is not based on new studies that change the fundamental understanding of perchlorate toxicity: the point of departure (POD) for both the 2004 and the draft 2011 PHG is the same—a benchmark dose level based on Greer *et al.* (2002). This POD is lower than the no observed effect level (NOEL) reported in Greer *et al.* (2002), which is based on the threshold for inhibition of iodide uptake into the thyroid gland, a *nonadverse* effect. Second, the draft PHG for perchlorate differs from all other PHGs developed by OEHHA in which the PODs are based on *adverse effects*. The draft document treats this POD for perchlorate as if it were an adverse effect. This is not scientifically defensible, nor has it been supported by other expert risk assessments.

When developing target exposure concentrations for contaminants in environmental media, the general approach is to identify the most susceptible population; if this population is protected, all other members of the public will also be protected. In the 2004 perchlorate risk assessment, OEHHA identified the pregnant woman and her fetus as the most susceptible population. The most critical difference between the 2004 risk assessment and the draft document is a change in the most susceptible population to the infant. This prompted OEHHA to increase the uncertainty factor (UF) from 3, applied in its 2004 risk assessment, to 10 in its 2011 draft risk assessment, and to use infant-specific body weight and water consumption factors in the new draft. However, OEHHA evaluated the infant in 2004 and used an UF of 3; there is no justification for raising the UF in the 2011 draft to 10. Furthermore, any additional layer of protection is unwarranted given the substantial protection associated with OEHHA's use of a NOEL as its POD.

In addition, OEHHA's identification of the infant as the most susceptible population contradicts the assessments of many other authoritative bodies, including the National Academy of Sciences National Research Council (NRC), the Centers for Disease Control (CDC) Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. EPA Office of Inspector General (OIG), which have evaluated the literature on perchlorate health effects and concluded that the most susceptible population is the pregnant woman and her fetus.

Conventional approaches to risk assessment rely on the pharmacological and toxicological concept that below a certain threshold, no adverse effect will occur. At the biological NOEL for perchlorate, there is no change of physiological function; hence at this dose there can be no increase in the risk of illness. Repeated research and reviews by a number of authoritative bodies have substantiated the identity of the perchlorate NOEL—thus, there is little uncertainty about this dose. Deriving a benchmark dose based on the NOEL and then applying an additional uncertainty factor to account for potentially susceptible populations further increases the margin of safety.

The change in susceptible population, and therefore the PHG, is largely based on OEHHA's interpretation of three studies—Steinmaus *et al.* (2010), Blount *et al.* (2006), and Steinmaus *et al.* (2007)—with the first receiving the most emphasis. These three studies stand alone against the preponderance of scientific literature (note that Blount *et al.* (2006) and Steinmaus *et al.* (2007) are based on the same data set and use a similar statistical approach such that these represent only one unique study). None of the cited studies demonstrate that the infant is more susceptible than the pregnant woman.

The findings of these studies are not consistent with other studies, and their experimental approach is in question. The Blount *et al.* (2006) and Steinmaus *et al.* (2007) studies report associations between urinary perchlorate and TSH or thyroid hormone measures in women with urinary iodine less than 100 µg/L; however, these studies are ecological in design and do not have detailed perchlorate exposure information on individuals and can not establish causation, nor have they been reproduced with independent data sets. The authors of these studies offer no scientifically justified mechanism of action to support their observations. Further, these studies examined public databases that were not designed for intricate assessment of thyroid function. Reliance on these studies are not in concordance with OEHHA's directive to use the best science for establishing a PHG.

In Steinmaus *et al.* (2010), the authors report an association between higher thyroid stimulating hormone (TSH) in newborn infants and prenatal maternal perchlorate exposure. Aside from methodological issues, the population being assessed is not the infant. The study population is the pregnant woman and her fetus. After birth and until after the time the TSH measurement is taken, the infant resides in the hospital without the source of water to which the paper attributes exposure (the maternal home address). The half life of perchlorate is short (eight to ten hours) and if the perchlorate were actually affecting TSH, the effect should decrease over time. However, this is not the trend that is reported.

Steinmaus *et al.* (2010) does not support the draft document's conclusion that the infant is the most susceptible population. Problematic methodological issues with the study stem from its ecological design and reliance on TSH measured during the first 24 hours of life, which is not advised by numerous medical organizations since this is a time of rapid fluctuation in infant TSH levels. Other issues include its failure to adjust for gestational age, which the literature demonstrates is crucial in establishing the comparability of TSH levels across a large number of samples; reliance on TSH

assays designed for congenital hypothyroidism screening and not for population-based clinical studies; questions about the clinical significance of minimal statistical increases in TSH levels that are within the normal range of values; and lack of individual measurements of maternal perchlorate exposure. All of these issues make the thyroid tests difficult, if not impossible, to interpret. Collectively, these issues decrease the ability to draw appropriate scientific inference from these works.

A critical omission of the draft document is that it does not address other goitrogens that are routinely encountered as part of daily exposure, including nitrate and thiocyanate, that are found in a normal healthy diet and in drinking water. These goitrogens act on the thyroid by the same mechanism of action as perchlorate. The draft document should consider exposure to these compounds, just as, for example, U.S. EPA considers the total contribution to pesticide exposure by a particular class. The U.S. EPA OIG estimates that the contribution of perchlorate to iodide uptake inhibition (IUI) is about 1% of the total contributed by agents that affect the sodium iodide symporter (NIS)— the “pump” that transports iodine into the thyroid— which include nitrate, thiocyanate, perchlorate, and iodine deficiency. Addressing perchlorate alone would not significantly impact public health. As noted by ATSDR (2008), “Nitrate and thiocyanate are widely distributed in nature and, because both anions also inhibit RAIU [radioactive iodide uptake], as demonstrated by Tonacchera *et al.* (2004), should also be included in the discussion of the effects of inhibition of the NIS by anions.” Attempting to address the effects of IUI by focusing on a *de minimis* contributor, while not examining the major contributors (thiocyanate, nitrate, and iodine deficiency) is not scientifically justified.

The draft document should be substantially revised to include the strongest and most reliable scientific information available for perchlorate. In particular, the draft document should: (1) adopt the pregnant woman and her fetus as the most sensitive population; (2) if OEHHA decides to use the infant against advisement, adopt uncertainty factors appropriate to a NOEL-based point of departure and commensurate with the use of infant-specific body weight and water intake rates; and (3) take into account other factors that are more significant determinants of IUI (thiocyanate, nitrate, and iodine insufficiency).

In summary, OEHHA does not scientifically justify the change in the PHG or in the most susceptible population from the pregnant woman and her fetus to the infant. Further, the draft document does not address how reducing perchlorate concentrations in drinking water from 6 ppb to 1 ppb will result in additional public health benefit, particularly considering the substantially greater effects that other thyroid-active chemicals with the same mechanism of action have on the inhibition of iodide uptake.

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ABBREVIATIONS AND SELECTED DEFINITIONS

ATSDR	Agency for Toxic Substances and Disease Registry.
CDC	Centers for Disease Control.
<i>in vitro</i>	A study conducted outside a living organism in an artificial environment.
<i>in vivo</i>	A study conducted in a living organism.
IUI	Iodide uptake inhibition. Reduction of iodide uptake into the thyroid through the NIS.
LOAEL	Lowest Observable Adverse Effect Level. The lowest exposure level at which there is biologically significant increase in frequency or severity of adverse effects between the exposed population and its appropriate control group.
MCL	Maximum Contaminant Level. A federally enforceable standard set by EPA; the highest level of a contaminant that is allowed in drinking water.
µg/L	Microgram per liter. A unit of mass concentration defined as the concentration of one microgram of a substance per unit volume of the mixture equal to one liter; equivalent to a part per billion.
mg/d	Milligrams of chemical per day. Daily doses of a chemical are often described in these units; they are not normalized for weight.
mg/kg-d	Milligrams of chemical per kilogram of body weight per day. Daily doses of a chemical are often described in these units, which are normalized for weight. This is important as an identical dose in mg/d could be different in a 70 kg adult versus a 10 kg infant.
NHANES	National Health and Nutrition Examination Survey I, II.
NIS	Sodium iodide symporter. An ion pump that actively transports an iodide ion along with two sodium ions across the membrane into certain cells, particularly thyroid epithelial cells; perchlorate can transiently block this uptake.
NOAEL	No Observable Adverse Effect Level. The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects.
NOEL	No Observable Effect Level. An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control.

NRC	National Academy of Sciences National Research Council.
OEHHA	Office of Environmental Health Hazard Assessment (California).
OIG	Environmental Protection Agency Office of the Inspector General.
PBPK	Physiologically-based pharmacokinetic.
PHG	Public Health Goal. Drinking water goal set by the State of California.
POD	Point of Departure.
ppb	Part per billion.
RfD	Reference Dose. An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.
T3	Triiodothyronine. A thyroid hormone, more potent than T4; can be bound to other molecules and measured as total T3, or as the fraction available for the body to use as free T3, which is more biologically relevant.
T4	Thyroxine. A thyroid hormone, that is also is a precursor of T3, a more potent thyroid hormone; can be bound to other molecules and measured as total T4, or as the fraction available for the body to use as free T4, which is more biologically relevant.
Tg	Thyroglobulin. An iodine-containing protein found in the thyroid gland that is involved in the production of the T4 and T3 hormones.
TGL	Total goitrogen load. The combined exposure to all substances that cause IUI, particularly nitrate, thiocyanate, and perchlorate.
TSH	Thyroid stimulating hormone. A pituitary hormone that stimulates the production of thyroid hormones.
UF	Uncertainty Factor.
US EPA	United States Environmental Protection Agency.
USFDA	United States Food and Drug Administration.
WHO	World Health Organization.

1.0 INTRODUCTION

On January 7, 2011, California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) published notice of the 45-day public comment period on its *Draft Public Health Goal for Perchlorate in Drinking Water* (OEHHA, 2011). If finalized, the OEHHA (2011b) document would supersede the Public Health Goal (PHG) for perchlorate of 6 parts per billion (ppb) (equivalent to 6 µg/L) in drinking water that was published in March 2004 (OEHHA, 2004d). The 2011 document proposes a PHG of 1 ppb. The document that follows provides our comments on the draft PHG.

1.1 Overview of OEHHA's Draft Risk Assessment for Perchlorate

The calculation of the perchlorate PHG is as follows:

$$PHG (\mu g / L) = \frac{POD (\mu g / kg - d) \times BW (kg) \times RSC}{UF \times WC (L / d)}$$

The point of departure (POD) for both the 2004 and the draft 2011 PHG is the same: both use a benchmark dose level (BMDL) derived from the no observed effect level (NOEL) for iodide uptake inhibition in adult human volunteers identified in Greer *et al.* (2002). This POD is 3.7 µg/kg-d.

The POD is then divided by an uncertainty factor (UF) to account for population subgroups that may be more susceptible to the effects of perchlorate than healthy adults. Both the 2004 and draft 2011 PHGs use a UF of 10 because the Greer *et al.* (2002) study included only healthy adults and was of a short duration. However, in deriving the PHGs, the 2004 and 2011 documents differ in their determination of the most susceptible populations. In OEHHA (2004), while infants are identified as a susceptible group, other groups are identified as having more susceptibility, including the fetus, pregnant women, those with low intakes of iodine, and those exposed to substances that, like perchlorate, cause iodide uptake inhibition (IUI) into the thyroid. In OEHHA (2011b), enhanced susceptibility in infants is cited, such that the most susceptible population is changed from pregnant women to infants.

In analyzing effects on infants, OEHHA (2004) used a UF of 3 rather than 10, and the value that was calculated for the infant was thus higher than that calculated for the pregnant woman. OEHHA previously determined that a UF of 3 was appropriate because the use of infant-specific body weight and water intake rates accounted for much (6-fold) of the uncertainty. In 2011, OEHHA increased the UF applied to infants from 3 to 10, but continued to use the infant-specific body weight and water intake.

The resulting Acceptable Daily Dose (ADD) is translated into a drinking water equivalent by incorporating factors for relative source contribution (RSC), body weight (BW), and drinking water consumption (WC). Because the 2004 and 2011 PHGs assume a different most susceptible population, the values of these factors differ. It is differences in these values that account for the numeric difference in the 2004 and draft 2011 PHGs. Specifically,

- The 2004 PHG used a ratio of body weight to tap water consumption rate (BW/WC) based on the 95th percentile of the pregnant woman population, or 25.2 kg-day/L. The draft 2011 PHG uses a ratio based on the 95th percentile for infants age 0-6 months, or 4.3 kg-day/L.
- The 2004 PHG used a RSC of 60% because OEHHA believed that the daily exposure of pregnant women to perchlorate would be predominantly from contaminated drinking water, not from other sources such as food. The 2011 draft PHG uses a RSC of 73% based on the difference between the ADD and the estimated perchlorate intake from formula prepared using perchlorate free water, divided by the ADD.

The 2011 draft is not based on new science—it was derived using different values for BW, RSC, and WC because it assumes a different most susceptible population. However, in the derivation of this value, OEHHA fails to demonstrate that the current PHG is not health protective or that the proposed PHG offers additional health benefit.

OEHHA’s draft 2011 PHG is inconsistent with the weight of scientific evidence because:

- The review conflicts with the assessments of numerous authoritative bodies, including the National Academy of Sciences National Research Council (NRC), the Centers for Disease Control (CDC) Agency for Toxic Substances and Disease Registry (ATSDR), and the U.S. EPA Office of Inspector General (OIG).
- The change in susceptible population is largely based on OEHHA’s interpretation of three studies—Steinmaus *et al.* (2010), Blount *et al.* (2006), and Steinmaus *et al.* (2007) — which are not scientifically valid due to a number of methodological concerns and inconsistencies with other studies. In Steinmaus *et al.* (2010), the authors report an association between higher thyroid stimulating hormone (TSH) in newborn infants born to mothers who had an address in an area where perchlorate had been detected at greater than 5 ppb in drinking water. The other two studies report an association between women with low urinary iodine and thyroid hormone effects. These three studies are not in concurrence with the weight of evidence in the scientific literature.
- The approach OEHHA uses to develop the PHG for perchlorate differs substantively from that used to develop PHGs for other compounds, and suggests similar or greater toxicity of perchlorate compared to such agents as inorganic mercury, beryllium, simazine, and nickel.

We describe these points in greater detail in our comments that follow.

1.2 Organization of this Report

This document responds to OEHHA’s request for comments on the 2011 draft PHG. These comments are organized as follows:

- **Section 1.0, Introduction:** This section provides an overview of the PHG calculation, its key differences from the 2004 PHG, and the primary ways that OEHHA’s review is inconsistent with the weight of scientific evidence. It also provides a brief review of the State of California’s regulatory requirements for

developing PHGs and summarizes what authoritative bodies conclude about perchlorate and its mechanism of action.

- **Section 2.0, OEHHA’s Selection of Infants as the Most Susceptible Population is Not Supported by the Science:** This section summarizes the primary scientific concerns with OEHHA’s identification of the infant as the most susceptible population.
- **Section 3.0, Scientific Concerns About Assumptions Used in the Calculation of the PHG:** This section discusses concerns about specific numeric values used in the equation to calculate the PHG.
- **Section 4.0, Comparison of the PHG for Perchlorate to Other Environmental Agents:** This section compares the assumptions used by OEHHA to develop the draft 2011 PHG for perchlorate to those used to develop PHGs for other environmental agents.
- **Section 5.0, Conclusions:** This section summarizes our conclusions and recommendations.
- **Section 6.0, References:** This section provides the references used to conduct the evaluation.

1.3 Brief Review of Regulatory Requirements Set by the State of California

The Calderon-Sher Safe Drinking Water Act of 1996 requires OEHHA to review and update the risk assessments that form the basis for California’s PHGs, as appropriate, at least every five years. The PHGs must be based exclusively on scientific and public health considerations without regard to cost impacts.

OEHHA summarized the requirements of the Safe Drinking Water Act of 1996 (OEHHA, 2011b). OEHHA (2011b) states,

The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that can cause chronic disease shall be based upon currently available data and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.

7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

Of note, OEHHA also commits to using the best available science when it states that “PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature.”

1.4 Authoritative Bodies’ Conclusions About the Toxicology of Perchlorate

Data on the mechanism of action and toxicology of perchlorate are extensive, due in large part to its long use as a therapeutic agent to treat hyperthyroidism. In addition, several authoritative entities have conducted extensive scientific reviews of the toxicology of perchlorate, including the NRC, U.S. EPA, U.S. EPA OIG, and ATSDR. A summary of key conclusions from authoritative bodies about perchlorate’s health effects is provided below.

1.4.1 Perchlorate Health Effects and Mechanism of Action

Compared with many environmental pollutants, a strong breadth of scientific knowledge exists concerning the potential health effects of perchlorate exposure. The scientific literature on perchlorate represents more than six decades of scientific inquiry, largely because of perchlorate’s use as a drug during the 1950s and 1960s to treat hyperthyroidism (*e.g.*, as caused by Graves’ disease, a form of autoimmune hyperthyroidism resulting from autoactivation of the TSH receptor). Perchlorate is still used for treating some thyroid-related diseases, although it is no longer the drug of choice for hyperthyroidism. Perchlorate’s medicinal use provided scientists with valuable information about its toxicity. Where data gaps remained, animal and human studies were performed in the 1990s and early 2000s.

In sufficient doses, perchlorate can inhibit the uptake of iodide from the blood to the thyroid by reducing uptake through the sodium iodide symporter (NIS), a membrane-bound protein on the basal side of the thyroid cell (known as the thyrocyte). Other chemicals such as nitrate and thiocyanate are also able to cause IUI. In the thyroid, iodide is organified to produce thyroid hormones, and reduction in thyroid iodide stores could result in reduced production of thyroid hormones. A reduction in thyroid hormones is potentially most deleterious to the developing fetus. However, the thyroid has stores of iodide such that short-term fluctuations in iodide uptake will cause no effect. The reduction in iodide uptake must be sustained for months or more to deplete existing stores. Further, in situations when IUI is decreased, the body adapts by increasing the number and efficiency of the NISs to pump more iodide into the thyroid. This is an adaptive response by the body.

Since U.S. EPA began evaluating perchlorate in the early 1990s, scientists from government, industry, and academia have made important contributions to reducing uncertainties about the risks of perchlorate to public health. During this time, at least 12 toxicological studies in

animals were conducted according to U.S. EPA protocols. These included pharmacokinetic, subchronic, developmental, and immunotoxicology studies and a multigenerational reproductive study, using a range of doses. These studies were evaluated independently by U.S. EPA.

In 2005, by request of U.S. EPA and the Department of Defense, the NRC was asked to review the data on the health effects of perchlorate. The NRC conducted an examination of the perchlorate literature and evaluated the risks associated with ingestion of perchlorate at environmental levels. The report, published in 2005, concluded:

- At doses above environmental levels but below therapeutic doses, IUI is the only consistently documented effect of perchlorate exposure in humans.
- As shown in Greer *et al.* (2002), IUI is only observed at doses greater than 0.007 mg/kg-d (equivalent to 245 ppb in drinking water). IUI is a reversible biochemical phenomenon and is not an adverse effect. The NOEL for IUI reported in Greer *et al.* (2002) is consistent with other clinical studies.
- Extensive human and animal data demonstrate that no adverse effect will occur if no IUI occurs.
- Thyroid hormone (thyroxine (T4) or triiodothyronine (T3)) changes are not necessarily adverse because these levels adapt in response to various environmental stimuli (*e.g.*, temperature, illness, or diet) on a daily and seasonal basis.
- Basing the risk assessment on a dose at which no effect, including a nonadverse effect, occurs is conservative and health protective compared to using a dose at which adverse effects occur.

In a typical risk assessment, safety factors are applied to the no observable adverse effect level (NOAEL) or the lowest observable adverse effect level (LOAEL) to determine the reference dose (RfD)¹. However, as the NRC (2005) stated 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.” In recommending a perchlorate RfD based on a nonadverse effect, the NRC (2005) emphasized it was taking an unconventional and unusually cautious approach:

The committee emphasizes that its recommendation differs from the traditional approach to deriving the RfD. The committee is recommending using a nonadverse effect rather than an adverse effect as the point of departure for the perchlorate risk assessment. Using a non adverse effect that is upstream of the adverse effects is a conservative, health-protective approach to the perchlorate risk assessment.

To derive an RfD, the NRC (2005) divided the NOEL by a UF of 10 to account for potential variation in response by the most susceptible individuals in a population—hypothyroid or iodine-deficient pregnant women and their developing fetuses. Regarding database

¹ An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

uncertainty, NRC states “the database is sufficient, given the point of departure selected—one based on inhibition of iodide uptake by the thyroid,” and so no further UF was applied.

The 2005 NRC report is the capstone of the scientific literature on perchlorate. Since its release, no scientific evidence has been developed to suggest that doses lower than the Greer *et al.* (2002) NOEL will cause any effect. Most of the studies published since 2005 focus on characterizing rates of exposure to perchlorate by men, women, and infants. For example, some studies estimate exposures to infants through breast milk or from food. These studies are useful for conducting risk assessments; however, they do not change the foundational science regarding perchlorate’s mechanism of action or toxicity.

In addition, perchlorate is water soluble, has a half life in humans of approximately eight hours, and does not bioaccumulate. Because its half life is short, some have questioned whether exposure to perchlorate might cause an acute effect. Toxicologically speaking, “acute” describes effects seen after a single high dose or an exposure of less than 24 hours. No published study has demonstrated an effect after a single exposure to perchlorate at or near environmental levels. This is not unexpected considering, for example, that T4 has a half life of six to eight days and must be sufficiently depleted for adverse effects to occur.

Other agencies, *e.g.*, the ATSDR (2008), have conducted rigorous reviews of the scientific literature regarding the potential for adverse health effects from perchlorate exposure and have determined that doses equivalent to the current RfD of 0.7 µg/kg-d (equal to 24.5 ppb assuming a 70-kg adult drinking 2 L/d) are health protective. U.S. EPA OIG (2010) also reports:

The OIG Analysis also confirmed that EPA’s perchlorate RfD is conservative and protective of human health, but limiting perchlorate exposure does not effectively address this public health issue [mild to moderate iodine deficiency].

1.4.2 Prevention of Iodide Uptake Inhibition Prevents Downstream Events

Perchlorate’s reversible inhibition of the NIS is documented in many experiments, both *in vitro* and *in vivo*. Perchlorate competitively causes IUI; it neither mimics a hormone nor directly stimulates a response. The inhibition of the NIS exhibits the sigmoidal dose-response curve commonly observed in toxicology and pharmacology—as dose increases, so does the response. In Greer *et al.* (2002), IUI precedes the potential adverse effect of hypothyroidism by several biochemical steps. There is no scientific evidence to suggest that low doses of perchlorate will cause any other effect.

The biological action of perchlorate (its “mechanism of action”) on the thyroid gland, including the delineation of what is and what is not considered an adverse effect, is summarized in Figure 1.

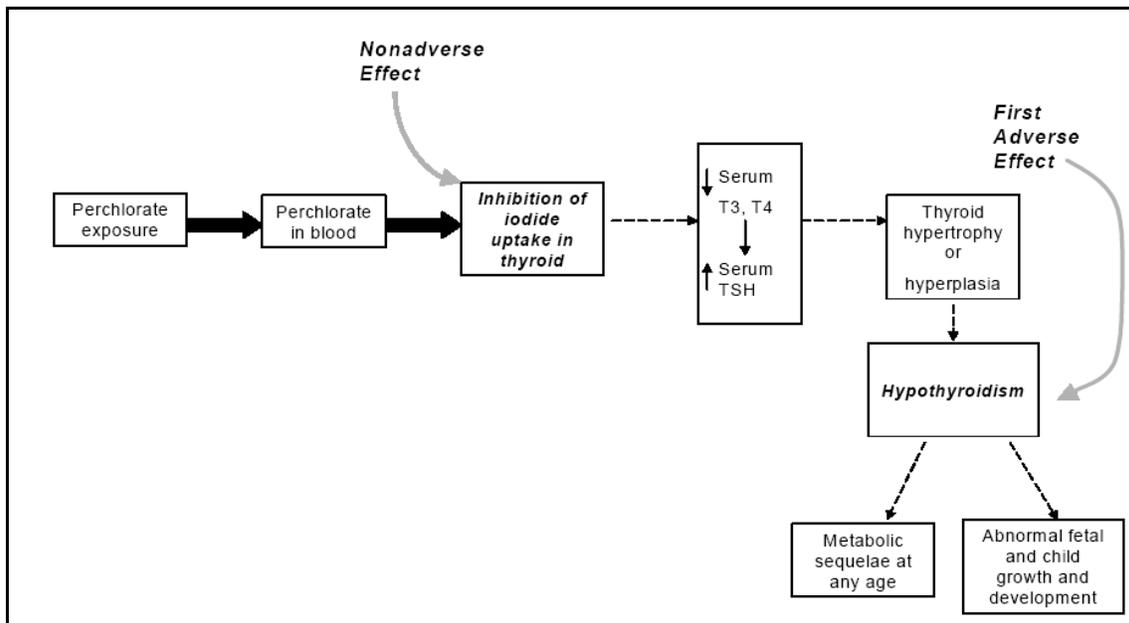


Figure 1. Mechanism of Action for Perchlorate as Summarized in the NRC (2005) Report*

*The NRC's suggested mode-of-action model of perchlorate toxicity in humans. Solid arrows represent outcomes that have been observed in humans with perchlorate doses greater than 0.007 mg/kg-d. Dashed arrows represent outcomes that have not been clearly demonstrated in humans exposed to perchlorate but that are biologically possible in the absence of adequate compensation. This includes the first proposed adverse effect, hypothyroidism. The thyroid response to increased serum TSH and an independent increase in thyroid iodide uptake would raise T3 and T4 production to normal and therefore usually prevent the later steps from occurring (NRC, 2005).

Regarding downstream effects, NRC (2005) states they

...have not been clearly demonstrated in humans exposed to perchlorate but that are biologically possible in the absence of adequate compensation. The thyroid response to increased serum TSH and an independent increase in thyroid iodide uptake would raise T3 and T4 production to normal and therefore usually prevent the later steps from occurring.

The NRC concluded that individuals with 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 could occur. In adults, that dose is estimated as being no lower than 30 mg/d (0.4 mg/kg-d for a 70-kg person, equivalent to drinking two liters of water with a perchlorate concentration of 15,000 ppb every day).

This estimate is based on doses given therapeutically without adverse effect. In therapeutic dosing, perchlorate is administered at high doses for extended periods. Historically, if perchlorate was used to treat a person with hyperthyroidism, high initial doses (6.2 to 20.5 mg/kg-d; Crooks and Wayne, 1960) were often given, sometimes followed by lower doses to maintain normal thyroid hormone levels. The treatment lasted a minimum of several weeks (depending on how responsive a patient was) to 22 years in one documented case (Connell, 1981). Most studies of therapeutic effects followed people for several months of treatment.

In a study reported by Wenzel and Lente (1984), eighteen patients were treated in the 1980s for Graves' Disease. Subjects were treated initially with 900 mg potassium perchlorate/d (9.2 mg/kg-d assuming a 70 kg adult²). As serum thyroid hormone concentrations declined (within two to four months following initiation of treatment), the dose of potassium perchlorate was reduced to between 40 and 120 mg/d (0.4 – 1.2 mg/kg-d for a 70-kg person) for the 12-month to 24-month maintenance period. The average dose for eight “late responders”³ was 93 mg/d, while 10 “early responders” had an average dose of 76 mg/d (Wenzel and Lente, 1984). During this time, all patients had normal serum T4 and T3 levels and most had normal levels of TSH receptor stimulating antibodies. No side effects were noted and all 18 patients were able to complete the study without developing hypothyroidism (NRC, 2005 citing Wenzel and Lente, 1984). The NRC has reviewed the study and supporting data and concluded that 0.4 mg/kg-d represents an appropriate point of departure for assessing adverse effects of perchlorate exposure.

The minimum dose estimated to cause an adverse effect (hypothyroidism) is approximately 57 times greater than the dose at which no effect, even IUI, occurs. In turn, the NOEL dose is approximately 40 times greater than the current PHG. As depicted in Figure 2, the current PHG has a large margin of safety and is adequately health protective and conservative.

Finally, the level of IUI that would be associated with exposure to perchlorate at the RfD is minimal compared to the IUI associated with other environmental goitrogens naturally present in many foods. Nitrate and thiocyanate are less

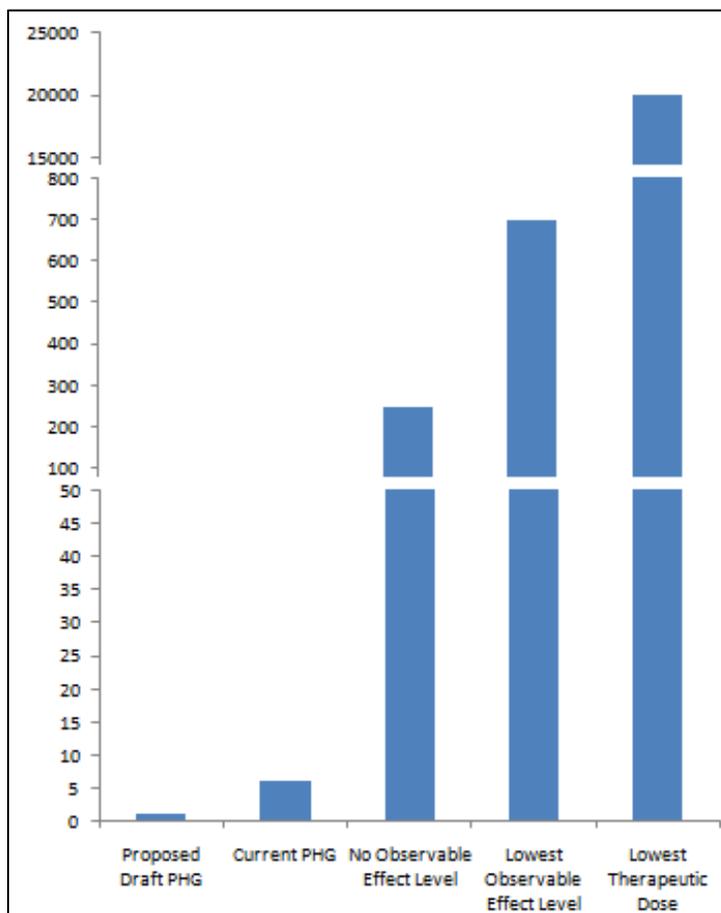


Figure 2. Comparison of Proposed and Current PHG, NOEL, LOAEL, and Therapeutic Doses of Perchlorate

² The calculation for perchlorate dose equivalence in studies that used potassium perchlorate therapeutically is as follows: $\text{Dose KClO}_4 \times \text{MW ClO}_4^- / \text{MW KClO}_4 = \text{dose ClO}_4^-$. MW $\text{ClO}_4^- = 99.45 \text{ g/mol}$; MW $\text{KClO}_4 = 138.55 \text{ g/mol}$.

³ The original research paper (Wenzel and Lente, 1984) does note some difficulty in controlling hyperthyroidism in five of the patients (prior to the minimum 12 month maintenance period). The authors hypothesize that one potential cause of this was excess iodine intake by these five patients, meaning diet was uncontrolled during the study period. Several chemicals found commonly in the diet, including nitrate, thiocyanate, fluoride, and iodide, also cause IUI. These chemicals have varying potencies, but can be expressed in perchlorate dose equivalents. Changes in the intake of these chemicals could affect the results of the study. However, dietary intake (in dose equivalents) of these other chemicals is likely to be much lower than the therapeutic doses of perchlorate given in the study. Therefore the potential confounding would be minimal.

potent (Tonacchera *et al.*, 2004) but more plentiful inhibitors of NIS activity than perchlorate (Belzer *et al.*, 2004). The potency of nitrate and thiocyanate relative to perchlorate has been demonstrated *in vivo* (Wyngaarden *et al.*, 1952, 1953; Greer *et al.*, 1966; Belzer *et al.*, 2004) and *in vitro* (Tonacchera *et al.*, 2004). The effects of perchlorate are much smaller than the effects of either nitrate or thiocyanate (Belzer *et al.*, 2004; U.S. EPA, 2010). The potential for perchlorate to cause IUI cannot be distinguished from the effects of other NIS inhibitors (De Groef *et al.*, 2006). Because exposure to nitrate and thiocyanate would continue even if perchlorate levels are reduced, OEHHA's attempt to isolate the effect of perchlorate has limited ability to provide an actual public health benefit.

1.4.3 The Pregnant Woman and the Fetus are the Most Susceptible Population

The fetus is understood to be the most sensitive population to perchlorate health effects. NRC (2005) states, "For the perchlorate risk assessment, potentially the most sensitive population is fetuses, particularly those of pregnant women who have hypothyroidism or iodide deficiency." It has also been well documented that in pregnant women who have untreated hypothyroidism, there may be effects on neurological development of the fetus (NRC, 2005). There is also a body of evidence supporting that untreated hypothyroidism, *e.g.*, as caused by primary congenital hypothyroidism, can affect neurological development in the newborn, although treatment is effective at preventing this if started within the first two to three weeks after birth (NRC, 2005). The fetus receives T4 from its mother and concentrations in the mother are similar to those in the fetus at birth (Abuid *et al.*, 1973).

In its extensive review of the literature in 2008 to define a Minimum Risk Level (MRL) for perchlorate, ATSDR concluded that the fetuses of pregnant women who might have hypothyroidism or iodide deficiency are the most sensitive population to perchlorate exposure. In 2010, U.S. EPA OIG (2010) also concluded that "the most sensitive population for cumulative risk assessment purposes is the iodide-sensitive fetus and neonate during gestation and lactation whose mothers are iodide deficient."

1.4.4 Conclusions Regarding Studies Cited to Support OEHHA's Draft PHG Document

While numerous authoritative bodies have reviewed the toxicology of perchlorate, OEHHA (2011b) disregards these scientific assessments. Appendix A summarizes key studies that OEHHA cited to support their risk assessment and the assessment of those studies by authoritative bodies. In brief:

- OEHHA asserts that studies of the association between perchlorate exposure and newborn thyroid hormone levels (*e.g.*, Kelsh *et al.*, 2003; Brechner *et al.*, 2000; Li *et al.*, 2000; Crump *et al.*, 2000) provide "a consistent body of evidence linking perchlorate exposure during pregnancy with changes in T4 or TSH levels in the newborn." However, authoritative bodies have carefully assessed these studies and determined that they show no association between perchlorate exposure and thyroid hormone levels. In the case of the Brechner *et al.* (2000) study, the study methods have been criticized.

- OEHHA cites Blount *et al.* (2006) and Steinmaus *et al.* (2007) as “key studies supporting two of the potential susceptibility groups identified by OEHHA (women with low iodine and women with high thiocyanate).” However, U.S. EPA OIG (2010) states,

“Until researchers can explain the increased toxicity of perchlorate by a verified biological mechanism, regulators should not use the Blount analysis as a basis for developing a perchlorate RfD, nor should it be used as the basis for establishing a drinking water limit,” and further, “the Steinmaus analysis does not corroborate the Blount analysis, because both studies use the same NHANES 2001-2002 dataset.”

- OEHHA indicates that in developing a PHG, the ADD is calculated by dividing the NOAEL, LOAEL, or BMDL by UFs. For perchlorate, OEHHA (2011b) states,

...we have chosen to estimate the ADD from the lower limit of the two-sided 95 percent confidence interval of the perchlorate dose estimated to cause a five percent reduction in iodide uptake in the thyroid gland based on the findings of Greer *et al.* (2002).

That is, OEHHA considers IUI as reported in Greer *et al.* (2002) to be an adverse effect. However, the NRC (2005) is clear that IUI should not be considered an adverse effect, stating,

The committee is recommending using a nonadverse effect rather than an adverse effect as the point of departure for the perchlorate risk assessment. Using a nonadverse effect that precedes the adverse effects is a conservative, health-protective approach to the perchlorate risk assessment, and the committee’s recommendations for uncertainty factors reflect the conservatism of the approach.

2.0 OEHHA'S SELECTION OF INFANTS AS THE MOST SUSCEPTIBLE POPULATION IS NOT SUPPORTED BY THE SCIENCE

The fundamental difference between the 2004 and draft 2011 PHG risk assessments is OEHHA's determination of the most susceptible population. However, OEHHA's selection of the infant as the most susceptible population in the 2011 risk assessment is not supported by the science.

OEHHA's justification for selection of the susceptible population is as follows:

- OEHHA contends that studies from California and elsewhere (Kelsh *et al.*, 2003; Brechner *et al.*, 2000; Buffler *et al.*, 2006; Steinmaus *et al.*, 2010; Li *et al.*, 2000; Crump *et al.*, 2000) provide evidence that TSH or thyroid hormone levels in infants were adversely affected by perchlorate at exposure levels much lower than the levels shown to cause no effects in healthy adults.
- OEHHA claims that new data suggests that many breast fed infants may not be receiving adequate iodine in their diets.
- OEHHA contends that young infants have low stores of thyroid hormone (less than one day's worth, compared to several weeks' worth in adults) (van den Hove *et al.*, 1999).
- New drinking water intakes per body weight from the U.S. EPA suggest that infants are likely to have greater perchlorate exposure per body weight than was assumed in the 2004 risk assessment.

Comments on these points are provided below.

2.1 The Draft Document is Incorrect that Cited Studies Provide Evidence that Thyroid Hormone Levels in Infants were Adversely Affected by Perchlorate at Low Exposure Levels

The draft PHG document cites studies by Kelsh *et al.* (2003), Brechner *et al.* (2000), Buffler *et al.* (2006), Steinmaus *et al.* (2010), Li *et al.* (2000), and Crump *et al.* (2000) as providing evidence that the infant is the most susceptible population. Our comments on Steinmaus *et al.* (2010) are addressed specifically in Section 2.2. The other studies are addressed in turn in subsequent sections. In evaluating these studies, a couple of general comments are particularly important.

First, it is important to recognize that the design of these studies (ecological) cannot determine causality. Rather, ecological studies provide important insights as they can identify associations which, in the context of what is understood about the mechanism of action of a chemical, can provide support to other toxicological or epidemiological studies.

Regarding ecological studies and perchlorate, the NRC (2005) states,

Ecologic studies can provide supporting evidence of a possible association but cannot themselves provide definitive evidence regarding cause... No studies have examined

the relation of perchlorate exposure and adverse outcomes, either in thyroid function or in neurodevelopment, among especially vulnerable groups, such as low-birthweight or preterm infants.

Second, the reported thyroid hormone and TSH measures in these studies are not clinically significant or indicative of adverse effects, and the draft document's interpretation of the majority of the studies differs from the finding of no association reported by the authors of the studies themselves.

2.2 Scientific Concerns Regarding Steinmaus *et al.* (2010)

The draft document places significant weight on Steinmaus *et al.* (2010), largely justifying the change in susceptible population from the pregnant woman to the infant based on this paper. Steinmaus *et al.* (2010) assessed the association between maternal drinking water perchlorate exposure during pregnancy and newborn TSH levels, using data collected for other purposes.

Steinmaus *et al.* (2010) correctly points out that,

...currently it is unknown whether the effects seen here cause actual impacts on health and development. Further research is needed on this issue, and needed to evaluate the possible role that iodine, thiocyanate, nitrate, and other thyroid-active agents may have played in these findings.

While Steinmaus *et al.* (2010) adds to the database of literature, a number of scientific issues challenge the interpretation of the results. The authors state that many of these issues would result in nondifferential misclassification (*i.e.*, occurring in the same proportion in each group) that would bias the results towards the null; however, the authors do not discuss other confounders such as issues with study methodology or collection that could result in differential misclassification (*i.e.*, occurring in different proportions in each group), that leads to a greater apparent association between the measured variables than is truly caused by these variables.

The following specific issues will be expanded on in this section:

- Any effects seen in the neonate would have resulted from exposure to the pregnant woman and her fetus. The results *reinforce* the pregnant woman and her fetus as the most sensitive population.
- TSH measurement bias and uncertainty was not considered.
- A cross sectional ecological study design does not collect individual exposure data and cannot demonstrate causality.
- Differences between “exposed” and “unexposed” groups are not clinically significant and no adverse effect is measured.

- No measure other than TSH was presented in this study to understand thyroid status. Elevated TSH levels collected during neonatal screening must be followed up by analysis of other thyroid hormones such as T3, T4, free T3, or free T4.
- The recognized TSH surge in neonates makes measurement of TSH levels within the first 24 to 48 hours unreliable.
- Timing of collection of neonatal TSH samples varies greatly.
- The authors do not control for gestational age of neonates, a key variable affecting measured TSH levels.
- The Newborn Screening Program (NBS) was not intended for intricate assessment of thyroid function.
- Questions arise regarding the statistical characterization of the TSH data.
- OEHHA recognizes limitations of Steinmaus *et al.* (2010), yet still uses it as the basis for changing susceptible populations.

Further, as discussed in the next section, previous studies using the same data set and independent data sets do not support the conclusions of Steinmaus *et al.* (2010) (Sections 2.3 and 2.4), nor do animal studies and PBPK modeling (Section 2.5).

2.2.1 Effects seen in the neonate would have resulted from exposure to the pregnant woman and her fetus

Steinmaus *et al.* (2010) present TSH data collected by the California Department of Public Health (CDPH) NBS program and data on perchlorate levels in community water collected in the CDPH Drinking Water Program for the years 1997–1998. Neonatal TSH was measured in 497,458 infants born in 1998. Infants were classified as “exposed” if their mother’s residence was in a community with “estimated average perchlorate” concentrations higher than 5 ppb. Infants were classified as “unexposed” if “estimated average perchlorate” concentrations were 5 ppb or lower. This level was chosen because it is “near the California detection limit for reporting of 4 µg/L and the current California perchlorate regulatory standard of 6 µg/L.”

Thus, Steinmaus *et al.* (2010) clearly evaluate maternal and fetal exposure, not infant exposure. Although no individual exposure data are provided, the analysis assumes that the perchlorate exposure occurred at the mother’s home address. Once the pregnant woman arrives at the hospital, home exposure would have effectively stopped and exposure at a different location, the hospital, would have begun (no data are provided on hospital exposure). Within 24 hours of birth, the breast fed infant would have had no other source of exposure to perchlorate than fetal exposure, breast milk, and possibly colostrum (a protein rich fluid secreted prior to milk production). The authors conclude that for infants with TSH samples collected within 24 hours of birth, the odds ratios for TSH greater than 25 µIU/mL and greater than 15 µIU/mL in exposed communities were 1.53 ($P < 0.0001$) and 1.23 ($P < 0.001$), respectively, suggesting that perchlorate is associated with increased neonatal TSH levels. However, if taken to be true, this finding reflects a fetal or maternal exposure to perchlorate, and does not demonstrate that the infant is more susceptible.

Furthermore, assuming that perchlorate exposure is associated with the home address, after the mother leaves her home (and potential exposure source) and the child is born, the mother and neonate will continue to excrete perchlorate and serum perchlorate corresponding with prenatal exposure will decline rather quickly. Since the half life of perchlorate is short (approximately eight hours), after about three half-lives (approximately 24 hours), 87.5% of perchlorate will have been excreted. The pharmacokinetics of neonates are not substantially different from those of adults (Appendix B).

Figure 1 in Steinmaus et al. (2011) does not support increased odds of greater TSH in newborns from perchlorate-exposed communities compared to unexposed communities. After 24 hours, Steinmaus *et al.* (2010) report no association between perchlorate exposure and TSH levels greater than 25 μ IU/mL, which is consistent with a previous study (Buffler *et al.*, 2006), but do report an increased odds ratio between perchlorate exposure and TSH levels greater than 8 μ IU/mL. Without continued exposure, any increased odds ratio associated with perchlorate exposure should diminish after 24 hours. However, Steinmaus *et al.* (2010) continue to report an increased odds ratio up to 64 hours after birth. By 64 hours after birth, one would expect essentially no contribution of perchlorate from the mother's home. Since most perchlorate ingested at the home address would be excreted within 24 hours of arrival at the hospital, it is unlikely that the increased odds ratio presented by Steinmaus *et al.* (2010) is due to perchlorate in drinking water at the mother's home.

2.2.2 The uncertainty in neonatal TSH assays is not discussed

Many sources of error can affect TSH measurements. In evaluating the results of a TSH screening program, an important question is whether the TSH assay has the sensitivity, specificity, reliability, reproducibility, and positive and negative predictive value to differentiate true TSH levels and thyroid status. Steinmaus *et al.* (2010) do not comment on uncertainties with the assay results.

Like all measurements, measurements of TSH possess some degree of measurement uncertainty which must be understood if the results measured across different programs are to be comparable. Regarding measurement uncertainty,

When reporting the result of a measurement of a physical quantity, it is obligatory that some quantitative indication of the quality of the result be given so that those who use it can assess its reliability. Without such an indication, measurement results cannot be compared, either among themselves or with reference values given in a specification or standard. It is therefore necessary that there be a readily implemented, easily understood, and generally accepted procedure for characterizing the quality of a result of a measurement, that is, for evaluating and expressing its uncertainty (Medicare, 2010).

Furthermore, in the State of California, six to eight contract laboratories conduct neonatal TSH assays. This introduces variability into the NBS data set.

Current TSH assay techniques (enzyme-linked immunoassays, chemiluminescent assays, and fluoroimmunoassays) use nonradioactive labels and have improved sensitivity with the potential for better separation between normal and abnormal TSH concentrations. The State

of California currently use DELFIA Neonatal hTSH kits, which are intended for the quantitative determination of TSH in blood specimens dried on filter paper as an aid in screening newborns for congenital hypothyroidism. The analytical sensitivity of the solid-phase, two-site fluoroimmunoassay is typically better than 2 $\mu\text{IU/mL}$ blood (4.4 $\mu\text{IU/mL}$ serum). As a comparison, PerkinElmer produces a diagnostic TSH assay called DELFIA® TSH Ultra, with functional sensitivity below 0.007 $\mu\text{IU/mL}$. The TSH Ultra reports a range of 0.005 – 100 $\mu\text{IU/mL}$ (PerkinElmer, n.d.).

The CDC's National Center for Environmental Health has programs to help assure the quality and accuracy of data, with the Newborn Screening Quality Assurance Program (NBSQAP) focusing specifically on laboratory tests that are related to newborn screening. Laboratory standardization is achieved when test results with the same high levels of accuracy and precision can be reproduced across measurement systems, laboratories, and over time. Standardization depends on a rigorous process that uses reference materials or controls sent to laboratories across the nation to evaluate analytical equipment. The NBSQAP sends a sample of known quantity to laboratories, and the results are returned to CDC and assessed.

Acceptable ranges for results are ± 2 standard deviations, which corresponds to 95th percentile confidence limits. An illustration of the results of this screening is shown in Figure 3. The results of CDC's program for the AutoDELFLIA and the DELFLIA methods in February 2010 are found in Table 1.

Given this potential for variability, Steinmaus *et al.* (2010) must demonstrate that the precision, sensitivity, and specificity of the neonatal assays yielded results that can be used in a reliable manner in their assessment.

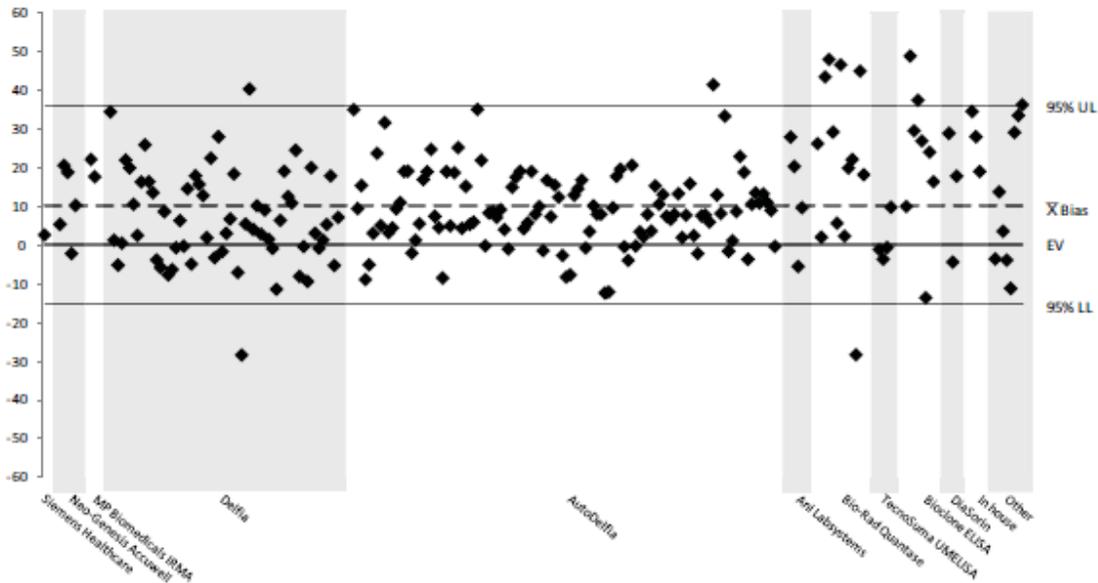


Figure 3. Bias Plot of TSH Values by Measurement Method *

*These data reflect measurements of control samples sent by CDC to state laboratories in the U.S. The units are $\mu\text{IU/mL}$. EV is the standard sent by CDC. \bar{X} Bias is the mean of the results for the CDC controls. Upper and lower 95th ile confident limits are presented. The overall statistics are for an aliquot of 7 $\mu\text{g/mL}$ — the CDC lab assayed 7 $\mu\text{g/mL}$, while the participant mean was 7.7 $\mu\text{g/mL}$, indicating a participant bias of 0.7 $\mu\text{g/mL}$ (CDC, 2010).

Table 1. Summary of Reference Sample Prepared by CDC and Assayed Using AutoDELPHIA or DELFIA.

Neonatal TSH Method	Control Amount ($\mu\text{IU TSH/mL Serum}$)	N (Lot 8XX; 9XX)	Mean (Lot 8XX; 9XX)	Total SD (Lot 8XX; 9XX)
AutoDELPHIA	25	789; 875	24.7; 28.2	2.9; 2.7
	40	786; 893	36.8; 46.1	3.8; 4.6
	80	784; 891	79.5; 92.5	8.8; 9.2
DELFIA	25	466; 493	25.5; 26.7	3.6; 2.9
	40	470; 492	37.2; 44.6	4.6; 4.6
	80	476; 489	80; 90.2	9.7; 9.0

These data reflect measurements of control samples sent by CDC to state laboratories in the U.S. The DELFIA and AutoDELPHIA (i.e., automated DELFIA) methods are presented. This is the current method used by the California NBS (CDC, 2010).

2.2.3 *The study lacks individual measures of perchlorate exposure*

A cross sectional study design does not allow for individual measures of exposure, necessitating broad assumptions about this characteristic. For example, to estimate perchlorate exposure, Steinmaus *et al.* (2010) assumes that the mother drinks mostly tap water, the tap water concentration is consistently above or below 5 ppb, and neither dietary impacts of perchlorate nor dietary or drinking water impacts of other thyroid-active compounds are significant. However, these assumptions very likely oversimplify perchlorate exposure, leading to misclassification. For example, the mother could have moved in and out of an exposure area during pregnancy, perchlorate concentrations within an area likely varied, the average diet could have contributed significantly to perchlorate exposure (Murray *et al.*, 2008), and other goitrogenic compounds such as nitrate and thiocyanate that are found in much greater quantities in the average diet likely contributed more significantly to IUI than perchlorate (Section 2.7).

2.2.4 *Minor differences in TSH levels are not likely clinically significant*

While Steinmaus *et al.* (2010) report some statistical differences in TSH measures between groups, these differences are not likely to be clinically significant. The authors do not report any adverse effect on the neonate, nor do they report any persistent effect. It is important to interpret statistical significance based on what is known about the biological system—there is a difference between “statistical significance and “clinical significance.” A “statistically significant” difference means it is unlikely that a difference between two variables (*e.g.*, measurements) is due to chance. However, because statistical significance is also a function of sample size, variability in the variables measured, and other parameters, a finding of statistical significance does not necessarily mean the difference is large, important, or biologically significant, that is, that the change in a variable or parameter is large enough to affect a clinical or medical condition (*e.g.*, development of hypothyroidism).

For example, Steinmaus *et al.* (2010) cite geometric means for neonate TSH levels (all available ages) in the ≤ 5 $\mu\text{g/L}$ and > 5 $\mu\text{g/L}$ groups as 4.03 $\mu\text{IU/mL}$ (SD = 5.84) and 4.35 $\mu\text{IU/mL}$ (SD = 4.83), respectively. The authors note that these values are statistically significantly different ($p < 0.001$); however, the clinical implication of this difference is not discussed and is almost certainly not significant. Differences are also apparent in other groups, though not explained and of no apparent clinical significance. For example, the geometric mean TSH level in Asians is 4.40 $\mu\text{IU/mL}$ (SD = 6.01), which is statistically significantly different from Hispanics (4.01 $\mu\text{IU/mL}$, SD = 6.28). The greatest influence on statistical significance for any of these variables is likely related to the sample sizes, which are very large. For example, the sample sizes for Hispanics, non Hispanics, and Whites are 241,357, 256,101, and 148,009 respectively.

2.2.5 *TSH is the only variable obtained to assess thyroid physiology*

The limitations of only using one thyroid parameter to assess thyroid physiology can not be overemphasized. While TSH is useful for screening in newborns, the NBS program retests TSH for verification and then, if TSH levels are still deemed elevated, more thyroid testing is undertaken. Thyroid hormones are key for growth, metabolism, and brain development, and the parameters included in studies of thyroid function in premature infants and neonates

include T3, cord T4, fT4, thyroglobulin (TGB), TSH, rT3, and in some cases, serum T4 (Williams *et al.*, 2004; Simpson *et al.*, 2005 ; Hume *et al.*, 2004). Testing for these variables allows for more precise understanding of thyroid state. Measuring the full complement of thyroid hormones also allows the determination of ratios, *e.g.*, TSH:fT4, which provide important information about thyroid response for a given sample period.

2.2.6 *The State of California’s Newborn Screening Program provides clear warning to state medical institutions not to take samples before 24 hours*

Shortly after birth, the pituitary of the newborn releases a surge of TSH. This is natural and expected. Over the next hours and days, if the baby was born with a properly functioning thyroid gland, TSH levels resolve to normal levels. If the thyroid gland is absent or its capacity severely limited, then serum TSH remains high or increases. Steinmaus *et al.* (2010) attempt to characterize thyroid status during this period using a limited publicly available data set that does not have the specificity or sensitivity needed to understand the true thyroid status.

As Steinmaus *et al.* (2010) correctly state:

Neonatal TSH levels normally surge within the first few hours after birth, peaking at about 2 hours after birth and steadily decreasing to normal long-term levels over the next 48 to 72 hours.

In utero, a fetus receives T4 from its mother and, at birth, maternal T4 and cord blood T4 are similar (Abuid *et al.*, 1973). At birth, the neonatal pituitary gland releases a surge of TSH that increases endogenous T4 and T3 (Abuid *et al.*, 1973). The early TSH surge ceases by approximately 30 minutes after birth, but a sustained hypersecretion of TSH persists through 24 to 48 hours after birth (Fisher and Odell, 1969). Because of this instability in TSH levels, screening for congenital hypothyroidism (CH) is recommended after 24 to 48 hours (AAP, 2006).

Lott *et al.* (2004) found that based on data from 161,244 newborns collected at the Newborn Screening Laboratory in Columbus, Ohio, approximately 20% of newborns with specimens collected within the first 24 hours had TSH levels ≥ 20 μ IU/mL, and concluded that “blood specimens collected within the first 24 h are undesirable because there are many babies with a high TSH who do not have hypothyroidism.” They found no confirmed cases of hypothyroidism in babies with a TSH of <29 μ IU/mL.

Newborn Screening Programs, including that in California, are adamant about when to obtain blood samples, requiring that a screening sample for TSH be collected at discharge from the hospital, prior to a red blood cell transfusion, or, for infants with extended hospital stays, on the sixth day of life (CDPH, 2010). The State of California states the following regarding its program:

Timing of the Collection (Section 6505):

...early collection **can** affect the results for other metabolic and hypothyroidism screening. It can result in a false positive for primary congenital hypothyroidism due to the biological phenomenon known as “neonatal surge.”[emphasis in original].

They further state,

Obtain a specimen from a newborn using the following requirements and guidelines:

- * Collect the specimen as close to time of discharge as is practical, but no earlier than two hours prior to discharge, when discharge occurs before six days of age.

- * Collect the specimen on the sixth day if the newborn remains hospitalized beyond five days of age.

- * The newborn should be at least 12 hours old since a neonate's metabolic system is still stabilizing before 12 hours of age.

And, in 2001, the California Department of Health Services stated (CDHS, 2001):

Beginning April 2001, as a result of new California State Regulations, newborns screened at less than 12 hours of age, for any reason, will have to be rescreened through the NBS Program. Included in this group are babies whose specimen collection forms have erroneous or missing information, without which the age at collection can not be determined. Licensed perinatal facilities will be required to collect another specimen from these babies on or before the sixth day of age. The NBS Program has routinely cautioned against early specimen collection. In 1995, the Program issued interim early testing guidelines specifically warning against specimen collection prior to 12 hours of age. While multiple mailings, newsletters, and other attempts to reinforce these recommendations have substantially reduced the number of newborns tested under 12 hours of age, some newborns are still being tested inappropriately.

Recognizing this dynamic period, Steinmaus *et al.* (2010) states:

We addressed potential confounding or effect modification due to this surge in several ways. First, because the surge occurs mostly within the first 24 hours of birth, all analyses were stratified on the basis of whether the collection age was greater or less than 24 hours. Second, possible residual confounding was addressed by adjusting for collection age within each of these strata. This was done by dividing collection age into five categories: 0 to 5 hours (the period of early very unstable TSH values), 6 to 19 hours (the period when mean TSH levels peaked in this data set), 19 to 32 hours (the period when TSH levels decline rapidly), 33 to 70 hours (the period when TSH levels decline more slowly), and 70 hours or more (the period when TSH levels are close to long-term levels).

However, despite attempting to address potential confounding issues, serious problems remain. For example, the authors provide no scientific rationale supporting the physiological relevance of their segregation periods, and there is no discussion on whether the authors attempted to verify collection times. Although Steinmaus *et al.* (2011) attempt to adjust for

time of collection, they use only one set of cutoffs to define elevated TSH that does not reflect the time of collection.

2.2.7 Timing of neonatal TSH sample collection varies greatly

The levels of TSH in the newborn are highly variable. To illustrate the dynamic changes in TSH levels, Table 2 provides screening result classifications for TSH levels based on hours post partum, from the State of Washington NBS program. TSH levels are noted as “normal” if they are below 20 $\mu\text{IU/mL}$ serum. As shown, levels up to 54.9 $\mu\text{IU/mL}$ are characterized as “normal” if they are measured within 12 hours of birth, and levels up to 44.9 $\mu\text{IU/mL}$ serum are characterized as “normal” if they are measured within 24 hours of birth. Levels just above 25 $\mu\text{IU/mL}$ serum are only considered potentially abnormal if they are measured at 37 to 48 hours following birth or later.

Regarding the effect of the surge on TSH analyses and the potential determination of CH, LaFranchi (2010) states, “programs need to consider the range of age of specimen collection in their newborn population. There is a rise in TSH levels after birth; serum TSH rises from cord blood levels of 1–20 mIU/L [$\mu\text{IU/mL}$] to peak around 60–80 mIU/L 30 min after delivery. TSH levels then fall over the next few days, and by a week of life are in the 1–8 mIU/L range typical of early infancy.”

A complicating trend in labor and delivery on measured TSH levels is the reduced time of hospital stay: the trend is strongly toward early discharge of mothers and infants (before 48 hours of age). With early hospital discharge, the first screening specimen commonly is obtained before 48 hours of age. Although not presented in Steinmaus *et al.* (2010), Buffler *et al.* (2006) (who evaluated the same population, see Section 2.3) report that 89.8% of the population in the >5 ppb perchlorate exposure group had their blood sampled prior to 24 hours following birth compared to 76.9% of the ≤ 5 ppb population. Thus, a greater percentage of the “exposed” population was sampled during the period of greater TSH variability.

Clearly, serious potential bias can be introduced in studies that interpret TSH levels measured during newborn screening programs if the study does not appropriately adjust the assumed normal TSH range for the times when samples are taken. Although Steinmaus *et al.* (2011), attempt to adjust for time of collection, this does not account for the wide variability that has been documented in several studies and NBS programs.

Table 2. Classification of Clinical TSH Values by Hours Postpartum

Screening Tests

The newborn screening test for CH measures the infant’s *thyroid stimulating hormone (TSH)* level using a fluoroimmunoassay technique. Results are classified in the table below.

Screening Result Classifications and Corresponding Follow-up Actions for CH

TSH μIU/mL serum	1 to 12 hrs	13 to 24 hrs	25 to 36 hrs	37 to 48 hrs	49 to 504 hrs	> 504 hrs
0 - 14.9	Normal	Normal	Normal	Normal	Normal	Normal
15 - 19.9	Normal	Normal	Normal	Normal	Normal	Borderline
20 - 24.9	Normal	Normal	Normal	Normal	Borderline	Borderline
25 - 29.9	Normal	Normal	Normal	Borderline	Borderline	Borderline
30 - 44.9	Normal	Normal	Borderline	Borderline	Borderline	Borderline
45 - 54.9	Normal	Borderline	Borderline	Borderline	Borderline	Borderline
55 - 59.9	Borderline	Borderline	Borderline	Borderline	Borderline	Borderline
60 - 99.9	Borderline	Borderline	Presumptive	Presumptive	Presumptive	Presumptive
≥ 100	Presumptive	Presumptive	Presumptive	Presumptive	Presumptive	Presumptive
Typical Follow-up Actions						
Normal Results		Borderline Results		Presumptive Results		
Results are mailed to specimen submitter. No follow-up is required.		NBS waits for the routine second specimen or contacts health care provider to recommend newborn screening specimen as soon as possible. If previous abnormal, health care provider is contacted by phone to recommend <i>diagnostic testing</i> . Results are also mailed to submitter.		Health care provider is immediately contacted by phone to recommend a repeat newborn screening specimen and/or <i>diagnostic testing</i> as soon as possible. Results are also mailed to submitter.		

Source: WSDOH, 2008

2.2.8 Neonatal TSH values are not adjusted for gestational age, a requirement for comparing neonatal TSH values

As shown in Table 3, TSH, T4, T3, and fT4 change considerably from prenatal to postnatal periods. Controlling for gestational age is not critical in newborn screening programs since measured concentrations are (ideally) compared to age-specific reference ranges and, if “normal” levels are exceeded, further testing is conducted. However, characterization of gestational age is critical if the intent is to compare TSH levels across members of the population. It is well established in the literature that in studies comparing prenatal or postnatal thyroid hormone function, researchers must consider gestational age at the time of at birth (Williams *et al.* 2004; Simpson *et al.*, 2005; Hume *et al.*, 2004). However, Steinmaus *et al.* (2010) does not control for gestational age.

Of note, other factors (*e.g.*, disease state, see Table 4) can also affect TSH and thyroid hormone levels, and could influence population comparisons.

Table 3. Summary of TSH and Thyroid Hormone Levels in Prenatal and Postpartum Newborns, and Pregnant and Non-pregnant Women.*

Stage of life	TSH (μ IU/mL)	T4 (nmol/L)	T3 (nmol/L)	fT4 (pmol/L)
Prenatal infant (week of gestation)				
23-27	6.8 \pm 2.9	70 \pm 26	0.30 \pm 0.23	16.5 \pm 5.3
28-30	7.0 \pm 3.7	81 \pm 26	0.44 \pm 0.32	18.6 \pm 5.5
31-36	7.9 \pm 5.2	98 \pm 29	0.54 \pm 0.36	19.2 \pm 4.3
37-42	6.7 \pm 4.8	118 \pm 25	0.92 \pm 0.53	18.1 \pm 5.0
Postpartum infant (days)				
Day 7	2.6 \pm 1.8	163 \pm 37	2.27 \pm 0.77	34.7 \pm 7.3
Day 14	2.5 \pm 2.0	138 \pm 18	2.57 \pm 0.48	26.1 \pm 3.6
Day 28	1.8 \pm 0.9	125 \pm 28	2.70 \pm 0.49	21.2 \pm 4.4
Pregnant woman				
23-27	2.7 \pm 2.4	155.9 \pm 49.5	2.3 \pm 0.9	17.5 \pm 8.8
28-30	1.96 \pm 1.7	147.7 \pm 34.3	2.2 \pm 0.7	15.6 \pm 4.6
31-36	2.24 \pm 1.6	149.7 \pm 34.9	2.3 \pm 0.8	15.4 \pm 3.3
37-42	2.07 \pm 1.7	140.6 \pm 30.1	2.4 \pm 0.7	13.5 \pm 3.0
Non pregnant woman				
	1.36 \pm 0.9	99.0 \pm 22.45	2.13 \pm 0.4	15.3 \pm 2.5

*Data are the presented as mean \pm SD. Data obtained from Williams *et al.* (2004) and Hume *et al.* (2004)

Table 4. Summary of Effect of Disease on Thyroid Hormone Values Postpartum at Days 7, 14, and 28, Relative to Reference Values.*

Postnatal Day of Life	TSH (μ IU/mL)		T4 (nmol/L)		T3 (nmol/L)		fT4 (pmol/L)	
	Ill	Reference	Ill	Reference	Ill	Reference	Ill	Reference
Day 7	9.4 \pm 19	7.0 \pm 3.2	85 \pm 51	94 \pm 21	1.06 \pm 0.75	0.59 \pm 0.33	25 \pm 9.9	18.6 \pm 4.1
Day 14	45 \pm 65.6	6.6 \pm 3.2	76 \pm 45	95 \pm 29	0.92 \pm 0.86	0.49 \pm 0.26	16.6 \pm 6.9	18.3 \pm 5.7
Day 28	2.9 \pm 2.6	7.2 \pm 2.2	62 \pm 44	112 \pm 20	0.92 \pm 0.94	0.81 \pm 0.22	17.0 \pm 6.7	18.7 \pm 3.3

*Data obtained from Simpson *et al.* (2005). Infants had severe diseases, including respiratory distress syndrome, chronic lung disease, cerebral pathology, and enterocolitis. All infants had intensive care support as required including intermittent positive ventilation, correction of fluid electrolyte, blood glucose, acid-base balance, drug treatment, blood transfusions, and enteral feeding. Reference values are from cord equivalent gestational age infants that had remained *in utero*. Data are the presented as mean \pm SD.

Birth weight can sometimes be used as a surrogate for gestational age, but it is imprecise and not preferred. The degree of consideration of birth weight by Steinmaus *et al.* (2010) is limited. They report the following regarding their assessment of birth weight:

Subjects in this data set meeting the following criteria were excluded: ... (5) birth weight less than 250 or more than 7500 g...

First, bivariate analyses were done to investigate associations between TSH levels and sociodemographic factors such as gender, ... and birth weight.

Using logistic regression, we calculated both unadjusted ORs and ORs adjusted for ... birth weight (entered as less than or more than the 5th or 95th percentiles, respectively, each coded as 0 or 1)...

Entering birth weight and mother's age as continuous variables or in quartiles had little impact on results.

Confining analyses to birth weights between 2500 and 4000 g, or excluding those with collection ages less than 4 hours (the period when TSH levels peak in healthy children) or very high TSH levels (ie, >200 $\mu\text{U}/\text{mL}$), also had little effect on results.

Despite the comments in the paper that birth weight “had little impact,” some of the largest differences in geometric means reported in the study are shown for birth weight groupings (Figure 4). The geometric mean for low, medium, and high birth weight percentiles are 2.29 $\mu\text{IU}/\text{mL}$, 4.18 $\mu\text{IU}/\text{mL}$, and 3.76 $\mu\text{IU}/\text{mL}$, respectively (the reported SDs are 9.43, 5.50, and 5.88, respectively).

TABLE 2. Geometric Mean TSH Levels by Sociodemographic Characteristics

	<i>n</i>	Geometric Mean	SD	<i>P</i>
Birth weight (percentile)				
Low (<5th)	20,853	2.29	9.43	<0.001
Medium (5th–95th)	451,666	4.18	5.50	Ref
High (>95th)	24,939	3.76	5.88	<0.001

Figure 4. Geometric Mean TSH Levels by Birth Weight, as Reported in Steinmaus *et al.* (2010).

2.2.9 The Newborn Screening Program was not intended for intricate assessment of thyroid function

The purpose of a newborn screening program is to identify newborn infants at risk for specific diseases. The NBS program screens newborns for 76 disorders⁴ including cystic fibrosis, primary congenital hypothyroidism, hemoglobin disorders, and variant hypothyroidism. Routine newborn screening of TSH (or T4) was initiated by public health agencies to identify those infants that are born with primary CH so that they can be treated. Obtaining accurate TSH levels during this physiologically active post partum period can be challenging, and spurious results are common.

The state of California Newborn Screening Program defines congenital hypothyroidism as "...a deficiency of the thyroid hormone caused by the failure of the thyroid gland to develop normally. As a result, the thyroid gland does not produce enough thyroid hormone to meet the body's needs." To screen for potential CH, the state of California uses a TSH cut off of 25 $\mu\text{IU/mL}$; above that number, infants will be rescreened. With confirmation of a second high TSH, the pediatrician and or parents will be notified and the infant will undergo a diagnostic evaluation. If found to have CH, the newborn will begin treatment. The screening assay needs the sensitivity and specificity to identify newborns at risk from those not at risk. However, it is not a diagnostic device.

2.2.10 Questions arise regarding the statistical characterization of TSH data

To characterize TSH levels in different segments of the population, Steinmaus *et al.* (2010) report geometric means. The authors discuss the use of the geometric mean in the Methods section; the discussion is rather short and uninformative. It states: "Since TSH levels were not normally distributed, geometric means were calculated and differences between groups were assessed using the Wilcoxon rank-sum test." In the results section, the papers reports "The overall geometric mean TSH in all neonates combined was 4.06 $\mu\text{IU/mL}$ (SD = 5.75 $\mu\text{IU/mL}$)." Note that "SD" is not defined specifically in the paper; however, it is universally used to mean "standard deviation."

Several questions arise. First, the authors' use of the geometric mean is unclear. The sample geometric mean of a data set X_1, \dots, X_n is

$$\left(\prod_{i=1}^n X_i \right)^{1/n}$$

In calculating the geometric means, zero or negative values can be used; however, one would not expect TSH values to be equal to or below zero. The geometric mean is always less than or equal to the sample arithmetic mean.

⁴ The full list of disorders screened for can be found here: <http://www.cdph.ca.gov/programs/nbs/Documents/NBS-DisordersDetectable121509.pdf>.

The standard deviation (SD, also written as σ) is the square root of the arithmetic mean.⁵ Thus, it appears that the authors are mixing apples and oranges in characterizing the TSH data. For example, the statement “The overall geometric mean TSH in all neonates combined was 4.06 $\mu\text{IU/mL}$ (SD = 5.75 $\mu\text{IU/mL}$)” would mean that a significant portion of the TSH values was below zero. As written, it does not make either statistical or scientific sense to have a significant number of TSH values equal to or below the value of zero (Caffo, 2006).

The definition of significant figures is the digits in a decimal number that are warranted by the accuracy of the means of measurement (Farlex, 2011). Significant figures imply a level of precision. In Steinmaus *et al.* (2010), the geometric means for population groups are shown with three significant figures, implying a relatively high level of precision. For example, the geometric means for Hispanics and non Hispanics are 4.01 $\mu\text{IU/mL}$ and 4.10 $\mu\text{IU/mL}$; the difference of 0.09 $\mu\text{IU/mL}$ is characterized as statistically significant ($p < 0.001$). However, while the geometric means for Hispanics and Whites appear to be the same (the geometric mean for both is 4.01 $\mu\text{IU/mL}$), they are noted as being significantly different ($p = 0.04$). Based on the apparent precision of the means shown, they do not appear to be different.

2.2.11 OEHHA recognizes the serious limitations of Steinmaus *et al.* (2010) and still uses it as the basis for changing susceptible populations

OEHHA acknowledges that TSH levels collected prior to 24 hours may be unstable, however they state,

As discussed above, although TSH measurements collected within the first 24 hours of birth may not be the most appropriate for screening for PCH, levels collected during this time may be the most relevant for assessing associations between maternal drinking water perchlorate concentrations and changes in neonatal thyroid hormone levels that are less severe than those typically seen with PCH.”

OEHHA continues,

As discussed above, since the half-life of perchlorate and thyroid hormones is relatively short, this [excluding measurements collected before 24 hours] may have limited the ability of this study to find an association between maternal perchlorate exposure during pregnancy and neonatal thyroid hormone levels.

Steinmaus *et al.* (2010) report a statistically significant association only for data collected before 24 hours. For infants for whom data was collected after 24 hours, the association was only significant if the TSH level that was considered “high” was dropped from the standard 25 $\mu\text{IU/mL}$ to 8 $\mu\text{IU/mL}$. This suggests that after 24 to 36 hours, there was no effect on infants. It appears that after 24 hours, the TSH had normalized and there is no association

⁵ There is a method to estimate for geometric means. As noted above with standard deviation of the mean, one adds or subtracts the standard deviation from the mean in order to estimate fractions of included data. One can estimate fractions of data, although with a different meaning using the log standard deviation (q). In this case one would multiply or divide. In general, one would expect 68.3% of the data set to be between $X_{\text{geo}} \times q$ and $X_{\text{geo}} \div q$ and 95.4% of your data would be between $X_{\text{geo}} \times q^2$ and $X_{\text{geo}} \div q^2$. If that was the case, and using geometric mean of 4.06, then for 95.4% between approximately -2 and 8, which does not make sense given the information in the manuscript.

with perchlorate tap water concentrations near the mother's home. This is consistent with the data reported previously by Buffler *et al.* (2006).

The reported statistical association is based on a low concentration of perchlorate in municipal drinking water (5 ppb). The equivalent dose associated with this concentration (0.00014 mg/kg-d, assuming exposure of a 70 kg person to 2 L per day) is below the dose at which no effect occurs (*i.e.*, the NOEL for IUI from Greer *et al.*, 2002 was 0.007 mg/kg-d). It is biologically implausible that an effect occurred at this dose. The TSH level at 24 hours is irrelevant, as it is not evidence of an adverse or a persistent effect.

2.3 Buffler *et al.* (2006) is Based on the Same Data Set as Steinmaus *et al.* (2010), and Does Not Report an Association Between Perchlorate Exposure and TSH

Steinmaus *et al.* (2010) reanalyzes the same dataset previously analyzed by Buffler *et al.* (2006), who assessed the association between maternal drinking water perchlorate exposure (at 5 ppb or lower, or above 5 ppb) during pregnancy and newborn TSH levels. However, in Buffler *et al.* (2006), the authors were clear that "Given the documented physiologic postnatal surge of TSH before 24 hr of age, we restricted the TSH analyses to those newborns with an age at specimen collection \geq 24 hr." They found no association between living in an area with perchlorate greater than 5 ppb and increased TSH.

2.4 Other Studies Cited by OEHHA Do Not Support Their Assertions About the Association Between Perchlorate Exposure and TSH

In addition to Buffler *et al.* (2006) and Steinmaus *et al.* (2010), OEHHA cites several other studies to augment its assertion that there is a "consistent body of evidence linking perchlorate exposure during pregnancy with changes in thyroid hormone levels in the newborn," including Kelsh *et al.* (2003), Brechner *et al.* (2000), Li *et al.* (2000), and Crump *et al.* (2000). OEHHA states these studies "...provide evidence that perchlorate is associated with more subtle changes in newborn thyroid hormone levels." However, closer examination of these studies does not support these assertions.

First, none of the studies provide individual perchlorate exposure data. Assumptions about perchlorate exposure to the neonate are based on the average concentration in the community in which the child was born and in which the mother was assumed to reside during her pregnancy. As discussed in Section 2.2.3 regarding Steinmaus *et al.* (2010), variations in perchlorate exposure for specific locations or with time are not accounted for, nor were the potential contributions of exposure to perchlorate in food or exposure to other goitrogens.

Second, as discussed in Section 2.1, the design of these studies (ecological) does not allow determination of causality. Regarding the specific ecologic studies identified by OEHHA (with the exception of Steinmaus *et al.*, 2010, which had not been published at the time), NRC (2005) stated,

... given the limitations of ecologic data in inferring causation, the available epidemiologic evidence is not consistent with a causal association between perturbations of thyroid hormone and TSH production in normal newborns (that is, not low-birthweight or preterm) and exposure during gestation to perchlorate in

drinking water at up to 120 ppb (120 µg/L). Most studies do not show either significantly lower T4 concentrations or significantly higher TSH concentrations among infants born in geographic areas in which the water supply has measurable perchlorate.

Third, of the studies listed, only one (Brechner *et al.*, 2000) is suggestive of an adverse effect. However, there are weaknesses in the design of this study that limit its interpretation. The NRC (2005) states the following regarding Brechner *et al.* (2000):

The actual adjusted concentrations for each city were not reported. Neonatal T4 values did not differ significantly between Yuma and Flagstaff after adjustment for race or ethnicity. However, follow-up testing of TSH is done only in infants with the lowest 10% of T4 concentrations, so the absence of differences in T4 concentrations between the cities is not especially informative inasmuch as only the lowest part of the entire distribution was compared. ...perchlorate exposures in infants' mothers were not directly measured; in fact, drinking-water concentrations of perchlorate were not derived from the same period as the newborn screening results.... Information on birthweight or gestational age was not available, so those potential confounders could not be addressed in the analysis. Low birthweight infants are more likely to have lower T4 concentrations without the magnitude of the TSH surge observed in term infants (Mercado *et al.* 1988). Thus, it is possible that more of the infants with low T4 who had a follow-up blood test for TSH in Flagstaff were low-birthweight infants who would not have had as great a TSH surge, keeping the mean and median concentrations in Flagstaff lower than in Yuma.

ATSDR (2008) states:

Lamm (2003) reanalyzed the study and compared TSH neonatal values of Yuma and two cities near Yuma, Somerton and San Luis, which get their water from a different source than the city of Yuma. The water from Somerton and San Luis is assumed to have no perchlorate contamination. Lamm's analysis showed no significant difference in TSH values between newborns from Yuma and Somerton/San Luis, suggesting that the results of Brechner *et al.* (2000) reflected regional differences, possibly related to the difference in altitude (7,000 feet) between Yuma and Flagstaff.

In each of the remaining studies, the study authors indicate there is no association between elevated perchlorate exposure in drinking water and thyroid hormone or TSH changes, when the analyses are based on measurements collected after hormone levels have stabilized.

To make the claim that these studies provide evidence of an association, OEHHA reanalyzed data that have been deemed "uninformative" (*i.e.*, Buffler *et al.*, 2006 stated, "Because of the physiologic postnatal surge of TSH, the results for newborns screened before 24 hr were uninformative for assessing an environmental impact") and excluded other studies in the literature that were not supportive of its hypothesis. OEHHA (2011b) presented a summary of conclusions regarding these studies in its Table 13. However, when evaluating the data as interpreted by the authors of each study, the weight of evidence clearly indicates a lack of association between exposure to perchlorate in drinking water and changes in thyroid hormone or TSH levels. OEHHA's conclusions are compared to those of the authors in Table 5; this table clearly shows that the authors' conclusions are not supportive of those drawn by OEHHA.

Table 5. Comparison of Study Results as Presented by OEHHA (Table 13) and the Study Authors (findings unresponsive of OEHHA are highlighted in blue) (Page 1 of 4)

Study	Location	Endpoint and Exposure Measure	OEHHA Interpretation Based on <24 hr Data, Exposed Compared to Unexposed Group		Author Interpretation, Exposed Compared to Unexposed Group	
			T4	TSH	Neonatal T4	Neonatal TSH
Studies cited by OEHHA (2011b) as providing “evidence of an association” between maternal perchlorate exposure and thyroid hormone levels in newborns						
Brechner <i>et al.</i> (2000)	Yuma and Flagstaff, AZ (1,542 newborns)	Neonatal TSH (Oct. 1994-Dec. 1997); ClO ₄ ⁻ in dw in Yuma (supplied by Colorado River below Lake Mead, with ClO ₄ ⁻ = 6 ppb (Aug. 1999)) and Flagstaff (not supplied by Colorado River, with ClO ₄ ⁻ =ND (Sept. 1999))	OR for low T4 = 1.18 (p = 0.006)	Mean TSH = 27% higher	Data not presented, nor is collection time. The false positive rate for T4 screening before TSH is ~0.3% compared to 0.08% for TSH screening prior to T4 (AAP, 2006)	Median first TSH level for Yuma (19.9 mU/L) significantly higher than Flagstaff (13.4 mU/L). The effect of age in days on TSH was highly significant (p<0.001). Only infants with low T4 were tested. Authors state, “...the individual <i>t</i> tests were not significant...” for infants in the 0-1 day group
Crump <i>et al.</i> (2000)	Chile (11,967 newborns)	Neonatal TSH (Feb.1996-Jan. 1999); ClO ₄ ⁻ in dw at “high” (100 to 120 ppb; Taltal), “low” (5 to 7 ppb; Chañaral), or non-detectable (< 4 ppb; Antofagasta) concentrations	No data	Mean TSH ~ 45% higher	No data	No association; Decreased TSH with exposure to 100-120 ppb (mean 2.4 μIU/mL) compared with city with no ClO ₄ ⁻ in dw (mean 3.4 μIU/mL) (opposite expected direction).

Table 5. Comparison of Study Results as Presented by OEHHA (Table 13) and the Study Authors (findings unresponsive of OEHHA are highlighted in blue) (continued, Page 2 of 4)

Study	Location	Endpoint and Exposure Measure	OEHHA Interpretation Based on <24 hr Data, Exposed Compared to Unexposed Group		Author Interpretation, Exposed Compared to Unexposed Group	
			T4	TSH	Neonatal T4	Neonatal TSH
			Kelsh <i>et al.</i> (2003)	Redlands, San Bernadino, and Riverside Counties, California (83,462 w/ TSH levels)	Neonatal T4 and TSH (if T4 was lower than prescribed threshold (usu. 9.0 µg/dL) or in lowest 5% of daily tray samples) (1983-1997); ClO ₄ ⁻ in dw in Redlands (“exposed”; ND-9 mg/L in 2001-2002, avg. < 1 mg/L) or San Bernadino or Riverside Counties (“unexposed”)	OR for low T4 = 1.18 (p < 0.0001)
Z. Li <i>et al.</i> (2000)	Las Vegas and Reno, Nevada	Neonatal T4 (Apr 1998-June 1999) for infants with serum T4 in lowest 10th %ile, BW of 2,500-4,500 g, and in first 4 d of life; ClO ₄ ⁻ in drinking water in Las Vegas from Lake Mead (monthly ClO ₄ ⁻ in finished water < 4 ppb for eight of the months-15 ppb).	Mean T4 ~ 22% lower*	No data	No difference in mean T4 level	No data

Table 5. Comparison of Study Results as Presented by OEHHA (Table 13) and the Study Authors (findings unresponsive of OEHHA are highlighted in blue) (continued, Page 3 of 4)

Study	Location	Endpoint and Exposure Measure	OEHHA Interpretation Based on <24 hr Data, Exposed Compared to Unexposed Group		Author Interpretation, Exposed Compared to Unexposed Group	
			T4	TSH	Neonatal T4	Neonatal TSH
			Steinmaus <i>et al.</i> (2010)	California (497,458 newborns)	Neonatal TSH (Jan-Dec. 1998); ClO ₄ ⁻ in dw ≤5 ppb and >5 ppb, based on average in mother's city of residence (1997-1998).	No data
Studies Deliberately Excluded by OEHHA						
Amitai <i>et al.</i> (2007)	Israel	Neonatal T4 measured 36-48 hrs after birth; ClO ₄ ⁻ in dw: ≥340 ppb, 42-94 ppb, or <3 ppb	Not considered	Not considered	No difference between groups with perchlorate up to ≥340 ppb	No data
X. Li <i>et al.</i> (2000)	Las Vegas and Reno, Nevada	Neonatal TSH (Dec. 1998-Oct. 1999) for infants with T4 in lowest 10th %ile, BW of 2,500-4,500 g, and in first month of life (excluding the first day of life, when TSH levels are unstable); ClO ₄ ⁻ in dw in Las Vegas from Lake Mead (monthly ClO ₄ ⁻ in finished water < 4 ppb for three of the months-15 ppb).	Not considered	Not considered	No data	No effect up to 15 ppb. Overall TSH mean: Las Vegas = 11.5 μIU/mL; Reno = 12.5 μIU/mL; TSH mean using data for days 2-7 only: Las Vegas = 12.8 μIU/mL; Reno = 12.8 μIU/mL; Only infants with low T4 were tested.

Table 5. Comparison of Study Results as Presented by OEHHA (Table 13) and the Study Authors (findings unresponsive of OEHHA are highlighted in blue) (continued, Page 4 of 4)

Study	Location	Endpoint and Exposure Measure	OEHHA Interpretation Based on <24 hr Data, Exposed Compared to Unexposed Group		Author Interpretation, Exposed Compared to Unexposed Group	
			T4	TSH	Neonatal T4	Neonatal TSH
			Tellez <i>et al.</i> (2005)	Chile	Neonatal TSH, Tg, free T4; ClO ₄ ⁻ in dw at “high” (avg. 114 ppb; Taltal), “low” (6 ppb; Chañaral), or ND (<4 ppb; Antofagasta) concentrations	Not considered
Studies Not Considered by OEHHA						
Buffler <i>et al.</i> (2006)	California (342,257 newborns)	Neonatal TSH (Jan-Dec. 1998); ClO ₄ ⁻ in dw ≤5 ppb and >5 ppb, based on average ClO ₄ ⁻ in mother’s city of residence in 1997-1998	Not considered	Not considered	No data	No association; for newborns screened ≥ 24 hr, the adjusted POR for high TSH was 0.73 (95% CI, 0.40–1.23). “Because of the physiologic postnatal surge of TSH, the results for newborns screened before 24 hr were uninformative for assessing an environmental impact.”

BW — Body weight; CI — Confidence interval; dw — drinking water; ND — Nondetect; OR — Odds ratio; POR — Prevalence odds ratio

*This approximation by OEHHA is based on Z. Li *et al.*, 2000, Table 3 on the first collection day. No N is provided. It is noteworthy that for collection on day 2, the mean is approximately 18% higher (the opposite direction)

Further, in two of these studies (Brechner *et al.*, 2000; Kelsh *et al.*, 2003), the respective states' newborn screening programs analyzed an initial sample for T4 with collection of a follow up for TSH if the T4 level was below a cutoff or was in the group's (usually daily) lowest fifth or 10th percentile. Thus, TSH measurements were only available for patients with lower T4 levels. Of note, the American Academy of Pediatrics reports a higher false positive rate for primary CH when T4 screening is done before TSH, or about 0.3% compared to 0.08% when TSH is collected first (AAP, 2006).

Regarding the Crump *et al.* (2000) study, contrary to the impression provided by OEHHA, the study's authors report:

A study was conducted to investigate the potential effects of perchlorate in drinking water on thyroid function in newborns and school-age children. A total of 162 school-age children and 9784 newborns were studied in three proximate cities in northern Chile that have different concentrations of perchlorate in drinking water: Taltal (100 to 120 µg/L), Chanaral (5 to 7 µg/L), and Antofagasta (non-detectable:< 4 µg/L). Among school children no difference was found in thyroid stimulating hormone levels or goiter prevalence among lifelong residents of Taltal or Chanaral compared with those of Antofagasta, after adjusting for age, sex, and urinary iodine. Neonatal thyroid-stimulating hormone levels were significantly lower in Taltal compared with Antofagasta; this is opposite to the known pharmacological effect of perchlorate, and the magnitude of difference did not seem to be clinically significant. These findings do not support the hypothesis that perchlorate in drinking water at concentrations as high as 100 to 120 µg/L suppresses thyroid function in newborns or school-age children.

Of this study, the NRC (2005) reports:

The committee thinks that the study of Crump *et al.* (2000) had important strengths. Although individual exposures were not assessed, it is one of the few studies that measured perchlorate in drinking water in samples taken directly from the environment of the children studied, such as homes and schools. In addition, it was possible to compare assessments of thyroid function, other end points, and potential risk factors obtained in a systematic manner from all participants and adjusted for a number of important covariates, and participation was high. All laboratory assessments were done at the same facility, and assessments of thyroid status and other measures were done by observers unaware of the perchlorate exposure of the children. All newborn screening tests in Chile are done at a single laboratory.

Numerous critiques of the Crump *et al.* (2000) study have been done either directly by the U.S. Environmental Protection Agency (EPA) in its risk-assessment document or by others at the request of EPA (Park 2001; Marcus 2003).... The committee considers this criticism adequately addressed by the authors.

Fourth, even if one assumes that TSH measurements before 24 to 48 hours of life are meaningful, there is no evidence that TSH levels in those with assumed higher exposure to perchlorate remain different after TSH levels stabilize. The scientific evidence supports there

being no difference after 24 to 48 hours (Buffler *et al.*, 2006, Kelsh *et al.*, 2003, X. Li *et al.*, 2000, Tellez *et al.*, 2005).

Fifth, for those studies that report T4, OEHHA's assertions about the significance of T4 changes are based on questionable reinterpretation of the data presented by the study authors. Specifically, OEHHA claims,

If perchlorate were simply causing an increase in TSH by some extra-thyroidal process (that is, without first causing a low T4), then this increase in TSH would be expected to cause an *increase* in T4 levels. This was not seen. Instead, a perchlorate-associated *decrease* in T4 levels was seen in several studies. This suggests that the TSH effects are due to a perchlorate-associated inhibition of T4 production and a direct action on the thyroid gland.”

However, for these studies (Brechner *et al.*, 2000; Kelsh *et al.*, 2006; and Z. Li *et al.*, 2000), OEHHA drew its conclusions regarding T4 using statistics taken from the authors' tables and not from individual data, since these were not reported by the authors. Consequently, OEHHA could not adjust for factors understood to contribute to changes in thyroid hormones (*e.g.*, birth weight).

In one reanalysis (based on Z. Li *et al.*, 2000), OEHHA approximates T4 levels collected on the first day and suggests that T4 levels in a perchlorate exposed population are 22% lower than in a nonexposed population. The validity of this “statistic” is uncertain because there is no information regarding the size of the population sampled on the first day and, utilizing the same approximation technique, the difference on the second day would be nearly equal in the opposite direction—that is, T4 levels would be estimated to be 18% higher in the perchlorate exposed population.

Finally, OEHHA deliberately excluded three studies from their analysis (Amitai *et al.*, 2007; X. Li *et al.*, 2000; and Tellez *et al.*, 2005). However, each of these studies provides additional evidence that exposure to perchlorate in drinking water did not affect T4 or TSH levels in newborns.

OEHHA excluded Amitai *et al.* (2007) because “< 10% of newborns had thyroid hormones measured in first 36 hr after birth.” Amitai *et al.* (2007) conducted an ecological epidemiological study in Israel designed 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 with drinking water perchlorate levels associated with “very high exposure” (10 to 100-fold greater compared with levels in the U.S.; ≥ 340 ppb), “high exposure” (42-94 ppb), or “low exposure” (<3 ppb). 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, a factor that could affect T4 measures in infants. The authors report no statistical differences between neonatal T4 levels among the groups.

At a satellite meeting of the Society of Toxicology (SOT) (Seattle 2008), Amitai *et al.* provided a follow-up to their previously reviewed 2007 publication (Amitai, 2008). They located a subset of the original study population and evaluated the children using the Bayley

Scales of Infant Development to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants ages 0 to 3. They found that there was no difference between the groups of children.

This study adds data regarding exposure and outcome on critical neurological endpoints. First, exposures were to pregnant women and their developing fetuses. Second, these are the highest concentrations of perchlorate ever reported in a study in which the public, including the most susceptible subpopulation was exposed. For example, 340 ppb, assuming consumption of 2 L of water per day, yields a dose that is almost 3-fold greater than the NOEL from Greer *et al.* (2002); this dose would likely cause a small degree of IUI even without contribution from nitrates and thiocyanates in food. Third, the endpoints assessed are measures of neurological development, the endpoints of greatest concern when assessing thyroidal influence. The results presented thus far point to no adverse effect on neurological development from exposures to levels of perchlorate that exceed 340 ppb. Dr. Amitai intends to publish this and additional results during 2011 (Pleus, 2010. Personal Communication).

OEHHA excluded X. Li *et al.* (2000) because “TSH measurements collected on first day after birth were excluded.” X. Li *et al.* (2000) compared TSH levels for newborns living in Las Vegas, Nevada, with up to 15 ppb perchlorate in drinking water, to those for newborns in Reno, which had no detected perchlorate in drinking water. The TSH dataset was composed of TSH levels for newborns in Las Vegas and Reno for the period December 1998-October 1999 who had serum T4 levels in the lowest 10th percentile, a birth weight of 2,500-4,500 g, and were in their first month of life (excluding the first day of life when TSH levels are unstable). The source of drinking water for inhabitants of Las Vegas is Lake Mead—during the 11-month period of the study, monthly perchlorate measurements of finished water in Las Vegas ranged from non-detect (< 4 ppb) for three of the months, up to 15 ppb. Multiple linear regression analyses were conducted to assess the affect of age, sex, and residence in Las Vegas or Reno on TSH levels. This study had sufficient statistical power to detect the effects of age and gender on the newborn blood hormone levels, but detected no effect associated with perchlorate exposure. The authors found no differences in blood TSH in newborns living in Las Vegas compared to those in Reno, and no association was found between neonatal TSH levels and perchlorate levels in the drinking water in this study.

OEHHA excluded Tellez *et al.* (2005) because “45% of women from the exposed city delivered in the unexposed city and the iodine levels were very high.” Tellez *et al.* (2005) conducted a longitudinal epidemiologic study of the effects of exposure to environmental perchlorate, found naturally at high levels in Chilean soils and water supplies, on the thyroidal status of pregnant women and neonates. Some have questioned the relevance of this study to the U.S. population since Chile has historically had high levels of dietary iodide supplementation. However, this supplementation has decreased to levels that are similar to U.S. levels. In this study, the levels of iodine in breast milk were not associated with perchlorate exposure, and the levels of maternal iodine in urine were intermediate to those found in NHANES I and NHANES III and consistent with World Health Organization (WHO) recommendations. 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.”

OEHHA also did not include the study by Buffler *et al.* (2006) in their table. As discussed previously, Buffler *et al.* (2006) evaluated the same dataset that Steinmaus *et al.* (2010) later reanalyzed, and concluded that there was no association between living in an area with perchlorate greater than 5 ppb and increased TSH. Buffler *et al.* (2006) were very clear that “Given the documented physiologic postnatal surge of TSH before 24 hr of age, we restricted the TSH analyses to those newborns with an age at specimen collection \geq 24 hr.”

2.5 Animal Study Data and PBPK Modeling Do Not Demonstrate that the Neonate is More Susceptible

In addition to studies of human populations, studies in animals do not demonstrate that the neonate is more susceptible to perchlorate exposure. Clewell *et al.* (2003) developed a physiologically based pharmacokinetic (PBPK) model to reproduce measured perchlorate distribution in the lactating and neonatal rat and predict resulting effects on iodide kinetics from competitive inhibition at the NIS, using physiological and kinetic parameters obtained from literature and experiment, and systemic clearance and Michaelis-Menten (M-M) parameters estimated by fitting simulations to tissue and serum data. Neonatal iodide kinetic parameters were determined by the fit of the model to the data obtained from directly dosing the pup. Comparison of predicted dosimetrics across life-stages in the rat indicates that neonatal thyroid iodide uptake inhibition is similar to the adult and approximately 10-fold less than the fetus.

Despite the increased dose to the neonate (0.07 vs. 0.01 mg/kg-day in the adult), Clewell *et al.* (2003) show that the postnatal day (PND) 10 pup serum average perchlorate concentrations were consistently lower than those of the adult. In fact, a comparison across life stages reveals that the serum perchlorate concentrations of the lactating dam were slightly higher than the male, pregnant, fetal or neonatal rat, suggesting that lactation may be the time period with the greatest internal perchlorate exposure. The authors surmise that while this increased serum concentration of perchlorate in the lactating rat is somewhat surprising considering the additional clearance route provided through the milk, it is likely due to increased serum binding. In spite of the multiple inhibition sites (mammary gland, milk and thyroid), inhibition in the neonatal thyroid was similar to that of the dam. This may be due to the fact that neonatal serum perchlorate levels are less than those of the dam. **The neonate shows less perchlorate-induced inhibition of thyroid iodide uptake compared to the other life stages in the rat.** Model estimates suggest that the fetal rat thyroid is most vulnerable to inhibition, with a 10-fold greater inhibition than the neonate at the lowest measured dose

2.6 Other Studies Do Not Support Higher Infant Sensitivity

Blount *et al.* (2009) published another study that was not cited in OEHHA (2011b), that evaluated the association between maternal (urine, serum) and fetal (cord blood) levels of perchlorate, thiocyanate, nitrate, and iodide compared to infant body weight, body length, and head circumference. All chemical agents were assumed to be derived from food or water. The study location was in New Jersey. The data were obtained from children born via C-section. They report no association between perchlorate, nitrate, and thiocyanate in cord blood and fetal birth weight, head circumference, and birth length. Blood levels of

perchlorate, nitrate, and thiocyanate were higher in the mother than fetus, while iodine was higher in the fetus.

2.7 New Evidence Presented for Other “Potential Susceptibility Groups” in Blount *et al.* (2006) and Steinmaus *et al.* (2007) Does Not Support the Draft Document’s Conclusions

OEHHA (2011b) cites studies by Blount *et al.* (2006) and Steinmaus *et al.* (2007) as support for the assertion that women with low iodine and/or high thiocyanate intakes are “potential susceptibility groups.” OEHHA (2011b) also used the data from Blount *et al.* (2006) to calculate a benchmark dose (BMD) to support the PHG calculated using the data from Greer *et al.*, using a relative decrease in T4 of 10% as the benchmark response (BMR). In these calculations, perchlorate exposure in terms of urinary perchlorate (in $\mu\text{g/L}$) was converted to perchlorate dose (in $\mu\text{g/kg-d}$) by using the urinary perchlorate per gram of creatinine concentrations (from Blount *et al.*, 2006) multiplied by creatinine output per day (g/d) and divided by body weight (in kg). However, evaluation of the Blount *et al.* (2006) and Steinmaus *et al.* (2007) studies, as well as the full database of perchlorate exposure studies, does not support OEHHA’s assertions.

Blount *et al.* (2006) and Steinmaus *et al.* (2007) used data from the same cross-sectional investigation of urinary perchlorate levels and serum levels of thyroid hormones in 2,299 men and women greater than or equal to age 12 years who took part in the 2001-2002 National Health and Nutrition Examination Survey (NHANES). Blount *et al.* (2006) examined the relationship between urinary levels of perchlorate and serum levels of TSH and total T4 in men and women. They observed that perchlorate was not a significant predictor of T4 or TSH levels in men, but that in women with urinary iodine concentrations $<100 \mu\text{g/L}$, perchlorate was a significant negative predictor of T4 ($p < 0.0001$) and a positive predictor of TSH ($p = 0.001$), and in women with urinary iodine $\geq 100 \mu\text{g/L}$, perchlorate was a significant positive predictor of TSH ($p = 0.025$) but not T4 ($p = 0.550$). The authors note that 37% of the women had urinary iodine levels below $100 \mu\text{g/L}$, the urinary iodine level used by WHO to define iodine deficiency in a population. Regarding this study, OEHHA (2011b) states, “these effects highlight the importance of gender and iodine status when assessing the potential impacts of perchlorate.”

Steinmaus *et al.* (2007) asserted that in women with urinary iodine levels $<100 \mu\text{g/L}$, the association between the logarithm of perchlorate and decreased T4 was greater in smokers ($p = 0.0005$) than in nonsmokers ($p = 0.04$), and that for high, medium, and low urinary thiocyanate tertiles, the regression coefficients were -1.67 ($p = 0.0009$), -0.68 ($p = 0.09$), and -0.49 ($p = 0.11$). Clear interactions between perchlorate and smoking were not seen with TSH, with T4 in women with urinary iodine levels $\geq 100 \mu\text{g/L}$, or in men. The authors concluded that the results suggest that thiocyanate in tobacco smoke and perchlorate interact in affecting thyroid function.

Despite OEHHA's reliance on these studies to support their PHG, these studies do not provide sufficient evidence to support OEHHA's assertions, for a number of reasons. The following specific issues will be expanded on in this section:

- The studies do not demonstrate causation.
- The reported results on T4 have not been seen in other studies.
- Spot urine samples do not support identification of iodine deficient individuals.
- Spot urine samples are unreliable indicators of longer term perchlorate exposure status.
- Thyroid hormone and TSH levels are expected to show temporal variability.
- Thyroid hormone changes were not clinically significant and perchlorate doses were below the NOEL.
- The reported difference in response between men and women is not seen in other studies.
- Relative exposure to other iodide uptake inhibitors is likely much greater than perchlorate, and findings in this regard reported by Blount et al. (2006) are inconsistent with the literature.
- T4 measurements alone may be insufficient to assess thyroid effects.
- OEHHA ignores or discounts the results of several studies that conflict with the findings of Blount et al. (2006) and Steinmaus et al. (2007).

2.7.1 The studies do not demonstrate causation

As discussed previously, association does not equal causation. Any study must give careful consideration to the parameters and variables that are measured or available and how these variables relate to the outcome of interest. NHANES is designed to collect a broad range of data about the health and diet of people in the United States. While the p values are well below 0.05, signifying that the probability the findings were due to chance is fairly low, this simply means there may be an inverse association between T4 and perchlorate in women in the <100 µg/L group. It does not mean that perchlorate *caused* the lower T4 (and the physiologic/mechanistic data for perchlorate, coupled with data from other animal and human studies, suggest it does not). Rather, an independent variable that is associated with both T4 and perchlorate could be responsible for the apparent association.

The type of study conducted by Blount and colleagues (*i.e.*, a cross-sectional study) 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 measurement of the full set of variables that could influence thyroid measurements, including iodide exposure as reflected in repeated 24-hour urinary iodide measurements, any association should be examined carefully for reliability.

While NHANES collected data on some parameters relevant to thyroid function, these parameters fall short of providing sufficient information to clearly understand thyroid health.

Only two clinical measures of thyroid function were taken (total serum T4 and TSH). Missing were free T4, thyroid binding globulin (TBG), and T3 among others. Further, NHANES did not collect data on the incidence of autoimmune thyroiditis (also known as Hashimoto's 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). Other potential causes of a decrease in T4 could include consumption of goitrogenic foods, seasonal changes, hormonal fluctuations (*e.g.*, menopause, hormone replacement, birth control, pregnancy), use of certain prescription drugs (*e.g.*, antidepressants, cholesterol lowering drugs, corticosteroids), and stress or illness (Shomon, 2003), some of which were reported and measured in the study, and some which were not.

As also stated by ATSDR (2008):

It should be noted that none of these biomarkers is specific to perchlorate; other antithyroid agents such as carbimazole can have similar effects. It should also be noted that TT4 is not routinely measured for clinical significance as it is too impacted by other biological variables. Although the specific amount of change in these biomarkers associated with a demonstrably adverse effect has not been established, changes in these parameters can be considered to indicate potential impairment of health.

2.7.2 The reported results on T4 have not been seen in other studies

Other studies have not shown the effects reported by Blount *et al.* (2006) and Steinmaus *et al.* (2007) on T4 at the perchlorate levels measured in the NHANES study. For example:

- Greer *et al.* (2002) show that perchlorate administered at a dose of 0.5 mg/kg-d (about 17,000 ppb in drinking water) for two weeks to adult volunteers causes iodide uptake to be inhibited by approximately 70%. This level of inhibition did not cause changes in serum thyroid hormone levels in men or women, or changes in any of the more than 15 blood chemistry values that were measured. They estimated a true no effect level for inhibition of iodide uptake ranging from 0.0052 mg/kg-d to 0.0064 mg/kg-d, equivalent to a drinking water level of 180 to 220 ppb.
- Observations in perchlorate plant workers (Gibbs *et al.* 1998; Lamm *et al.* 1999) show that perchlorate exposure at levels associated with a relatively high percentage inhibition of thyroidal iodide uptake (equivalent to up to 0.49 mg/kg-d) produced no adverse health effects. These populations included individuals of both sexes exposed for many years (most over 5 years).
- In long-term exposure of children to perchlorate in drinking water at concentrations as high as 120 ppb (for their entire lifespan), no changes in thyroid hormones were observed (Crump *et al.* 2000).
- Brabant *et al.* (1992) found that a dose of about 13 mg/kg-d in humans for 4 weeks (about 455,000 ppb in drinking water) caused a decrease in free T₄, a decrease in intrathyroidal iodine, and a decrease in TSH, but did not deplete thyroidal iodide. This study suggests a LOAEL for thyroid hormone changes in humans.

- Pearce *et al.* (2010) found no association between urinary perchlorate concentrations and serum TSH or free T4 in women, including those with urinary iodide less than 100 µg/L. In one of the study populations, the median of urinary perchlorate concentrations was 5 µg/L (range 0.04–168 µg/L, in Turin, Italy) and in the other it was 2 µg/L (range 0.02–368 µg/L, in Cardiff).

2.7.3 *Spot urine samples do not support identification of iodine deficient individuals*

Use of the <100 µg/L urinary iodine cutoff to define iodine deficiency is arbitrary since it is based on spot urine samples that are highly variable and provide unreliable estimates of daily iodine excretion. The reported effects of perchlorate on thyroid measures disappear when segregation based on iodine status is removed.

The median urinary iodine concentration for the study population evaluated by Blount *et al.* (2006) (n =1,111) is 133 µg/L. 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 that population* (WHO, 2004). Thus, assuming that iodine deficiency exists *within individuals* in the NHANES is inappropriate—the WHO criteria are not meant to diagnose iodine deficiency in individuals (Borak, 2005). Rather, the best way to establish individual urinary excretion of iodine (or any analyte) is to collect 24-hour samples (Bourdoux, 1997). However, this is logistically prohibitive in a large survey like NHANES.

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). In fact, because of these variables, urinary iodine concentrations in even 24-hour samples can vary up to three fold from one day to another (Rasmussen *et al.*, 1999).

WHO chose the 100 µg/L spot sample concentration threshold for populations not on the basis of metabolic studies linking this level to iodine deficiency, but because this level very roughly corresponded to a daily iodine excretion across a population of 150 µg/d, which is thought to correspond to a threshold for iodine deficiency; however, in general, individual spot urine samples frequently grossly underestimate 24-hour urinary excretion (Als *et al.*, 2003). In a study using measurements from 110 people, spot urinary iodine measurements underestimated 24 hour urinary excretion by an average 30-35%, and the percentages of values below threshold levels corresponding to no, marginal, mild, intermediate, moderate or severe iodine deficiency were all significantly higher in the spot samples when compared to the 24 hour samples (Als *et al.*, 2003). In another study in which spot urine samples were collected monthly from 16 individuals for 13 months, urinary iodine concentrations were highly variable, with the coefficient of variation (the ratio of the standard deviation to the mean) for specific individuals ranging from 22-86% (Andersen *et al.*, 2008).

All of these factors suggests that a single urine sample is insufficient to determine long-term iodine status (Rasmussen *et al.*, 1999). Further, per Andersen *et al.* (2008), “The thyroid gland has the capacity to store large amounts of iodine, unaffected by short-term low iodine

intake. In addition, iodine excretion in the individual reflects the iodine intake over a short period of time prior to collection, and urinary iodine excretion varies considerably. Thus, short-term estimation of individual iodine intake may likely be inaccurate.”

During the NHANES study, serum samples for perchlorate, T4, and TSH and urine samples for iodine were collected at either “morning” (46%), “afternoon” (36%), or “evening” examination sessions (18%); specific sample time of day is not reported in NHANES. However, time of sample collection can significantly impact measured concentrations: a highly significant circadian rhythm in urinary iodine concentration has been reported, with nadirs in early morning and peaks in the evening (Als *et al.*, 2003), and iodine concentrations in afternoon samples were shown to better estimate 24-hour urinary excretions than concentrations in morning samples (Vanacor *et al.*, 2008). Iodine excretion also shows seasonality, with higher 24-hour excretion in spring and summer than in fall and winter (Andersen *et al.*, 2001).

Fasting times were also highly variable in the NHANES data. 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, a priority was not placed on compliance with this recommendation—the protocol states that the “greater goal [is] completing as many components as possible within the time constraints of the session with phlebotomy as the highest priority component.” In actuality, reported lengths 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, of participants did not meet the fasting requirement. Variability in these parameters could have influenced measured spot urine concentrations.

2.7.4 Spot urine samples are unreliable indicators of longer term perchlorate exposure status

The same factors that make spot urines for iodide unreliable can impact spot urines for perchlorate. As such, the high- and-low perchlorate groups in the Blount *et al.* (2006) and Steinmaus *et al.* (2007) may likewise be misclassified. Further, spot urine samples do not provide a good measure of longer term perchlorate exposure status, and are not likely to correlate with T4 measured at the same time. Even if perchlorate exposure was briefly sufficient to cause transient IUI, because the half life of circulating T4 is estimated to be 6.7 days (Russell *et al.*, 2008), an expected lag in response between iodide deficiency and effect on thyroid hormone of more than a week would be expected. Thus, if exposure to perchlorate or other IUI-causing agents (e.g., nitrate or thiocyanate) fluctuates, a spot measurement of perchlorate in urine would not be expected to correlate with a spot serum T4 measurement taken concurrently.

2.7.5 Thyroid hormone and TSH levels are expected to show temporal variability

Thyroid hormone levels are also variable. TSH and thyroid hormone, particularly free T3, levels in serum demonstrate circadian rhythms. 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). TSH is considered

to reach a maximum in the early morning between 2 and 4 am and a nadir in the afternoon/evening between 4 and 8 pm, but there is considerable interindividual variability (Russell *et al.*, 2008). In humans, serum TSH concentrations shortly before sleep were reported to be about 50-100% greater than at waking (Fisher, 1996), and early morning values were reported to be greater than later morning values (Surks *et al.*, 2005). Free T3 data showed a similar profile to TSH but with smaller amplitude and a time lag of approximately 90 minutes (Russell *et al.*, 2008). Because the half life of circulating T4 is longer than that of T3 (6.7 days, compared to 0.75 days for T3), T4 shows very little diurnal rhythm (Russell *et al.*, 2008). Still, thyroid hormone levels reflect seasonality, with higher serum T4 in autumn and winter than in spring and summer (Andersen *et al.*, 2001).

2.7.6 Thyroid hormone changes were not clinically significant and perchlorate doses were below the NOEL

In Blount *et al.* (2006) and Steinmaus *et al.* (2007), perchlorate did not lower (nor was it associated with) thyroid hormone levels outside the normal range of values. In fact, the perchlorate exposure levels considered in these studies are below those that caused measurable IUI in other studies. Blount and colleagues (Blount *et al.*, 2007) used the NHANES urinary perchlorate data to estimate a 95th percentile daily perchlorate dose for adults of 0.234 $\mu\text{g}/\text{kg}\text{-d}$ with a confidence interval of 0.202 – 0.268 $\mu\text{g}/\text{kg}\text{-d}$. By comparison, Greer *et al.* (2002) estimated a true no effect level for inhibition of iodide uptake ranging from 5.2 $\mu\text{g}/\text{kg}\text{-d}$ to 6.4 $\mu\text{g}/\text{kg}\text{-d}$. The doses estimated by Blount *et al.* (2007) are also lower than the current U.S. EPA RfD of 0.7 $\mu\text{g}/\text{kg}\text{-d}$. If individuals did not have iodine deficiency or measurable IUI, they would not be expected to have substrate deficiency for thyroid hormone synthesis.

Mendez *et al.* (2009) used probabilistic modeling to estimate the total dose of perchlorate from food and drinking water using three drinking water scenarios based on U.S. EPA Unregulated Contaminant Monitoring Rule 1 (UCMR1) data. The highest estimated dose through water and food was 0.15 $\mu\text{g}/\text{kg}\text{-d}$ at the 95th percentile, well below the RfD of 0.7 $\mu\text{g}/\text{kg}\text{-d}$. When compared to the NHANES 2001-2002 estimates based on urinary output (used by Blount *et al.*, 2007), they found that intraday variability contributed to an overestimation of dose based on the NHANES data. Interestingly, they also found that the urinary excretion of perchlorate in NHANES 2003-2004 was significantly lower than in 2001-2002. Two data points do not represent a trend, but this lower urinary excretion may represent lower doses through food and water in subsequent NHANES studies.

2.7.7 The reported difference in response between men and women is not seen in other studies

The observed differences in thyroid response between men and women reported in Blount *et al.* (2006) and Steinmaus *et al.* (2007) are not explained, and could be due to another unexplained variable. Comparable differences have not been observed in other studies of perchlorate exposure (*e.g.*, Greer *et al.*, 2002; Braverman *et al.*, 2006; Gibbs and Van Landingham, 2008; Lamm *et al.*, 2007; Pearce *et al.*, 2010). Specifically:

- Greer *et al.* (2002) evaluated five women and five men in each of the 0.02, 0.1, or 0.5 mg/kg-d groups, and six women and one man in the 0.007 mg/kg-d group. No sex difference in RAIU at a given perchlorate dose was seen.
- Braverman *et al.* (2006) exposed 13 healthy volunteers, including nine women, to 0, 0.5, or 3.0 mg/d of potassium perchlorate (equivalent to 0, 250, and 1500 ppb in drinking water, assuming ingestion of 2 L/d by a 70 kg adult) for six months, and measured RAIU and serum T3, free T4, TSH, and Tg concentrations. No effect on thyroid function or significant change in RAIU was seen. No sex related difference in any of the parameters was reported.
- Lamm *et al.* (2007) reanalyzed the NHANES 2001-1002 dataset (the same dataset used by Blount *et al.* (2006) and Steinmaus *et al.* (2007)) with two critical differences. Lamm *et al.* (2008) normalized urinary iodine using urinary creatinine and limited the study population to women of childbearing age (15-44 years old). Regarding creatinine normalization, OEHHA states, “The fact that the perchlorate-T4 association goes away in analyses stratified by I/Cr ratios suggests that the use of I/Cr leads to much more, not less, misclassification of true iodine status;” however, it is also likely that there is no association with such low environmental perchlorate exposures. OEHHA cites several studies suggesting that creatinine normalization is not appropriate for urinary iodine, but does not evaluate others that demonstrate creatinine normalization is useful (Manz, 2000; Thompson, 2001; Hoption Cann, 2007). Both Blount *et al.* (2006) and Steinmaus *et al.* (2007) include women up to age 85+ years. The levels of T4 fall as one ages, so the inclusion of this population may bias the association. These data are further discussed in Lamm (2008) (see Figure 6, below).
- Pearce *et al.* (2010) published the results from a large study of 22,000 pregnant women living in Cardiff, Wales and Turin, Italy. The authors measured iodine, thiocyanate, and perchlorate in spot urine samples and TSH, free T4, and thyroperoxidase (TPO) in blood in a subset of 2,640 participants. They further stratified the study population to look at women with urinary iodine less than 100 µg/L (999 women). The authors conclude, “There were no associations between urine perchlorate concentrations and serum TSH or FT4 in the individual euthyroid or hypothyroid/ hypothyroxinemic cohorts.”
- Tellez *et al.* (2005) evaluated pregnant women in Chile with dramatically higher urinary perchlorate levels when compared to Blount *et al.* (2006) (the mean urinary perchlorate level in Taltal was 133 µg/L). No increases in Tg or TSH and no decreases in free T4 among either the women during early pregnancy (16.1 ± 4.1 weeks) or late pregnancy (32.4 ± 3.0 weeks) related to perchlorate in drinking water were seen.

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), and 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). However, thyroid autoantibodies were not measured in the 2001-2002 NHANES. In analysis of data from NHANES III, a significant association between

female gender and elevated serum TSH levels disappeared when controlled for thyroid peroxidase antibodies (Hollowell *et al.*, 2002).

2.7.8 *Relative exposure to other iodide uptake inhibitors is likely much greater than perchlorate and findings reported by Blount et al. (2006) are inconsistent with the literature*

Because perchlorate, nitrate, and thiocyanate compete with iodide for uptake by the NIS, the combination of these chemicals in food or water will affect iodide uptake to the thyroid as well as absorption into the body from the gut. Of the four chemicals, perchlorate has the highest affinity for the NIS; however, the other three are more abundant in the diet. In Blount *et al.* (2006), some reported associations of urinary iodide uptake inhibitors and serum T4 or TSH were opposite what would be expected if associations were causal. These include the strongly negative association between urinary thiocyanate and serum TSH in males, the positive association between urinary nitrate and serum T4 in females, and the negative association between urinary thiocyanate and serum T4 in females.

In the FDA Total Diet Study, the dose range of perchlorate was 2.4 to 9.1 µg/person-d and the dose range of iodine was 144 to 353 µg/person-d (Murray *et al.*, 2008). Similar to biomonitoring studies (Blount *et al.*, 2006) and dose models (Belzer *et al.*, 2004), the OIG Report (U.S. EPA, 2010) found that perchlorate accounted for less than 1% of the predicted IUI in the total goitrogen load. Based on NHANES 2001-2002, the average urinary concentration of perchlorate was 4.8 µg/L, which was much lower than the urinary concentrations of nitrate, thiocyanate, and iodine at 55,017 µg/L, 2,042 µg/L, and 200 µg/L, respectively. Figure 5 demonstrates the relative contribution of perchlorate to total goitrogen load based on the NHANES 2001-2002 database.

One characteristic of this relationship is that as the perchlorate dose increases from food, so does intake of nitrate, thiocyanate, and iodine. Two things become clear from Figure 5. First, as iodine level increases, so does total cumulative dose. Second, perchlorate contributes, by far, the smallest amount to the total cumulative dose.

Other studies do not support OEHHA’s (2011) assertion that “human data show that perchlorate can interact with other contaminants to produce a greater effect (Blount *et al.*, 2006, Steinmaus *et al.*, 2007).” Authoritative bodies that have examined this issue have concluded that thiocyanate, nitrate and perhaps other substances compete with iodine and cause IUI by the same mechanism of action as perchlorate; however, nitrate and thiocyanate are present in a healthy diet in amounts greater than that of perchlorate. US EPA OIG used a cumulative risk assessment to evaluate the effects of perchlorate, nitrate, thiocyanate, and iodine deficiency on the thyroid and found that perchlorate accounts for less than 1% of an adult’s typical total goitrogen load. Tarone *et al.* (2010) also concluded that perchlorate is responsible for less than 1% of IUI when considering exposure to nitrate and thiocyanate based on five studies in the US and other Western populations.

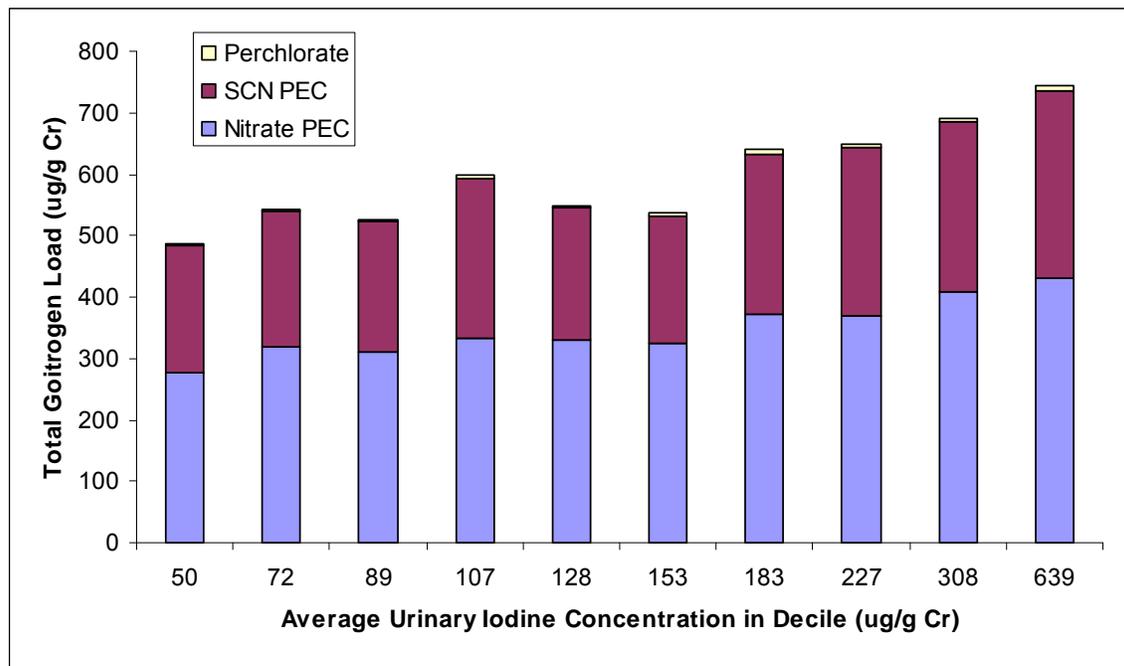


Figure 5. Total Goitrogen Load in Perchlorate Equivalence Based on Urinary Measurements from Women Ages 12 and Older from NHANES 2001-2002*

*Perchlorate equivalence is based on the relative ability to inhibit iodide uptake. Data presented are extracted from NHANES 2001-2002 and represent the approximate population used in Blount *et al.* (2006, n=1162). SCN: thiocyanate; PEC: Urinary perchlorate equivalence (SCN PEC = thiocyanate / 8.8; Nitrate PEC = nitrate / 150).

2.7.9 T4 measurements alone may be insufficient to assess thyroid effects

Measurement of total serum T4 could be influenced by serum protein variations, although a significant relationship between serum albumin and serum T4 and TSH was not seen in Blount *et al.* (2006). However, as mentioned in the Blount *et al.* (2006), serum albumin only accounts for 15-20% of T4 binding. Measurement of free T4, as was done by Tellez *et al.* (2005), is a better metric for assessing thyroid effects.

As discussed above in Section 2.2.2, hormone assays can be affected by multiple factors, some of which may alter the measurement without affecting the true hormone value (Ravel 2000; Larsen 2003). The accuracy of serum hormone levels is important when determining the putative effects of environmental contaminants, which are typically found at low or background levels (Brucker-Davis, 1998). For example, drugs (prescription and over-the-counter), hormones (estrogens), and pesticides have been demonstrated to impact T4 measurement, usually by displacing thyroid hormone from its binding site and accelerating the metabolism of the free hormone (Ravel 2000; Larsen 2003). Analytical methods also impact the reliability of analysis. Currently, serum total thyroid hormone (TT4) is measured by competitive immunoassay methods that are non-isotopic and use enzyme fluorescence or chemiluminescence molecules as signals (Davies, 2003). Enhancements to our understanding of thyroid measurements, however, have improved dramatically in the last two decades. Development of such techniques as ultra-centrifugation and equilibrium dialysis

(ED) have become “gold standards” for measuring true thyroid hormone values, as they overcome the inherent negative bias associated with assays that don’t account for potential binding issues (Sapin and d’Herbomez, 2003).

2.7.10 OEHHA ignores or discounts the results of several studies that conflict with the findings of Blount *et al.* (2006) and Steinmaus *et al.* (2007)

OEHHA’s discussion largely ignores other studies that do not support the assertions of Blount *et al.* (2006) and Steinmaus *et al.* (2007), as discussed earlier, or discounts the results of those studies for various methodological reasons.

Regarding Pearce *et al.* (2010) for example, OEHHA (2011b) notes,

It is unknown if perchlorate levels were adjusted for creatinine, which is commonly used to adjust for urine dilution. Failure to adjust for urine dilution can potentially cause misclassification of exposure and bias results towards the null. Urine thiocyanate levels were low, much lower than those commonly found in the U.S. The median urine thiocyanate levels in Cardiff and Turin were 470.5 and 372.5 µg/L, respectively. In the NHANES study discussed above (Steinmaus *et al.*, 2007), the strongest perchlorate-thyroid hormone associations were found with thiocyanate levels in the upper tertile (*i.e.*, above 1800 µg/L), and clear associations were not found with thiocyanate levels below 751 µg/L. In this regard, the Pearce *et al.* (2010) findings are consistent with those of Steinmaus *et al.* (2007).

Although Pearce *et al.* (2010) is discounted by OEHHA, this is the largest study published to date of the association between urinary perchlorate and thyroid hormone. It evaluates the same relationship as Blount *et al.* (2006) and Steinmaus *et al.* (2007); however, it is based on an independent data set. Pearce *et al.* (2010) used a larger population (n = 1,641) of pregnant women while Blount *et al.* (2006) and Steinmaus *et al.* (2007) was based on a smaller population (n = 1,111) of women over age 12. The authors reported there was no association between urinary perchlorate and thyroid function, even in iodine deficient pregnant women. In an additional study, Pearce *et al.* (2011) found no association between urinary perchlorate and thyroid function in pregnant women in Los Angeles, California and Cordoba, Argentina. Urinary perchlorate levels were greater than in Blount *et al.* (2006) and Steinmaus *et al.* (2007) and the authors were clear that they did adjust for urinary creatinine. This is an additional independent data set from Blount *et al.* (2006), Steinmaus *et al.* (2007), and Pearce *et al.* (2010).

Regarding the discrepancies between the findings cited in Blount *et al.* (2006) and Steinmaus *et al.* (2007) and some other studies, OEHHA (2011b) states, “some occupational and clinical studies, although they involved higher perchlorate exposure levels, may have missed the effects seen in Blount *et al.* (2006) and Steinmaus *et al.* (2007) because they did not specifically investigate susceptible groups.” These “susceptible groups” presumably include women with urinary iodine levels below 100 µg/L and people with high thiocyanate. However, as discussed above, classification of study participants as iodine deficient based on a single spot urine sample is erroneous, and other studies do not support the increased susceptibility of people with high thiocyanate exposure.

Further, more extensive criticisms regarding study design and statistical methodology have been published by ATSDR (2008) and Tarone *et al.* (2010). Regarding Blount *et al.* (2006), 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). Tarone *et al.* (2010) conducted a multiple regression analysis of the same data used by Blount *et al.* (2006) and Steinmaus *et al.* (2007), and express concerns regarding the information that can be derived from the type of data Blount *et al.* (2006) and Steinmaus *et al.* (2007) used and report that these papers are unable to determine a causal relationship.

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.* (2006) with an adjustment for urinary creatinine. Lamm *et al.* considered a subset of women from the Blount *et al.* (2006) study who were of childbearing age (15-44 years old; the Blount *et al.*, 2006 study group included all women over the age of 12) as well as the interaction of thiocyanate and nitrate, both in urine. They found no significant association between perchlorate and total T4, including in women with urinary iodine less than 92 µg/g (Figure 6).

Serum Thyroxine and Iodine Uptake Inhibitors, by Terciles of UICr*, weighted Data, NHANES 2001-2002, WCBA***			
UICr	Low Tercile (<92.0 µg/g*)	Middle Tercile (92.0-163.7 µg/g)	High Tercile (> 163.7 µg/g)
Perchlorate	-0.13 (p=0.89)	-0.35 (p=.07)	-1.09 (p=0.01)
Thiocyanate	-0.30 (p=0.31)	0.71 (p=0.29)	-0.98 (p=0.02)
Nitrate	1.55 (p=0.06)	-2.31 (p=0.02)	0.06 (p=0.94)

* Cr-Adj. Urine Iodine (µg iodine / gram creatinine)
 ** Regression coefficient (beta)
 *** Women of Childbearing Age

Source: Lamm *et al.*, 2008

Figure 6. Urinary Reanalysis Results of the NHANES Data

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.* (2007) and Lamm *et al.* (2008), both presented at the Seattle SOT meeting.

3.0 SCIENTIFIC CONCERNS ABOUT ASSUMPTIONS USED IN THE CALCULATION OF THE PHG

As discussed in Section 1.1, the PHG is the product of a mathematical equation that incorporates five variables:

$$PHG (\mu g / L) = \frac{POD (\mu g / kg - d) \times BW (kg) \times RSC}{UFs \times WC (L / d)}$$

None of the factors used to derive the 2011 PHG is scientifically supportable. In the draft PHG, the combined effect of these factors results in OEHHA further lowering the PHG without demonstrating how these adjustments will result in an increased public health benefit.

Concerns about specific assumptions OEHHA uses in the calculation of the PHG are discussed below.

3.1 The Point of Departure is Based on a Conservative No Observed Effect Level in Humans with Additional Uncertainty Factors Applied

The POD for the PHG is based on the scientific study by Greer *et al.* (2002). This POD is divided by an uncertainty factor (UF), which is largely based on speculation.

In Greer *et al.* (2002), thirty-seven healthy adults were exposed to 0.007, 0.02, 0.1, or 0.5 mg/kg-d perchlorate in drinking water daily for two weeks. The study reports:

The lowest dose producing no statistically significant inhibition of uptake was 0.007 mg/kg-day. Thus, in this study, 0.007 mg/kg-day (7 μ g/kg-day) was a NOEL for inhibition of RAIU.

The NRC (2005) stated that the resulting NOEL reported "...is consistent with findings of similar studies in humans." Thus, not only was Greer *et al.* (2002) a scientifically rigorous study, but its results were bolstered by other studies also conducted in humans.

In its proposed PHG, OEHHA states that Greer *et al.* (2002) was chosen

...because it was an experimental study in humans where subjects were given known doses of perchlorate and evidence of a dose-response relationship was seen with a critical outcome, iodide uptake in the thyroid.

Although the study reported an already conservative NOEL, OEHHA performed a benchmark dose (BMD) analysis to derive an estimate of the BMD lower bound (BMDL) that reflects a reduction in radioactive iodide uptake of 5%. According to OEHHA, using a BMDL "helps to account for the uncertainty inherent in a given study and according to the U.S. EPA, 'assures (with 95 percent confidence) that the desired response is not exceeded' (U.S. EPA, 2000)." This BMDL of 3.7 μ g/kg-d is approximately half the NOEL for inhibition of RAIU determined in the Greer *et al.* (2002) study.

Yet even with this BMDL analysis to account for uncertainty, OEHHA further added an uncertainty factor of 10. OEHHA states:

We chose an uncertainty factor of 10 to help account for interindividual variability in the population that was not captured by the Greer *et al.* (2002) study. The Greer *et al.* study included only 37 healthy adults so the variability of the study data is likely to be smaller than that in the general population. Furthermore, the study population did not specifically examine individuals with low iodine intake, pregnant women, infants, or people with the other potential susceptibility factors.

The increase in UF is the largest numerical factor in the PHG equation which results in the proposed PHG of 1 ppb. OEHHA evaluated the infant in 2004 and used a UF of 3 rather than 10 (as they applied to the pregnant woman). OEHHA (2004) stated,

A smaller uncertainty factor of three is used for the infant. This is because traditionally, an uncertainty factor of 10 is used to account for interindividual variability, which is assumed to include a factor of approximately three (or half a log unit) for differences in toxicokinetics and another three for differences in toxicodynamics. In the case of infant exposure estimation, an infant specific BW/WC ratio was used, which accounted for up to a 6-fold difference in toxicokinetics between infants and adults. It should be noted that the differences in toxicokinetics between infants and adults might have been over-estimated. Using PBPK modeling, U.S. EPA (2002) concluded that uptake and elimination kinetics of perchlorate are such that the resultant time-integrated perchlorate concentrations in blood (area under the curve) for adults (70 kg) and children (15 kg) should be about the same. Due to these considerations, a full interindividual variability factor of 10 for infants did not appear warranted.

In its proposed PHG, OEHHA (2011b) continues to use an infant-specific BW/WC, yet also increases the UF from 3 to 10. They did this even though no new information on the toxicokinetics of perchlorate is presented in the draft document that would provide scientific support for a change (e.g., Appendix B demonstrates that there is no difference in the pharmacokinetics of perchlorate in the neonate). Further, as noted above, the draft document provides no scientific support that the infant, in general, is more sensitive than the pregnant woman. The decision by OEHHA to increase the UF to 10 is, in effect, double counting the toxicokinetics and is a much cruder estimation of toxicokinetics than the use of BW/WC for a specific life stage. The revised draft document should either provide reliable scientific support or it should return to the previous UF of 3.

Regarding use of the data from Greer *et al.* (2002), NRC (2005) states,

... the committee recommends that inhibition of iodide uptake by the thyroid, which is the key biochemical event and not an adverse effect, should be used as the basis of the risk assessment. Inhibition of iodide uptake is a more reliable and valid measure, it has been unequivocally demonstrated in humans exposed to perchlorate, and it is the key event that precedes all thyroid-mediated effects of perchlorate exposure.

The committee emphasizes that its recommendations differ from the traditional approach to deriving an RfD. The committee is recommending using a nonadverse

effect rather than an adverse effect as the point of departure for the perchlorate risk assessment. Using a nonadverse effect that precedes the adverse effects is a conservative, health-protective approach to the perchlorate risk assessment, and the committee's recommendations for uncertainty factors reflect the conservatism of the approach.

Some have criticized the NOEL dose of Greer *et al.* (2002). For example, Zewdie *et al.* (2010) states,

Using standard sample size calculation approaches (Green 1979; Sokal and Rohlf 1995) the minimum difference in RAIU in the Greer study's low perchlorate dose group that could be discriminated from the baseline mean value of 18.1% (SD = 8.2) was $\pm 40\%$ at an alpha of 5%... Thus, the Greer *et al.* (2002) study was unable to reliably detect up to a 40% change in RAIU at the lowest dose tested.

Zewdie *et al.* (2010) appear to perform a *post hoc* sample size calculation for just the low dose group. It is unclear why the paper chose to disregard the entirety of the dose response data and focus on the standard deviation of the mean of one group, as this evaluates only differences among individuals instead of evaluating the existence of the effect. Furthermore, it is not clear how the paper calculates the range "(10.9-25.3%)" under any set of assumptions.

The raw data from Greer *et al.* (2002) have been broadly available since 2002 and to state and federal agencies since before 2002. The data for the low dose group are summarized in Table 6. The standard deviation (SD) of differences is $SD(X-Y) = \sqrt{SD(X)^2 + SD(Y)^2 - 2 * \text{covariance}(X,Y)}$ and, based on the Zewdie *et al.* (2010) comments, it appears that they assume that covariance = 0. However, the original statistical analysis was conducted directly on the paired differences, which is a more appropriate method to address the question of a difference in RAIU before and after administration of any test substance. This avoids the need to make assumptions as Zewdie *et al.* (2010) did.

These data are plotted in Figures 7 and 8. Examination of the RAIU data show good correlation between the 8-hour and 24-hour measures for each subject. This includes pre-exposure to perchlorate, 14-day exposure to perchlorate, and the difference between pre and post exposure (Figure 7).

Figure 8 presents a different view of the data by summarizing individual and group statistics for percent RAIU relative to baseline at 8 and 24 hours. These data clearly demonstrate that for this group of subjects with 14 days of exposure, there is no difference in RAIU after taking the 0.007 mg/kg-d dose.

Given this analysis, the NOEL is transparent and logical. Further, the Greer *et al.* (2002) data are supported by a 14-day clinical study conducted by Lawrence *et al.* (2000, 2001) and a six month study conducted by Braverman *et al.* (2006).

Table 6. Presentation of the raw data from Greer *et al.* (2002) for the low dose (0.007 mg/kg-d) group.

Thyroid ¹²³I Uptake (% of ingested ¹²³I in cpm)

Dose (mg/kg-d)	Sex	Baseline		Exposure		%Change		Difference	
		8-hr	24-hr	8-hr	24-hr	8-hr	24-hr	8-hr	24-hr
0.007	f	8.2	12.2	8.7	13.2	106.1	108.2	0.5	1.0
0.007	m	13.0	21.5	12.8	18.7	98.5	87.0	-0.2	-2.8
0.007	f	6.8	10.0	8.7	13.9	127.9	139.0	1.9	3.9
0.007	f	7.6	12.7	8.0	13.5	105.3	106.3	0.4	0.8
0.007	f	16.6	22.0	10.2	17.8	61.4	80.9	-6.4	-4.2
0.007	f	25.4	33.7	16.3	24.7	64.2	73.3	-9.1	-9.0
0.007	f	10.5	14.5	9.8	13.4	93.3	92.4	-0.7	-1.1
Mean		12.6	18.1	10.6	16.5	93.8	98.2	-1.9	-1.6
SD		6.6	8.3	2.9	4.3	23.8	22.0	4.1	4.2
Count		7	7	7	7	7	7	7	7
u(Mean)		2.5	3.1	1.1	1.6	9.0	8.3	1.6	1.6
U95(Mean)		6.1	7.7	2.7	4.0	22.0	20.4	3.8	3.9
Ratio		84.6	91.0	-1.9	-1.6	Diff			
u(Ratio)		19.0	18.1	2.7	3.5	u(Diff)			
U95(Ratio)		37.8	36.0	6.7	8.6	U95(Diff)			

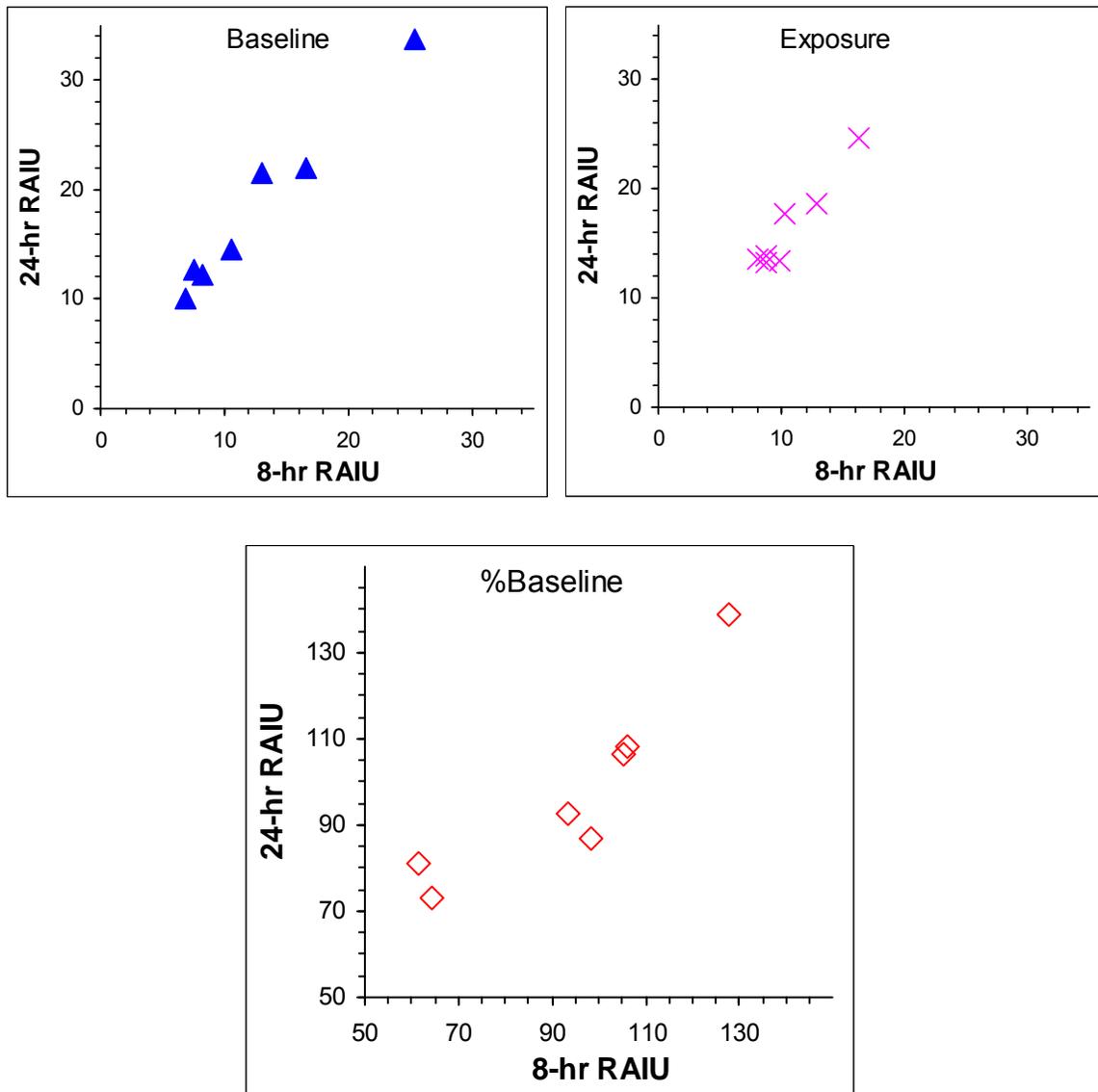


Figure 7. Plot of the Baseline, Difference Between Exposure and No Exposure, and % Baseline for Each Subject for 24-hr v 8-hr RAIU at the NOEL Dose of 0.007 mg/kg-d After 14 Days of Intake.*

*See Greer *et al.*, 2002 for details. These are values for RAIU with administration of the low dose of perchlorate. One would assume that these values would reflect the influence of perchlorate from the diet as well as other inhibitors of iodide uptake such as nitrate and thiocyanate.

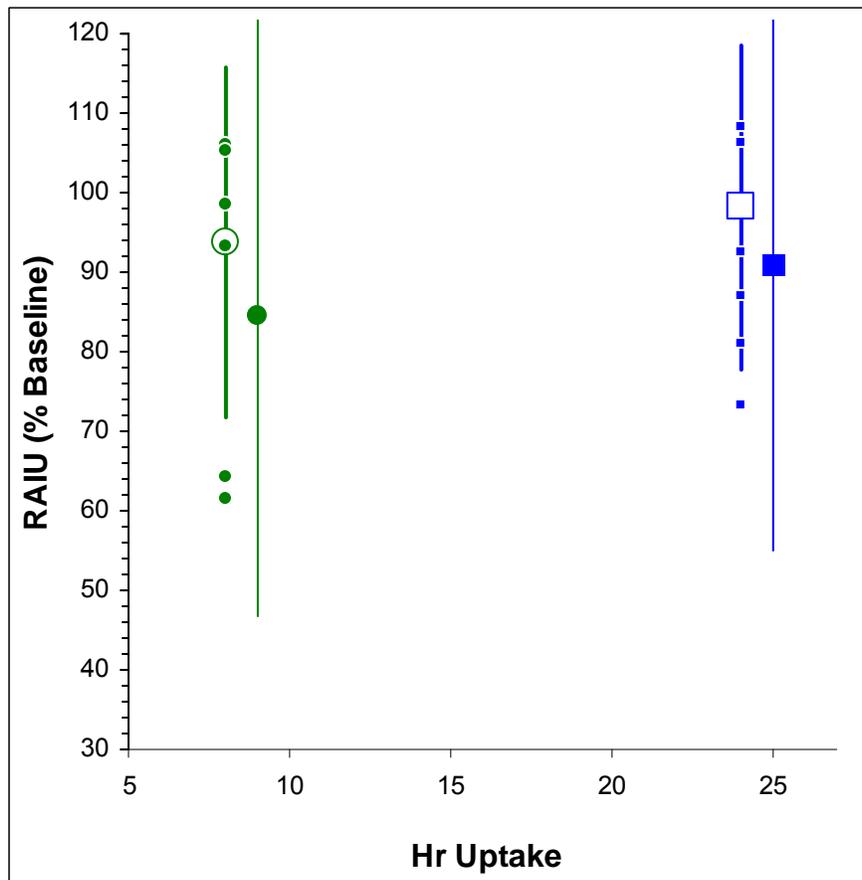


Figure 8. Summary Plot of the Data in Figures 7.*

*The plot of the difference between exposure and no exposure for each subject at 24-hr (blue lines) and 8-hr RAIU (green lines) for each subject, as well as a summary of the group statistics (thin blue and green lines) at the NOEL dose of 0.007 mg/kg-d after 14 days of intake. See Greer *et al.*, 2002 for details. One would assume that these values would reflect the influence of perchlorate from the diet as well as other inhibitors of iodide uptake such as nitrate and thiocyanate.

3.2 The RSC is Redundant

OEHHA applied an RSC of 0.73 to account for exposures to perchlorate from different media in addition to water (*e.g.*, food). The RSC reflects the portion of the total acceptable daily dose (ADD) not derived from food, and is based on mean values of estimated intake from the FDA Total Diet Study (Murray *et al.*, 2008) for adults and a study that estimated intake based on formula in infants (Schier *et al.*, 2009).

Considering the critical study for the POD, use of an RSC is redundant. Greer *et al.* (2002) did not control dietary intake of perchlorate or other natural agents such as nitrate, thiocyanate, or iodine. Perchlorate has been detected in many foods, including but not limited to milk, lettuce, and cantaloupes (Murray *et al.*, 2008). Thus, the dose of perchlorate that the study subjects in Greer *et al.* (2002) received from drinking water was in addition to background intake from diet. If the background dose of perchlorate from all sources was 0.02 to 0.234 $\mu\text{g}/\text{kg}\text{-d}$ (the 5th and 95th percentile estimated doses from Blount *et al.*, 2007)),

then the administered doses in Greer *et al.* (2002) underestimate the subjects' true exposure by this amount and the POD in the PHG equation already incorporates a RSC.

3.3 Conservative Assumptions are Embedded in Drinking Water Intake Rates and Body Weights

For the body weight and water consumption rate factors, OEHHA's PHG (2011) calculation uses the upper 95th percentile water consumption rate:body weight ratios for infants age 0-6 months (U.S. EPA, 2004). These ratios are based on water intake from all sources, including direct water intake (used for drinking) and indirect water intake (used in the final preparation of foods at home or restaurants). This calculation implicitly assumes that the body's disposition of perchlorate is directly proportional to body weight. There are no scientific data to support these assumptions.

The drinking water intake rates are based on data collected in the USDA Continuing Study of Food Intakes by Individuals from 1994-1996 and 1998. This was a large study of dietary recall and was reported by U.S. EPA (2004) and Kahn and Strahlka (2008); Dr. Kahn and Ms. Strahlka were the primary authors of U.S. EPA (2004). Kahn and Strahlka (2008) uses the same data set as reported in U.S. EPA (2004), however, for children, data are presented for finer age groups. For example, in U.S. EPA (2004), infants under one year old were grouped by birth to six months and six to 12 months. In Kahn and Strahlka (2008), infants under one year are grouped by birth to one month, one to three months, three to six months, and six to 12 months.

The body weights presented by Kahn and Stralka (2008) are lower than those reported in 1996-2000 NHANES, as presented by U.S. EPA in the Child Specific Exposure Factors Handbook (U.S. EPA, 2008), and may not be representative of the general population. For one month olds, the Kahn and Stralka (2008) mean, 90th and 95th percentile body weights are 20, 45, and 24% lower than the NHANES 1996-2000 data. A higher body weight will result in a lower total dose, if intake is the same. The use of a lower body weight, as was done by OEHHA, serves to decrease the PHG.

Most importantly, Kahn and Stralka (2008) is a recall study; participants had to recall their own (or their infant's) consumption over two non-consecutive days. No direct measurements were taken. Recall studies are subject to bias from errors in memory when recalling the amounts consumed (*i.e.*, what, when, and how much did you eat or drink?). For example, it is possible and not unusual that the person reporting the infant intake estimated a high value for intake. If this occurred for one or two individuals in the study, the data would be skewed (as observed by inspection), overestimating the statistical estimation of the 90th and 95th percentile water consumption values.

Furthermore, a dietary recall study should represent the entire population, not just tap water consumers. OEHHA chose to use the data for consumers of water only, yet individuals commonly use water from other sources (*e.g.*, bottled, in juice or soda). Since OEHHA is interested in informing a maximum contaminant level (MCL), sources of water that would be regulated are municipal water systems. One effect of choosing not to use the analysis of all individuals is that the results are higher than if all data were used. For example, OEHHA proposes to use an intake rate of 234 mL/kg-d based on tap water consumers only. However,

the value would be 216 mL/kg-d based on all individuals. Use of the values for consumers only is not a science-based decision.

Another data set available that could be used for estimating water intake could be compiled from studies measuring nutrition from formula intake. These studies (including Fomon *et al.*, 1969, 1971, 1975, 1977, 1983, 1995, 1999; Ryu *et al.*, 1983; Shepherd *et al.*, 1988; Ekstrand *et al.*, 1994; Specker *et al.*, 1997) used tightly controlled measurement of actual formula intake with a consistent ratio of formula to water. These studies all present individual body weight and intake rates in infants based on actual measurements rather than recall. An example of how these data differ from those reported by U.S. EPA (2004) and Kahn and Strahlka (2008) is the difference in the intake rates of infants less than one year old. Kahn and Strahlka (2008), which present finer age groups, report the mean intake rate to be 137 mL/kg-d, with a 90% confidence interval of 109 to 166 mL/kg-d, but the formula measurement studies report a lower mean intake of 131 mL/kg-d with a 90% confidence interval of 128 to 133 mL/kg-d. This suggests that a more rigorously developed data set may offer a lower 95th percentile drinking water intake.

One way to gauge whether the 90th and 95th percentile values suggested in U.S. EPA (2004) are statistically reliable is a simple comparison to blood volume. The average adult has a blood volume of approximately 5 L and drinks approximately 1.4 L/d (about 1/3 of a gallon), or 27% of their blood volume (U.S. EPA, 1997). It is well understood that infants and children consume more than adults when normalized for body weight, but using the intake rates suggested by U.S. EPA, the 95th percentile one to six month old infant would consume 1.2 L/d or 130% of their blood volume. This is the equivalent to assuming that the average adult consumes just under two gallons, every day. Just as this quantity seems unlikely for an adult, the volume in U.S. EPA (2004) is unlikely for an infant.

It should also be noted that OEHHA bases its draft PHG on the exclusively formula fed infant, not the breast fed infant or an infant that receives both. A breast fed infant would not be directly exposed to municipal drinking water and rates of exposures via breast milk were not estimated in the OEHHA PHG document. However, in its justification for choosing the infant as the most susceptible population, OEHHA cites studies of low iodine in breast fed infants. OEHHA states,

The increased susceptibility in infants is also supported by data suggesting that many infants may not be receiving adequate iodine in their diets and that young infants have low stores of thyroid hormone (less than one days worth compared to several weeks worth in adults) (van den Hove *et al.*, 1999; Pearce *et al.*, 2007).

And further continues,

...new data suggests that many infants may not be receiving adequate iodine in their diets. In a study of nursing mothers in Boston, 47 percent of breast milk samples did not contain enough iodine to meet the infant iodine intake recommended by the Institute of Medicine (Pearce *et al.*, 2007). Since the mechanism of perchlorate toxicity is a reduced iodide uptake into the thyroid, perchlorate-related toxicity is likely to be greater in infants who are already deficient in iodine.

Formula is fortified with iodine and an exclusively formula fed infant would receive middle to higher iodine levels through formula and would not be expected to be iodine deficient (Schier *et al.*, 2009). The same study cited by OEHHA to determine the RSC (Schier *et al.*, 2009) reports,

Although the minimum levels of iodine would be insufficient based on exposure modeling, it is more likely that the true levels would approach somewhere in between (middle value). In this case, and in situations with higher iodine intakes, no infants would be expected to be iodine deficient.

It is not scientifically justifiable to base the draft PHG on the exclusively formula-fed infant and to further assume that such an infant will be iodine deficient when there is no research to support this.

4.0 COMPARISON OF THE PHG FOR PERCHLORATE TO OTHER ENVIRONMENTAL AGENTS

Compared to PHGs developed by OEHHA for other compounds, OEHHA's draft 2011 PHG for perchlorate is inconsistent with its own previous risk assessment methodology. Table 7 presents a summary of some non-cancer and cancer PHGs for perchlorate and other chemicals developed by OEHHA as of December, 2010. Briefly, this review shows:

- Most noncancer PHGs were developed based on a NOAEL or LOAEL from experiments of non-carcinogenic effects in animals, with a drinking water equivalent level based on an adult male and/or female weighing 70 kg or 60 kg, respectively. PHGs for some other compounds (*e.g.* arsenic, lead, and trichloroethylene (TCE)) were derived using the benchmark dose approach. However, in all cases, the point of departure was an adverse effect level.
- In general, OEHHA used standard risk assessment methodology by applying UFs representing the various extrapolations or sources of uncertainty to the NOAEL/LOAEL to estimate a dose that would be unlikely to cause adverse effects in the human population or susceptible subpopulations with a lifetime of exposure. These UFs were applied to PODs defined as adverse.

With the exception of perchlorate and thallium, the PHG calculation for all of the chemicals was based on an identified adverse effect and not a biochemical precursor event in the continuum of possible effects related to exposure (in the case of perchlorate) or a “biologically insignificant” effect (in the case of thallium).

For thallium, the POD was described as a NOEL (OEHHA, 2004e). The NOEL was calculated after a critical analysis of a 90-day study by Stoltz *et al.* (1986). Per OEHHA, the authors in this study concluded that “the incidence of alopecia in female rats at the highest dose of 0.25 mg/kg-day was due to normal self barbering or the normal cyclic pattern of hair growth in rodents” and that “the finding was biologically insignificant.” They further elaborated, “Alopecia is characteristic of thallium toxicity in both animals and humans, and in humans thallium was once used as a depilatory agent. More importantly, it appears that alopecia is part of a continuum of dermal morphological changes and is therefore an early sign of an adverse health effect.”

The NOEL used for thallium is not similar to the NOEL used for perchlorate. For perchlorate, OEHHA calculated a BMDL that was below the experimental NOEL for a nonadverse biochemical response reported in the Greer *et al.* (2002) study. For thallium, alopecia was visually apparent in dosed animals at 0.25 mg/kg-d, although it was not clear if the effect was due to thallium since histological examination of a skin area showing alopecia in four females revealed hair follicle atrophy in only one case at the highest dose level. Based on the absence of gross or histopathologic findings, a NOEL of 0.05 mg/kg-day (administered dose equals 0.0405 mg/kg-day) was identified from this study.

Table 7. Summary of PHGs for Perchlorate and Other Chemicals.

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)				Variables			
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Perchlorate (OEHHA, 2011b)	1 ppb	BMDL = 0.37 µg/kg-d	Infant		10				73	4.5 kg, BW/WI = 4.3 kg-d/L	0.234 L/kg-d ^a
Perchlorate (OEHHA, 2004d)	6 ppb	BMDL = 0.37 µg/kg-d	Pregnant woman/ fetus		10				60	60 kg, BW/WI = 25.2 kg-d/L	0.044 L/kg-d ^a
1,1-Dichloroethylene (OEHHA, 1999a)	10 ppb	LOAEL = 9 mg/kg-d [2 yr rat (M/F) drinking water study, fatty changes in liver]	NA	3 (“the critical effect was not particularly severe”)	10	10		10	20	70 kg	4 Leq (inh + dermal)
1,2,4-trichlorobenzene(OEHHA, 1999b)	5.0 ppb	NOAEL = 14.8 mg/kg-d [95 d rat (M/F) drinking water study, enlargement of adrenal glands]	NA		10	10	10	10 (carcinogenic potential)	20	70 kg	4 Leq (inh + dermal)
Arsenic (OEHHA, 2004a)	0.004 ppb (based on cancer) 0.9 ppb (based on non-cancer)	For noncancer: Lower bound on effect levels (LED10), cumulative dose metric = 3 mg/L-yr/70 yrs [vascular effects in a human epidemiological study of cerebrovascular disease]	NA		10				20	NA	NA

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)				Variables			
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Barium (OEHHA, 2003b)	2.0 ppm	NOAEL = 0.21 mg/kg-d [absence of cardiovascular effects in humans]	NA		3				100	70 kg	2L
Benzene (OEHHA, 2001a)	0.15 ppb (based on cancer)	For noncancer: NOAEL = 0.087 mg/kg-d [hematological effects in U.S. male refinery workers exposed for up to 21 years]	NA		10				20	70 kg	4.7 Leq (inh + dermal)
	26 ppb (based on non-cancer)										
Beryllium (OEHHA, 2003a)	1.0 ppb	NOAEL = 0.15 mg/kg-d or BMD = 0.2 mg/kg-d (95% lower CL on 5% increase in lesions) [172 wk dog feeding study, gastrointestinal tract lesions]	NA		10	10		10 (data deficiency)	20	70 kg	2 L
								10 (carcinogenic potential)			
Bromate (OEHHA, December, 2009a)	0.1 ppb (based on cancer)	For noncancer: NOAEL = 1.1 mg/kg-d [100 wk rat (M) drinking water study, renal pelvis urothelial hyperplasia]	NA		10	10			20	NA	0.044 L/kg-d ^b
	50 ppb (based on non-cancer)										

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)				Variables			
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Cadmium (OEHHA,2006b)	0.04 ppb	NOAEL = 19 µg/d, [increased levels of urinary proteins in humans, a sensitive indicator of renal toxicity]	Woman		5			10 (carcinogenic potential)	20	60 kg	2 L
Carbofuran (OEHHA, 2000b)	1.7 ppb	NOAEL = 0.1 mg/kg-d [5 d/wk, 60 day adult rat (M) gavage study, testicular effects]	NA		10	10	3		20	70 kg	2 L
Chromium (hexavalent) (OEHHA, Draft December 2011a)	0.02 ppb (based on cancer) 2 ppb (based on non-cancer)	For noncancer: LOAEL = 0.2 mg/kg-d [2 yr rat (F) drinking water study, liver toxicity (mild chronic inflammation, fatty changes)]	Child, 1-10 years old ^d	10	10	10			80	NA	0.088 L/kg-d ^c
Dinoseb (OEHHA, 2010c)	14 ppb	LOAEL = 1 mg/kg-d [100 week adult mouse (M/F) feeding study, reproductive effects]	NA	10	10	10			80	60 kg	3.5 Leq (inh + dermal)
Diquat (OEHHA, 2000c)	15 ppb	NOAEL = 0.22 mg/kg-d [2 yr adult rat (M/F) feeding study, minimal lens opacities and cataracts]	NA		10	10			20	70 kg	2 L

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)			Variables				
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Endrin (OEHHA, 1999; OEHHA, 2008)	1.8 ppb	NOAEL= 1.0 ppm, or 0.025 mg/kg-d [2 yr dog feeding study, increased seizures and liver pathology]	NA		10	10			20	70 kg	2L
Fluoride (OEHHA, 1997a)	1.0 ppm	NOAEL = 1 mg/L [dental fluorosis in children]	Children						100	NA	NA
Lead (OEHHA, 2004c)	0.2 ppb	Lower level of concern = 2.86 µg/d, primarily based on Carlisle and Dowling (2006) and analysis of Lanphear <i>et al.</i> (2005)	Child (age unspecified)					3 (lack of a thres- hold, small sample size)	20	NA	1 L
Mercury, inorganic (OEHHA, 2005)	1.2 ppb	Intake from water of 2.86 µg/d = 1 µg/dL increase in blood lead = decrease of 1 IQ point in children NOAEL = 0.23 mg/kg-d [6 mo rat study, decrease in BW gain and increase in relative and absolute kidney wt at 0.46 mg/kg- d]	Pregnant women and their fetuses, infants, elderly		10	10	10		20	70 kg	2 L
Methoxychlor (OEHHA, 2010b)	0.09 ppb	LOAEL = 0.02 mg/kg-d [rat developmental study, effects on reproductive system of male offspring]	Pregnant women	10	10	10			20	60 kg	0.043 L/kg-d ^d

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)			Variables				
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Nickel (OEHHA, 2001b)	12 ppb	NOAEL = 1.12 mg/kg-d [rat development study, early pup mortality]	NA		10	10		10 (carcinogenic potential)	30	70 kg	2 L
Nitrate/nitrite (OEHHA, 1997b)	10 ppm as N	NOAEL = 10 mg nitrate-nitrogen/L [methemoglobinemia in infants] For nitrite, the NOAEL is multiplied by 0.1 to account for conversion of nitrate to nitrite by gastrointestinal tract bacteria	Infants A UF of one was applied because the NOAEL was of the critical effect (methemoglobinemia) in the most susceptible human subpopulation (infants)						NA	NA	NA
Oxamyl (OEHHA, 2009b)	26 ppb (based on non-cancer)	NOAEL = 0.06 mg/kg-d [acute oral study in adult male humans]	Infants and toddlers		10				100 (infants), and 20 (toddlers)	NA	0.0552 L/kg-day (infant) and 0.0457 L/kg-day (toddler)
Pentachlorophenol (PCP) (OEHHA, 2009c)	0.3 ppb (based on cancer) 5 ppb (based on non-cancer)	For noncancer: LOAEL = 1.0 mg/kg-d [decreased serum thyroid hormones in sheep (1-generation) and mink (multi-generation)]	Formula-fed infants <6 mo old	10	10	10			100 (for infant < 6 mo old)	4.5 kg	0.221 L/kg-d ^e

Chemical	PHG	Point of Departure	Assumed Susceptible Subpopulation	Uncertainty factors (UFs)				Variables			
				LOAEL to NOAEL	Intra-species	Inter-species	Sub-chronic to Chronic	Data-base	RSC (%)	BW	WI
Simazine (OEHHA, 2001c)	4 ppb (based on non-cancer)	NOAEL = 0.5 mg/kg-d [2 yr rat feeding study, reduced body weight]	NA		10	10		10 (carcinogenic potential)	20	70 kg	2 L
Styrene (OEHHA, 2010d)	0.5 ppb (based on cancer in rodents)	BMDL for non-cancer = 0.155 mg/kg-d [bronchiolar effects in male mice]	NA	10	10	3			20	60-70 kg	5.25 Leq
Thallium (OEHHA, 2004e)	0.1 ppb	NOEL = 0.0405 mg/kg-d [90d rat drinking water study, OEHHA determined the incidence of alopecia in F at 0.25 mg/kg-d was biologically significant]	NA		10	10	10	3 (steep dose-response curve)	20	70 kg	2 L
Trichloroethylene (TCE) (OEHHA, 2009d)	1.7 ppb (based on cancer)	For noncancer: BMD10 = 50 mg/kg-d [1 yr rat oral study, kidney nephropathy]	NA		10	10			20	70 kg	7.1 Leq
	1,000 ppb (based on non-cancer)										

BMD — Benchmark dose; BW — Body weight; CL — Confidence level; NA — Not available; WI — Water intake

Leq = To designate a combined liter equivalent value, assuming oral exposure is due to drinking 2L/day of water, followed by inhalation and dermal exposure.

^a Perchlorate – The 2011 and 2004 values are based on the upper 95th percentile of municipal water consumption for infants and pregnant women, respectively (U.S. EPA, 2004b).

^b Bromate – Based on the upper 95th percentile of municipal water consumption for the general population (U.S. EPA, 2004b).

^c Chromium – Upper 95 percentile water intakes for a child, adult woman, and adult male are 0.088, 0.054, and 0.053 L/kg-day, respectively (OEHHA, 2000).

^d Methoxychlor – Based on the upper 95th percentile consumption of community water supplies (tap water) for pregnant women (U.S. EPA, 2004b).

^e Pentachlorophenol – Based on the upper 95th percentile of consumption of community water supplies (tap water) for infants <6 months old (U.S. EPA 2004b).

PHGs for some chemicals such as arsenic, trichloroethylene (TCE), lead and styrene were derived using the BMD approach. For each of these compounds, the BMDL was based on an adverse effect (*e.g.*, for arsenic, the BMD is based on mortality from lung and urinary bladder cancers observed in epidemiological studies in Taiwan, Chile, and Argentina; for TCE, the BMD is based on kidney nephropathy in rats; for lead, a child-specific BMD is based on changes in blood lead concentrations, which are associated with decrements in IQ; for styrene, the BMD is based on bronchiolar effects in male mice). Again, for perchlorate, the BMDL was based on a nonadverse effect.

Further, the scientific support offered in the draft document for perchlorate is inconsistent with the preponderance of the weight of scientific evidence for perchlorate as well as OEHHA's assessment of other environmental agents. By proposing a PHG of 1 ppb, the draft document suggests that perchlorate is of comparable public health concern to other noncarcinogenic agents such as inorganic mercury (PHG = 1.2 ppb), beryllium (PHG = 1 ppb), and simazine (PHG = 4 ppb), and a greater concern than nickel (PHG = 12 ppb) (Figure 9). The proposed PHG for perchlorate is also similar to those for carcinogenic agents such as pentachlorophenol (PHG = 0.3 ppb) (Figure 10). These comparisons suggest that the draft PHG for perchlorate is not scientifically justified.

California, Public Health Goals for Non-cancer compounds

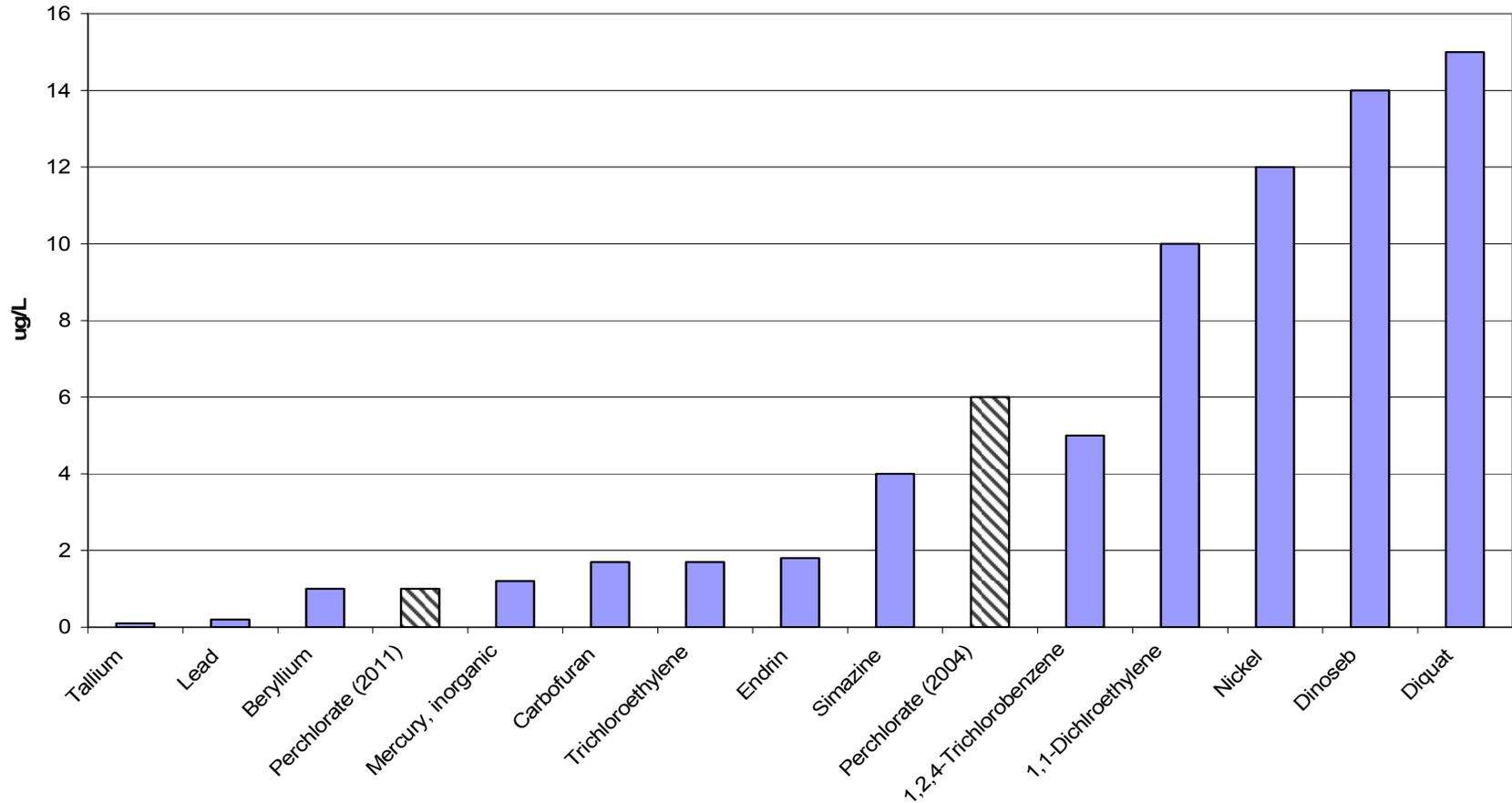


Figure 9. OEHHA PHGs Developed as of December of 2010, for Some Non-Cancer Compounds Compared to Perchlorate (2004) and the Proposed PHG for Perchlorate (2011).

California, Public Health Goals for carcinogenic compounds

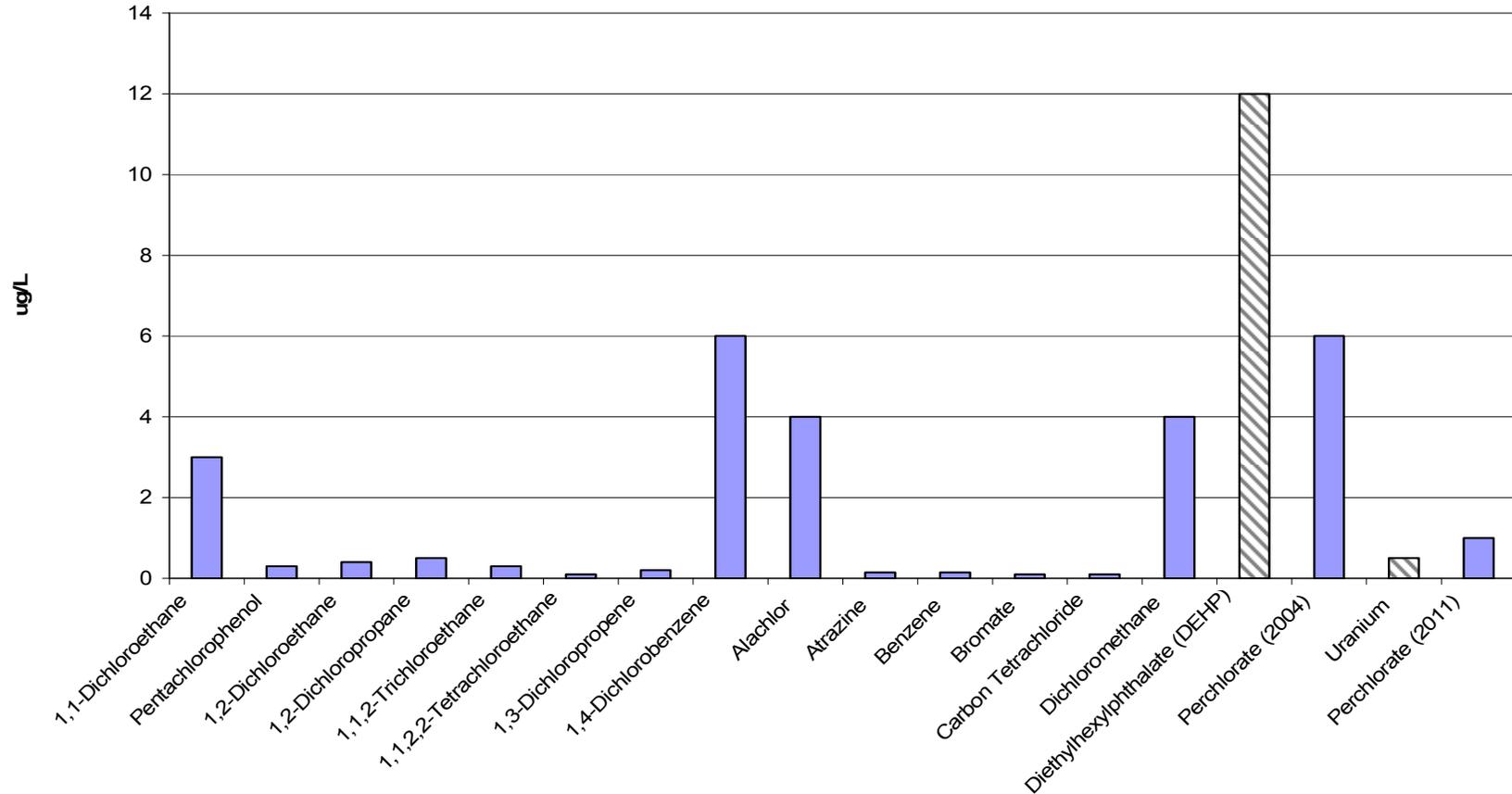


Figure 10. OEHHA PHGs Developed as of December of 2010, for Some Cancer Compounds Compared to Perchlorate (a non-cancer agent) (2004) and the Proposed PHG for Perchlorate (2011).

5.0 CONCLUSIONS

When developing target exposure concentrations for environmental media, the general approach is to identify the most susceptible population; if this population is protected, all other members of the public will also be protected. In 2004, OEHHA set a PHG for perchlorate of 6 ppb in drinking water, and identified the pregnant woman and her fetus as the most susceptible population. On January 7, 2011, OEHHA published a draft risk assessment for perchlorate that proposes a draft PHG of 1 ppb; the most critical difference is a change in the determination of the most susceptible population to the infant without providing a scientifically justified reason for doing so.

Since 2004, the scientific studies that have been published have not changed the fundamental scientific understanding of perchlorate toxicology. The weight of evidence, based on well-designed studies, continues to strongly demonstrate that doses needed to cause adverse effects are significantly higher than environmental levels.

In reviewing OEHHA's draft PHG and supporting documentation, the following points are clear:

- OEHHA's identification of the infant as the most susceptible population to perchlorate exposure conflicts with the work of many other authoritative bodies, including NRC, the U.S. EPA OIG, and ATSDR, who have concluded that the most susceptible population is the pregnant woman and her fetus.
- The change in susceptible population is largely based on OEHHA's interpretation of three studies—Steinmaus *et al.* (2010), Blount *et al.* (2006), and Steinmaus *et al.* (2007) — which are not scientifically valid due to a number of methodological concerns and inconsistencies with other studies. In Steinmaus *et al.* (2010), the authors report an association between higher thyroid stimulating hormone (TSH) in newborn infants born to mothers who had an address in an area where perchlorate had been detected at greater than 5 ppb in drinking water. The other two studies report an association between women with low urinary iodine and thyroid hormone effects. These three studies are not in concurrence with the weight of evidence in the scientific literature.
- The draft document does not address other goitrogens that the public encounters on a daily basis. These include nitrate and thiocyanate which are found in a normal healthy diet and in drinking water. The U.S. EPA OIG estimates that the contribution of perchlorate to inhibition of iodide uptake is about 1% of the total contributed by agents that act through this mechanism of action, including perchlorate. At this contribution level, addressing perchlorate alone can not make a significant contribution to public health. As noted by ATSDR (2008), "Nitrate and thiocyanate are widely distributed in nature and, because both anions also inhibit RAIU, as demonstrated by Tonacchera *et al.* (2004), should also be included in the discussion of the effects of inhibition of the NIS by anions." Attempting to address the effects of IUI by focusing on a *de minimis* contributor, while not examining the major contributors (thiocyanate, nitrate, and iodine deficiency) is not scientifically justified.
- All other PHGs developed by OEHHA are based on points of departure that are adverse effects. The PHG for perchlorate is based on a NOEL for a nonadverse effect—an interpretation that has been substantiated by many scientific authoritative assessments. However, the draft document treats the point of departure as if it were an adverse effect. This

is scientifically indefensible given the clear evidence to the contrary in the large database for perchlorate.

The draft document should be substantially revised to include the strongest and most reliable scientific information available for perchlorate. In particular, the draft document should: (1) adopt the pregnant woman and her fetus as the most sensitive population; (2) if OEHHA decides to use the infant against advisement, adopt uncertainty factors appropriate to a NOEL-based point of departure and commensurate with the use of infant-specific body weight and water intake rates; and (3) take into account other factors that are more significant determinants of IUI (thiocyanate, nitrate, and iodine insufficiency).

In summary, OEHHA does not scientifically justify the change in the PHG or in the most susceptible population from the pregnant woman and her fetus to the infant. Further, the draft document does not address how reducing perchlorate concentrations in drinking water from 6 ppb to 1 ppb will result in additional public health benefit, particularly considering the substantially greater effects that other thyroid-active chemicals with the same mechanism of action have on the inhibition of iodide uptake.

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APPENDIX A

**SUMMARY OF STUDIES USED IN OEHHA (2011B) AND THE ASSESSMENT
OF THOSE STUDIES BY AUTHORITATIVE BODIES**

Table A-1. Summary of Studies Used in OEHHA (2011b) and the Assessment of Those Studies by Authoritative Bodies

2011 Draft OEHHA Risk Assessment	Authoritative Evaluation
<p>Studies of perchlorate exposure and newborn thyroid hormone levels in Redlands (Kelsh et al., 2003); Arizona (Brechner et al., 2000); Nevada (Li et al., 2000a), Chile (Crump et al., 2000) (Table 13, page 50) provide “a consistent body of evidence linking perchlorate exposure during pregnancy with changes in T4 or TSH levels in the newborn.”</p>	<p>ATSDR/CDC, (ATSDR -Toxicological Profile for Perchlorates, September 2008):</p> <p>“Kelsh et al. (2003) also found no relationship between congenital hypothyroidism and exposure to perchlorate through the drinking water in a study of newborns (n=15,348) whose mothers resided in the community of Redlands, California, during the period 1983 through 1997 and who were screened by the California Newborn Screening Program. Analysis of the results showed an adjusted prevalence ratio for congenital hypothyroidism of 0.45 (95% CI, 0.06–1.64) and an odds ratio for elevated TSH of 1.24 (95% CI, 0.89–1.68) among all newborns screened and 0.69 (95% CI, 0.27–1.45) for newborns whose age at screening was ≥18 hours. Limitations of the study include the fact that data from a single year were used to characterize exposures over the entire 15 years of the study.”</p> <p>“Brechner et al. (2000) and Schwartz (2001) reported an association between high levels of perchlorate in the drinking water and elevated serum levels of TSH, but the methods used in the two latter studies have been criticized.”</p> <p>“Crump et al. (2000) also examined the effects of perchlorate on thyroid function in school-age children. For the most part, no significant alterations were reported.”</p> <p>The NRC (2005) states the following regarding Brechner et al. (2000):</p> <p>The actual adjusted concentrations for each city were not reported. Neonatal T4 values did not differ significantly between Yuma and Flagstaff after adjustment for race or ethnicity. However, follow-up testing of TSH is done only in infants with the lowest 10% of T4 concentrations, so the absence of differences in T4 concentrations between the cities is not especially informative inasmuch as only the lowest part of the entire distribution was compared. ...perchlorate exposures in infants’ mothers were not directly measured; in fact, drinking-water concentrations of perchlorate were not derived from the same period as the newborn screening results.... Information on birthweight or gestational age was not available, so those potential confounders could not be addressed in the analysis. Low birthweight infants are more likely to have lower T4 concentrations without the magnitude of the TSH</p>

surge observed in term infants (Mercado *et al.* 1988). Thus, it is possible that more of the infants with low T4 who had a follow-up blood test for TSH in Flagstaff were low-birthweight infants who would not have had as great a TSH surge, keeping the mean and median concentrations in Flagstaff lower than in Yuma.

ATSDR (2008) states:

Lamm (2003) reanalyzed the study and compared TSH neonatal values of Yuma and two cities near Yuma, Somerton and San Luis, which get their water from a different source than the city of Yuma. The water from Somerton and San Luis is assumed to have no perchlorate contamination. Lamm's analysis showed no significant difference in TSH values between newborns from Yuma and Somerton/San Luis, suggesting that the results of Brechner *et al.* (2000) reflected regional differences, possibly related to the difference in altitude (7,000 feet) between Yuma and Flagstaff.

Regarding the **Crump *et al.* (2000)** study, contrary to the impression provided by OEHHA, the study's authors report:

A study was conducted to investigate the potential effects of perchlorate in drinking water on thyroid function in newborns and school-age children. A total of 162 school-age children and 9784 newborns were studied in three proximate cities in northern Chile that have different concentrations of perchlorate in drinking water: Taltal (100 to 120 µg/L), Chanaral (5 to 7 µg/L), and Antofagasta (non-detectable:< 4 µg/L). Among school children no difference was found in thyroid stimulating hormone levels or goiter prevalence among lifelong residents of Taltal or Chanaral compared with those of Antofagasta, after adjusting for age, sex, and urinary iodine. Neonatal thyroid-stimulating hormone levels were significantly lower in Taltal compared with Antofagasta; this is opposite to the known pharmacological effect of perchlorate, and the magnitude of difference did not seem to be clinically significant. These findings do not support the hypothesis that perchlorate in drinking water at concentrations as high as 100 to 120 µg/L suppresses thyroid function in newborns or school-age children.

Of this study, the NRC (2005) reports:

The committee thinks that the study of **Crump *et al.* (2000)** had important strengths. Although

individual exposures were not assessed, it is one of the few studies that measured perchlorate in drinking water in samples taken directly from the environment of the children studied, such as homes and schools. In addition, it was possible to compare assessments of thyroid function, other end points, and potential risk factors obtained in a systematic manner from all participants and adjusted for a number of important covariates, and participation was high. All laboratory assessments were done at the same facility, and assessments of thyroid status and other measures were done by observers unaware of the perchlorate exposure of the children. All newborn screening tests in Chile are done at a single laboratory.

Numerous critiques of the **Crump et al. (2000)** study have been done either directly by the U.S. Environmental Protection Agency (EPA) in its risk-assessment document or by others at the request of EPA (Park 2001; Marcus 2003)... The committee considers this criticism adequately addressed by the authors.

ATSDR/CDC, (ATSDR -Toxicological Profile for Perchlorates, September 2008):

“**NRC (2005)** also suggested that the question of whether or not exposures to perchlorate at concentrations present in municipal drinking water are related to an increased incidence of maternal hypothyroidism during gestation could be addressed in the study of pregnant women in Chile (Téllez et al. 2005) and that using larger samples would improve the precision of the estimates.”

U.S. EPA OIG, 2010 (Appendix A): “Using EPA criteria, our review indicates that the whole mixture approach to risk assessment of perchlorate (*i.e.*, **Blount** analysis and the **Steinmaus** analysis) is not sufficiently developed and corroborated to be either the basis for developing a perchlorate RfD or the basis for establishing a potential perchlorate drinking water limit for the reasons cited in Appendix A.”

“Until researchers can explain the increased toxicity of perchlorate by a verified biological mechanism, regulators should not use the **Blount** analysis as a basis for developing a perchlorate RfD, nor should it be used as the basis for establishing a drinking water limit.”

Regarding **Tellez et al 2005**, OEHHA presented results of the mean thyroid hormone levels in three cities in northern Chile, Chañaral and Antofagasta. “As discussed in the section reviewing the neonatal findings of this study, maternal iodine levels were very high and it is possible that this may have protected the infants and the mothers from the impacts of perchlorate. Other factors that were also discussed above include: 1) similarity of the urinary perchlorate concentrations across the unexposed, low, and high exposure cities; 2) Cesarean-section rates were markedly different across cities. (Cesarean section rates may impact chemical-thyroid hormone associations (Herbstman et al., 2008)); 3) 45 percent of women from the exposed city gave birth in the unexposed city; and 4) there were few smokers (a common source of thiocyanate) and no data on thiocyanate levels.”

“**Blount and Steinmaus** are key studies supporting two of the potential susceptibility groups identified by OEHHA (women with low iodine and women with high thiocyanate) and thus were evaluated in further detail. OEHHA acknowledges that although some of the details of the findings of **Blount et al. (2006)** and **Steinmaus et al. (2007)** remain unexplained, a thorough analysis of the major tenets of causal inference show that the overall results are generally consistent with known mechanisms and not likely are due to chance, confounding, or other bias.”

“**Blount et al. 2006**, which shows that perchlorate-T4 association was seen in women with spot urinary iodine levels below 100 ug/L, and NHANES 2001-2 (geometric mean urinary iodine concentration in women was 126 ug/dL).”

Blount et al. (2006) was also used in benchmark dose calculations (Appendix 1).

“Therefore, the **Steinmaus** analysis does not corroborate the **Blount** analysis, because both studies use the same NHANES 2001-2002 dataset.”

“Glinoe 1995, 2005 and Delange 1994 studies indicate that iodide supplementation during pregnancy reduces the stress on the fetal thyroid so that the risk for developing subtle mental deficits is minimized.”

ATSDR/CDC (ATSDR,2008)(pg., 25): “Another potential susceptible population is women with urinary iodine levels <100 µg/L, as regression analysis of a population study by **Blount et al. (2006)** indicated that perchlorate exposure was correlated with decreased T4 and increased TSH. 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.” (Appendix A, pg. A-4).

Susceptibility of the fetus and young child (pg., 100): New studies that identify links between relatively small decreases in thyroid hormone levels during pregnancy and significant effects on cognition in the offspring child include **Kooistra et al. (2006), and Vermiglio et al. (2004)**.” “These findings are supported biologically by animal studies that have linked decreases in maternal thyroxine (T4) during pregnancy to permanent structural changes in the brains of the offspring (Lavado-Autric *et al.*, 2003; Auso *et al.*, 2004; Gilbert and Sui, 2008).”

“Some data suggests that many young children in the U.S. may not have an adequate iodine uptake (**Pearce et al. 2007**, single study in Boston area).”

U.S. EPA, OIG, 2010 (pg. 133) :

“Maternal hypothyroxinemia (*i.e.*, during pregnancy) is documented to be associated with mental deficits (*e.g.*, ADHD) in the children of those mothers (**Vermiglio 2004**).”

“The **Vermiglio 2004** study is one of four studies reporting that maternal hypo-thyroxinemia during early pregnancy results in neurodevelopmental deficits in children (Kooistra 2006). The Vermiglio study suggests that the rates of attention deficit and hyperactivity disorder (ADHD) could be significantly reduced by preventing iodide deficiency during pregnancy. Furthermore, the Vermiglio study suggests the possibility that providing a normal thyroid hormone level during gestation may allow a child with GRTH to have normal brain development by not challenging the mutated thyroid receptor-β gene.”

“Hypothyroxinemia is a common condition in pregnant women characterized by low maternal fT4 levels with normal TSH levels (**Kooistra 2006**).”

“Hypothyroxinemia is regarded to be without consequences for the mother and fetus (**Kooistra 2006**).”

NAS (2005): “fetuses and preterm newborns constitute the most sensitive populations, although infants and developing children are also considered sensitive populations. The expected high sensitivity of developing organisms is due to the important role played by thyroid hormones during development (Zoeller 2006).”

Calculation of the PHG (p. 107): The Acceptable Daily Dose (ADD) used to derive the PHG is calculated by dividing the NOAEL, LOAEL, or BMDL by UFs. OEHHA (2011b) states, “we have chosen to estimate the ADD from the lower limit of the two-sided 95 percent confidence interval of the perchlorate dose estimated to cause a five percent reduction in iodide uptake in the thyroid gland based on the findings of Greer *et al.* (2002).” That is, IUI as measured in Greer *et al.* (2002) is considered an adverse effect.

NRC (2005): “the committee recommends that inhibition of iodide uptake by the thyroid, which is the key biochemical event and not an adverse effect, should be used as the basis of the risk assessment. The NAS Committee recommended using the inhibition of NIS as the point of departure (POD) for human risk assessment. Inhibition of iodide uptake is a more reliable and valid measure, it has been unequivocally demonstrated in humans exposed to perchlorate, and it is the key event that precedes all thyroid-mediated effects of perchlorate exposure.

The committee is recommending using a nonadverse effect rather than an adverse effect as the point of departure for the perchlorate risk assessment. Using a nonadverse effect that precedes the adverse effects is a conservative, health-protective approach to the perchlorate risk assessment, and the committee’s recommendations for uncertainty factors reflect the conservatism of the approach.”

NOAEL vs. BMD (pg, 170) – “Although the committee recognizes that BMD modeling can be an improvement over the use of the NOAEL or LOAEL as a point of departure, there appears to be no consensus on the criteria for choosing one BMD approach over another. Because no clear justifications were provided with the individual analyses of the Greer *et al.* (2002) data that allowed selection of one set of results over another, the committee concluded that using the NOEL (0.007 mg/kg per day) 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.”

“The NOEL value from Greer *et al.* (2002) is a health protective and conservative point of departure is supported by the results of a 4-week study of higher doses in normal subjects (Brabant *et al.* 1992; see Chapter 2) and extensive human and animal data that demonstrate that there will be no progression to adverse effects if no inhibition of iodide uptake occurs.”

RSC for infants – “less than 6 months old was calculated based on the Schier *et al.* (2009) study, which measured perchlorate concentrations in 15 different powdered infant formulas (using perchlorate free water) and calculated mean perchlorate intake values based on the estimated daily ingested volume of formula.”

Shier *et al.*, 2009 study was not cited on OIG, EPA, ATSDR/CDC health effects perchlorate, and NAS, 2005.

U.S. EPA, OIG, 2010 (pg., 92) – Total NIS Inhibition Load Acting on Non-nursing Infants
 “The perchlorate exposure in non-nursing infants can be estimated using the results from the 2008 FDA Food Dietary Study. The 2008 FDA Food Dietary Study reports the total perchlorate intake from food for 6- to 11-month-old infants to be 0.26–0.29 µg/kg-day (*i.e.*, not including potential perchlorate exposure from water) (Murray 2008, Table 5). Since the perchlorate RfD is 0.6 µg/kg-day, the perchlorate exposure from food for 6- to 11-month-old infants of 0.26–0.29 µg/kg-day represents 37% to 41% of the perchlorate RfD. This suggests a RSC of about 60% for non-nursing infants.”

“Unfortunately, this estimated perchlorate RSC is derived using a single chemical risk assessment process that is characterized as being outdated. In other words, limiting only perchlorate to protect public health does not insure that the total NIS inhibition load acting on the non-nursing infants is “safe” because the NIS inhibition exposure from thiocyanate and nitrate in the food and water of the non-nursing infant is not considered.”

Risk characterization – support for changes (pg., 112)
 “Perchlorate was detected in the urine of every one of the 2,820 people tested (Blount *et al.*, 2007).”

U.S. FDA, 2009 and U.S. EPA, 2008a studies were not cited on ATSDR/CDC health effects/perchlorate, and NAS, 2005.

“Perchlorate found in foods - perchlorate was found in many commonly consumed foods including dairy products, fruits, and vegetables (U.S. FDA, 2009).”

U.S. EPA, OIG, 2010 (pg., 94) – Risk characterization of NIS stress “For perchlorate, a risk characterization cannot be done in humans because adverse health effects have not been clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 177). The excessive maternal exposure to the NIS stressor, the lack of iodide, is the principal NIS stressor in which adverse effects have been documented and reported in children born to mothers with low maternal TIU during pregnancy and nursing.”

“The U.S. EPA recently estimated that as many as 16.6 million people in the U.S. may be drinking water with perchlorate concentrations greater than 4 ppb (U.S. EPA, 2008a).”

U.S. EPA, OIG (pg., 33) – Human perchlorate exposure

“In California, from 2004 to April 2009, detectable levels of perchlorate were reported in 297 public drinking water sources (DPH, 2009).

Studies that provide evidence that thyroid hormone levels in infants were adversely affected by perchlorate at exposure levels that were much lower than the levels shown to cause no effects in healthy adults (Kelsh *et al.*, 2003; Brechner *et al.*, 2000; Buffler *et al.*, 2006; Steinmaus *et al.*, 2010; Li *et al.*, 2000a; Crump *et al.*, 2000).”

“In 2006, Blount assessed perchlorate exposure in the U.S. population using urinary biomonitoring data (Blount 2006a). The FDA TDS also estimated lower- and upper-bound average perchlorate intakes (*i.e.*, range of dietary perchlorate intake) for 2005-2006 from 5.4 to 6.8 µg/day from food in 25- to 30-year-old women (Murray 2008, table 5). Both the CDC biomonitoring and the FDA TDS datasets

“In a study of nursing mothers in Boston, 47 percent of breast milk samples did not contain enough iodine to meet the infant iodine intake recommended by the Institute of

Medicine (Pearce *et al.*, 2007).”

“Human data show that perchlorate can interact with other contaminants to produce a greater effect (Blount *et al.*, 2006, Steinmaus *et al.*, 2007).”

“New data available from the U.S. EPA show that drinking water intakes per body weight are higher in infants than previously thought. Consistent data from several large ecological studies suggest that perchlorate in drinking water may be associated with decreases in thyroid hormone levels in newborns (Kelsh *et al.*, 2003; Brechner *et al.*, 2000; Buffler *et al.*, 2006).”

“Recent evidence from NHANES 2001-2 and other sources suggests that women with low iodine intake also represent a susceptible group (Blount *et al.*, 2006).”

“In addition, given the importance of cognitive development and cardiovascular disease to the health and well-being of society, even very small changes in the overall mean population levels of these outcomes are likely to have profound impacts if the causative exposure and its related effects occur on a widespread basis (Miller *et al.*, 2009).”

indicate a low-level background exposure to perchlorate across the entire U.S. population.”

“A potential route of perchlorate exposure that is consistent with generating a uniform low background exposure level in the entire U.S. population is the contamination of the U.S. food supply with perchlorate. The FDA TDS identifies that vegetables and dairy foods combined account for between 46% and 59% of the total estimated intake of perchlorate by teenagers and adults, respectively (Murray 2008). The FDA’s perchlorate TDS identified that perchlorate is present at very low levels in food, primarily through the consumption of dairy and vegetables (Murray 2008, p 5).”

Perchlorate stressor, U.S. EPA OIG, 2010 (pg., 183)

– “Potentially reducing the maximum perchlorate exposure in drinking water from 24.5 ppb to 6.0 ppb or lower will not significantly improve the %TIU values in adults and, in particular, pregnant women.

The act of lowering perchlorate drinking water exposure below 24.5 ppp provides a negligible decrease in the size of the at-risk population. By contrast, implementing prenatal vitamins containing iodide has the potential to pull all 29% of the iodide-deficient pregnant women in the United States successfully above the critical %TIU(NOAE) value, and at a relatively low implementation cost.”

“Data from U.S. EPA, 2007 indicates that a significant portion of the U.S. population has a much **higher risk from NIS inhibition from nitrate from drinking water than the risk from NIS inhibition from perchlorate exposure** from drinking water at the RfD.”

ATSDR Tox Profile for Perchlorates September, 2008 (pg., 145). Comparative Toxicokinetics - Further research is necessary to determine how differences in thyroid physiology between humans and rats may affect the use of these models for human risk characterization and risk assessment.”

APPENDIX B

**PREDICTIONS OF THE PHARMACOKINETICS OF PERCHLORATE IN
FULL-TERM AND PREMATURE NEONATES**

**PREDICTIONS OF THE PHARMACOKINETICS OF PERCHLORATE IN
FULL-TERM AND PREMATURE NEONATES**

Prepared for:

*THE NATIONAL ACADEMY OF SCIENCES
Committee to Assess the Health Implications of Perchlorate Ingestion*

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LIST OF APPENDICES

APPENDIX 1. Clewell et al., (2004)

PREDICTIONS OF THE PHARMACOKINETICS OF PERCHLORATE IN NEONATES

The issue of pharmacokinetics of perchlorate has become an important topic because it is desired to predict the serum concentrations of perchlorate in neonates who might be exposed to perchlorate via breast milk. The following outlines what is known about the pharmacokinetics of perchlorate in adults, how one might scale the pharmacokinetics of perchlorate in adults to neonates, and lastly, how would one estimate the dose in a neonate required to achieve a target/threshold serum perchlorate concentration.

1.0 PHARMACOKINETICS OF PERCHLORATE IN ADULTS

1.1 Half-life

The half-life of perchlorate in humans has been estimated to be approximately 8 hours (Greer *et al.*, 2002) and the elimination of perchlorate follows first-order kinetics (Figure 1).

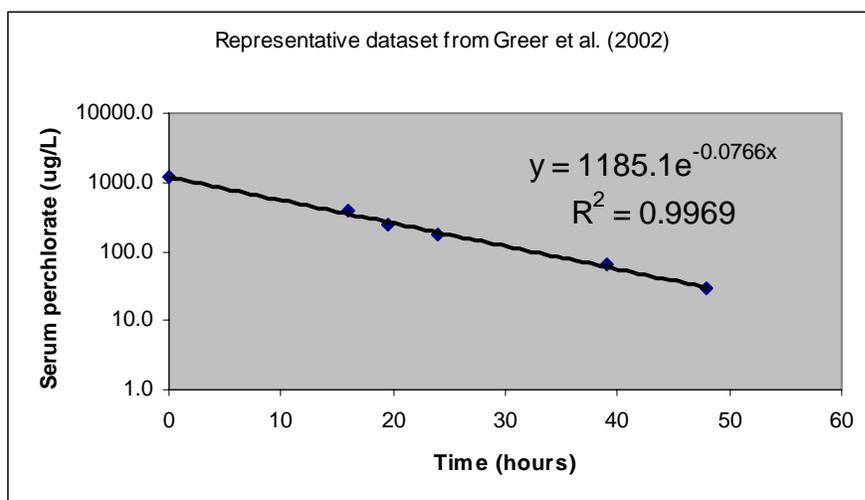


Figure 1: Representative post-dosing serum perchlorate versus time (data for Greer *et al.*, 2002)

1.2 Steady-state serum levels

The Greer *et al.* (2002) study allows for the assessment of the relationship between daily dose and steady-state serum levels of perchlorate. The Greer study involved daily doses of 0.007, 0.02, 0.1 and 0.5 mg/kg/day. Serum perchlorate levels were only detectable in the 0.1 and 0.5 mg/kg/day dose groups. Volunteers were dosed for 14 consecutive days, and serum perchlorate concentrations were measured on days 3, 4, 8 and 14 into the dosing period. Given the half-life of 8 hours, pseudo steady-state should have been obtained after approximately five half-lives (i.e., 40 hours). Therefore, the serum perchlorate concentrations were averaged for days 3 through 14 to obtain an estimate of steady-state serum perchlorate levels for the 0.1 and 0.5 mg/kg/day. The geometric mean of steady-state serum perchlorate concentration (C_{ss}) was 612.5 $\mu\text{g/L}$ ($n=5$) and 123.7 $\mu\text{g/L}$ ($n=3$) for the 0.5 and 0.1 mg/kg/day dose groups, respectively. Using these two dose groups plus the zero-dose origin, the relationship between C_{ss} and average daily dose (ADD) appears to be strongly linear (Figure 2).

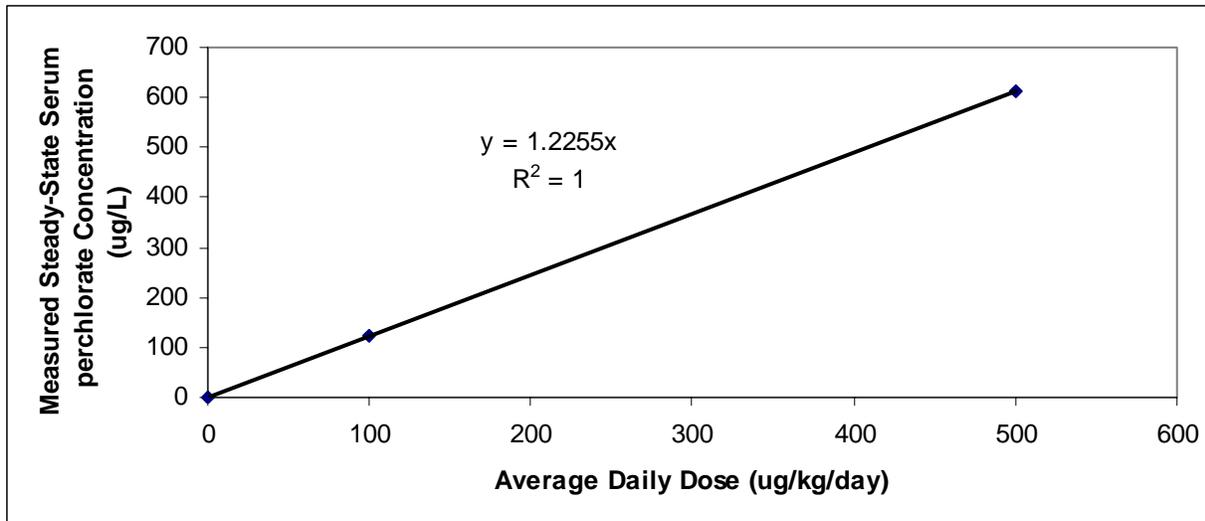


Figure 2: Steady-state serum perchlorate concentration versus average daily dose (data from Greer *et al.*, 2002)

The relationship between C_{ss} and perchlorate daily dose (ADD) in the adult is calculated to be;

$$C_{ss} (\mu\text{g/L}) = \text{ADD} (\mu\text{g/kg/day}) \times 1.23 (R^2 = 1). \quad (1)$$

Since the serum concentrations of perchlorate were non-detects in the exposure groups below 100 $\mu\text{g/kg/day}$, it is not known for sure if there are departures from linearity at doses below 100 $\mu\text{g/kg/day}$. However, another data point actually exists for children exposed to lower levels of perchlorate. Gibbs *et al.* (2004) report on the concentration of perchlorate among school aged children in Taltal, Chile who were exposed to 100 – 120 $\mu\text{g/L}$ perchlorate in their drinking water. They found an average serum concentration of 5.3 $\mu\text{g/L}$ and calculated average daily dose as 4.7 μg perchlorate/kg/day (based on urinary excretion data). Using the relationship above obtained for the Greer participants, the steady-state serum concentration would be predicted to be 5.8 $\mu\text{g/L}$ for an average daily dose of 4.7 μg perchlorate/kg/day. This is in close agreement with what was observed. Therefore, it can be assumed that this relationship between C_{ss} and ADD holds true for a broad range of ADDs and perhaps for school-aged children as well.

The linear relationship between C_{ss} and ADD provides additional evidence to suggest that the kinetics of perchlorate in humans follows first-order kinetics and can be adequately described using a linear one-compartment model.

1.3 Clearance

Steady-state clearance (CL) is defined as the volume of blood cleared of drug or compound per unit time; the units are volume per time (sometimes clearance may be expressed in units that have been scaled to body weight, ml/min-kg). Clearance is the one parameter that determines the dose rate required to achieve a desired blood concentration.

For a linear one-compartment model, C_{ss} is related to ADD and CL at steady-state as (Gibaldi and Perrier, 1982):

$$CL = \frac{ADD}{C_{ss}} \quad (2)$$

Because both ADD and C_{ss} are known from the Greer study, CL can be easily calculated. For the 100 (n=3) and 500 $\mu\text{g}/\text{kg}/\text{day}$ (n=5) dose groups, the geometric mean of CL was 0.8 L/day-kg (for both dose groups) (ADD/ C_{ss}) or 39.7 and 44.3 ml/min ((ADD*BW)/ C_{ss} *1/24*1/60*1000), respectively. Since the glomerular filtration rate (GFR) in an adult is approximately 100-125 ml/min, the elimination of perchlorate in an adult appears to be approximately 30 - 44% of GFR.

1.4 Apparent Volume of Distribution

The apparent volume of distribution (Vd) is a physiological adjustment factor used to relate serum concentration to intake. It has no physiological meaning. Studies across species and within humans have shown that the Vd for a specific compound scales across species as a linear function of body weight.

For a one-compartment model, it can be shown (Gibaldi and Perrier, 1982) that clearance is equal to:

$$CL = k \times Vd \quad (3)$$

Where k is the first-order elimination rate constant (1/hr):

Rearranging,

$$Vd = \frac{CL}{k} \quad (4)$$

From equations (2) and (3), it can be shown that:

$$C_{ss} = \frac{ADD}{k \times Vd} \quad (5)$$

Also, k is related to $t_{1/2}$ (half-life) as:

$$k = \frac{\ln(2)}{t_{1/2}} \quad (6)$$

Therefore, Vd can be calculated from:

$$Vd = \frac{ADD \times t_{1/2}}{C_{ss} \times \ln(2)} \quad (7)$$

Given that ADD, C_{ss} and t_{1/2} are known for perchlorate from the Greer study, Vd can be easily calculated. The geometric mean of Vd for both the 100 and 500 µg/kg/day dose groups was determined to be 0.39 L/kg.

2.0 METHODS TO SCALE PHARMACOKINETICS OF PERCHLORATE

It is desired to calculate the C_{ss} of perchlorate in a neonate being exposed to perchlorate through the diet or via breast milk. To calculate C_{ss} in a neonate requires scaling the pharmacokinetics of perchlorate from the adult to the neonate. This can be achieved using either a one-compartment PK model or a more complex physiologically based pharmacokinetic (PBPK) model.

2.1 One-Compartment PK Model

It has already been established that the kinetics of perchlorate are reasonably described using a one-compartment model in the adult. It is reasonable to assume this would also be true in the neonate. The rapidly growing neonate is, however, more complex simply because of the rapidly changing body weight. However, since perchlorate has a relatively short half-life (*i.e.*, approximately 8 hours in the adult), steady-state is achieved a couple of days after perchlorate exposure begins. Thus, the rate of elimination via the kidney is much more rapid than the rate at which body weight changes. Therefore, using a one-compartment model without considering changes in body weight should introduce only minor differences between predicted and actual half-life. Thus a one compartment model is appropriate for a first approximation of the kinetics of perchlorate in the neonate.

To estimate C_{ss} using a one-compartment model, it can be seen from equation (5) above and a rearrangement of equation (2);

$$C_{ss} = \frac{ADD}{CL} \quad (8)$$

that one must either scale both k (and thus t_{1/2}) and Vd or simply scale CL. Scaling Vd and t_{1/2} is perfectly valid, but requires many assumptions about both how the compound is distributed in the body and how it is eliminated. There are no hard and fast methods of accurately scaling these terms from adults to neonates (Alcorn and McNamara, 2002). It is simpler to scale CL, but also requires knowledge of the mechanisms of elimination and how this might scale from adults to neonates.

Several research groups have attempted to determine the differences between adults and children in terms of how they absorb, distribute, metabolize and eliminate compounds (*i.e.*, pharmacokinetics). Datasets for drugs provide a lot of valuable information for assessing these issues. Ginsberg and coauthors (Ginsberg *et al.*, 2002; Hattis *et al.*, 2003; Ginsberg *et al.*, 2004) and Alcorn and

McNamara (2002) have analyzed the historical literature on clearance in adults and children for a variety of drugs, some of which are eliminated via the urine with little to no metabolism (thus providing a potentially relevant comparison to perchlorate).

2.1.1 Ginsberg *et al.* (2002)

Ginsberg *et al.* (2002) identified four drugs that are not metabolized, are eliminated via renal clearance, and have information on clearance in both adults and children (dataset available at <http://www2.clarku.edu/faculty/dhattis/CDFORPRINT.XLS>). These are furosemide, gentamicin, ticarcillin, and vancomycin. However, further examination of the literature on these drugs reveals that furosemide is extensively metabolized via phase II biotransformation (Kearns, 1993). The table below presents the ratios of child to adult clearance (units of ml/min-kg) for the three drugs that are not metabolized and are cleared solely by the kidney.

Table 1: Comparison of Clearance Rates of Drugs in Children and Adults that Are Not Metabolized and Cleared Solely Via the Kidney (data from Ginsberg *et al.*, 2002)

Drug ^a	Age Range	CL _{neonate} /CL _{adult}	n (Neonates)	Reference ^b
Gentamicin	Premature and full term neonates (1-84 days postnatal)	1.0	17	Rodvold, 1993 and Kirkpatrick, 1999
Ticarcillin	1 mo - 2 yr	1.6	16	Reed, 1998
Vancomycin	Premature, 4-17 days postnatal	0.8	11	Jarrett, 1993 and Cutler <i>et al.</i> , 1974
GEOMETRIC MEAN		1.1		

^a It should be noted that the values in this table have not been independently verified from the primary literature.

^b The first reference is for the infant data, the second (if listed) is for the adult data.

Therefore, using the database of Ginsberg *et al.* to scale the pharmacokinetics of perchlorate from the adult to the neonate, one would predicted that the average neonate perchlorate C_{ss} is 91% (1/1.1) of that of an adult for the same ADD (in terms of ug/kg/day), with a range of 0.63 times (1/1.6) to 1.25 times (1/0.8) the adult C_{ss} for the equivalent ADD.

2.1.2 Alcorn and McNamara (2002)

Alcorn and McNamara (2002) undertook a similar effort to Ginsberg and coworkers to determine the relative difference in the rate of clearance of drugs in children and adults. They identified two drugs common to the Ginsberg study as being indicative of compounds that were not metabolized and were excreted solely in the urine: gentamicin and vancomycin. However, the studies that Alcorn and McNamara referenced for their analyses were different than those cited by Ginsberg. Therefore, their results are not duplicative and add strength to each others' results. The individual values for the ratio of clearances presented by Alcorn and McNamara are not reproduced here. The average infant

to adult ratio of clearances for both drugs was 0.95, with the ratios for gentamicin and vancomycin being 0.89 and 1.0, respectively. The range of ratios was 0.57 (gentamicin) to 1.50 (vancomycin). There was no discernable age trend for these ratios (Alcorn and McNamara, 2002).

Alcorn and McNamara (2002) also noted:

“For renal clearance due to glomerular filtration, data normalized to bodyweight (kg) showed a limited maturational trend, suggesting that adult renal clearances normalized to bodyweight might reasonably predict infant renal clearances in the first 6 months of life.”

This suggests that the infant has the same perchlorate C_{ss} as an adult for the same ADD (ug/kg/day). Based on the results from Alcorn and McNamara (2002), the infant is predicted to have a perchlorate C_{ss} 1.05 times (1/0.95) higher than an adult C_{ss} exposed to the same ADD (ug/kg/day). Based on the range of ratios reported by Alcorn and McNamara, the infant is predicted to have a C_{ss} from 0.67 times (1/1.50) to 1.75 times (1/0.57) the adult C_{ss} exposed to the same ADD (ug/kg/day). Using this dataset to estimate the differences between neonates and adults suggests that a reasonable PK uncertainty factor (UF) would be 1.05 with a range of 0.67 to 1.75.

2.2 PBPK Models

Scaling the pharmacokinetics of perchlorate relying on historical values for drugs generates some uncertainty. Another approach for scaling the pharmacokinetics from the adult to the neonate is to use a PBPK model for perchlorate. Such a model also could be used to simulate the breast milk concentrations of perchlorate in an exposed mother and the subsequent kinetics of perchlorate in the nursing infant. A PBPK model already exists for the rat. Some of these types of calculations have already been conducted for the rat.

Clewell *et al.* (2003) used the rat PBPK model to predict the serum perchlorate concentration in an adult male, lactating dam (and their nursing pups) associated with consuming different concentrations of perchlorate in drinking water. Their simulations indicate that the nursing pup (of a dam drinking perchlorate) would have almost identical serum perchlorate levels as the adult male rats drinking the same perchlorate drinking water concentration as the pup's dams, despite the pups having a higher perchlorate dose. Their simulations also indicate the lactating dam will have higher serum perchlorate levels despite having the extra route of elimination. Clewell *et al.* suggest that the lactating dam has extra serum proteins that bind the perchlorate and result in elevated serum levels.

Rebecca Clewell was contacted to see if she could simulate, using a human PBPK perchlorate model, what the nursing infant's serum perchlorate levels might be for an exposure to an infants' mother. Rebecca Clewell was able to conduct these simulations with a preliminary human PBPK model for perchlorate. Her results are provided in Appendix 1.

Briefly, Rebecca Clewell and her coauthors used the human PBPK model to simulate the average serum perchlorate concentration in an adult male, a lactating mother and her nursing infant exposed to equivalent perchlorate drinking water concentrations. They found that, like in the rat, the nursing infant will have an average serum perchlorate concentration less than the lactating mother, but will have a serum perchlorate level almost twice that of an adult male exposed to the same drinking water concentration as the lactating mother is exposed to (see Appendix 1). Unlike the one-compartment

model used to scale only the pharmacokinetics of perchlorate in the infant, the PBPK model takes into account both the pharmacokinetics of perchlorate in the infant and the pharmacokinetics of perchlorate in a lactating mother and the resulting concentrations of perchlorate in her milk.

3.0 SUMMARY AND CONCLUSIONS

This report has shown several means of utilizing what is known about the pharmacokinetics of perchlorate in the adult to predict what the pharmacokinetics of perchlorate might be in the neonate. The method that relies on a one-compartment model and scaling clearance suggests that the neonate (and even the premature infant) may have a serum perchlorate concentration that is no more than approximately 1.75 times that of an adult being exposed to the same daily dose of perchlorate.

The preliminary PBPK modeling results developed by Clewell et al., which already incorporates exposure via the mother's milk, suggests that a nursing infant will have approximately two times the serum perchlorate levels of an adult male exposed to the same drinking water concentration as the lactating mother.

This insight provides greater assurance that the pharmacokinetics of neonates and premature infants is not substantially different from that of adults. It also provides a scientifically credible upper-bound on the magnitude of any uncertainty factor for the pharmacokinetic differences of neonates and premature infants that may be included in a recommended Reference Dose (RfD).

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APPENDIX 1

Clewell et al. (2004)

Development of PBPK Models for Iodide and Perchlorate Distribution in Human Gestation and Lactation

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BACKGROUND

In order to aid in the quantitative evaluation of the effect of ClO_4^- on normal iodide homeostasis, the laboratory at Wright Patterson Air Force Base has developed a PBPK model for the distribution of perchlorate and iodide in the human during the perinatal period. This PBPK model was developed from the suite of models published previously for the adult male, pregnant, lactating, fetal and neonatal rats, as well as the adult (non-pregnant) human (Merrill *et al.*, 2003; Merrill *et al.*, submitted; Clewell *et al.*, 2003a,b). (See Figure 1 for a schematic of the approach used to develop this model). Since these PBPK models are based on actual physiology, it is possible to integrate data across both species and life stages by accounting for changes in body weight, tissue volume fractions, organ blood flows and changing physiology (*e.g.* protein binding characteristics). Uptake, distribution and clearance kinetics are described within the model using well-established concepts, including partitioning, permeability, and saturable Michealis-Menten kinetics for active transport processes. Biological interactions between the competing anions are described using mathematical equations and chemical specific parameters determined from *in vivo* and *in vitro* studies, such as the affinity of iodide for the sodium-iodide symporter (NIS). Thus, the majority of the parameters used in the models are based on experimentally determined data, thereby reducing uncertainty associated with extrapolating risk predictions to human populations with models developed for the rat, where there is much more data. It is our contention that only through a pharmacokinetic model, which incorporates experimentally determined physiology, chemical specific kinetics, and biochemical interactions, can the total database be used to its fullest extent.

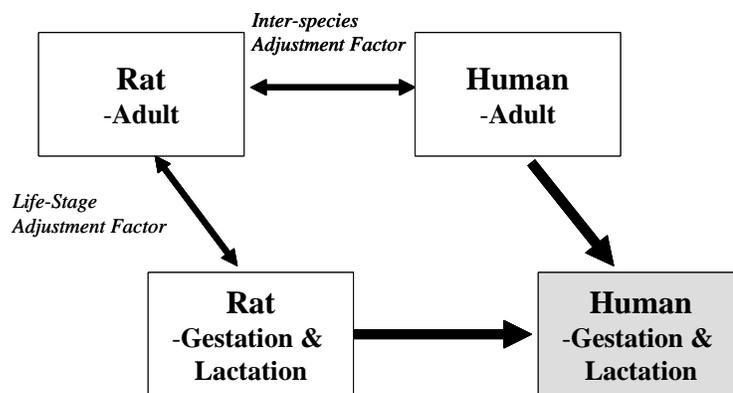


Figure 1. Approach for development of human perinatal PBPK models.

METHOD

Model Structure.

Model structure was identical in the rat and human. Thus, it was possible to use the validated model structures for the rat during gestation and lactation for the perinatal human (Clewell *et al.*, 2003a,b). Only the physiological parameters and those kinetic parameters shown to have significant species differences were altered.

Parameterization.

Physiological parameters were based on the growth equations of Gentry *et al.* (2002) and various published data sets. The fitted polynomials were implemented in ACSL (Advanced Continuous Simulation Language, Aegis Technologies Group, Inc.). All tissue volume and blood flow values were adjusted with respect to the changing parameters.

Limited iodide and no ClO_4^- data were available in the literature to determine the kinetic parameters in the human gestation and lactation models. However, the relationship of the parameters in the previously validated models allows the estimation of parameters in the lactating and pregnant human that have not been measured or which cannot be easily measured. For example, thyroidal uptake of iodide showed significant species differences in the male rat and human models, resulting in maximum capacities for follicular uptake (V_{maxcTF_i}) of 5.9×10^4 and 1.5×10^5 ng/L-h in the male rat and adult human, respectively. Additionally, the validated value for V_{maxcTF_i} in the pregnant rat (4.0×10^4 ng/L-h) was slightly lower than that of the male rat. By applying the male rat:human parameter value ratio to the value used for the pregnant rat, it is possible obtain a reasonable estimate of the corresponding parameter in a pregnant human, as is demonstrated in the equation given below.

$$\frac{V_{maxcTF_i}(\text{male human})}{V_{maxcTF_i}(\text{male rat})} \times V_{maxcTF_i}(\text{pregnant rat}) \approx V_{maxcTF_i}(\text{pregnant human})$$

Thus, the estimated value for V_{maxcTF_i} would be 1.01×10^5 ng/L-h. This same process was applied to obtain chemical-specific parameters for both I^- and ClO_4^- in the human gestation and lactation models (examples shown for the lactating mother in Table 1). In this way, kinetic parameters that could not otherwise be determined were estimated by available data. Physiological parameters were taken from literature data based on the approach of Gentry *et al.*, 2002.

Table 1. Calculating Kinetic Parameters with Species and Gender Adjustments

IODIDE	Lactating Rat	Male Rat	Male Human	Lactating Human
$ClUc_i$ (L/hr-kg)	0.05	0.05	1.0	1.0
$VmaxcTF_i$ (ng/L-hr)	5.0×10^4	5.4×10^3	1.5×10^5	1.4×10^5
$VmaxcTL_i$ (ng/L-hr)	8.0×10^6	4.0×10^6	1.0×10^8	2.0×10^8
PERCHLORATE				
$ClUc_p$ (L/hr-kg)	0.07	0.07	1.3	1.3
$VmaxcTF_p$ (ng/L-hr)	1.5×10^3	1.0×10^3	5.0×10^4	7.5×10^4
$VmaxcTL_p$ (ng/L-hr)	1.0×10^4	2.5×10^5	5.0×10^4	1.3×10^5

Some parameters are expected to vary significantly over time in the fetus and neonate, including: thyroid NIS activity and kidney function. The values for the maximum capacity of thyroid iodide uptake by NIS in the fetus and neonate ($VmaxcTF_i$) were determined by visually fitting the model simulation to the available radioiodide data at the various weeks in gestation and daily during the first post-natal week. Partition coefficients and the parameters for the sequestration of iodide in the colloid were kept consistent with the adult values. This approach is consistent with the method used in the rat and non-pregnant human models to account for inter-individual variations in thyroid parameters. Due to the lack of perchlorate data, the fetal thyroid ClO_4^- parameters were calculated using the same $ClO_4^-:I^-$ ratio as the adult human thyroid. After the first post-natal week, the thyroid activity ($^{125}I^-$ uptake and serum hormone levels) was not significantly different from that of the adult. The parameters were therefore scaled allometrically ($BW^{0.75}$) from the adult values after post-natal day 7. Glomerular filtration (GFR) was adjusted using the equations given by Gentry *et al.* (2003) to account for the initial decrease (1st day) and subsequent increase in renal function in the neonate and young child. Assuming age-related differences in iodide excretion were due primarily to differences in renal function, the urinary clearance value was then set to the same value used in the non-pregnant adult human mode (Merrill *et al.*, *submitted*).

RESULTS

After setting the model kinetic parameters based on the relationship of the male rat and human models and the pregnant to male rat models, as described in the Methods section, the human gestation and lactation models were run against a variety of data sets from acute radioiodide studies in human gestation (Figure 2) and lactation (Figures 3-4). Using the above approach for parameter estimation, the model produced highly representative predictions for human radioiodide kinetics. The iodide and perchlorate models share the same structure and the rat models successfully describe kinetics of both these anions. In addition, these models were successfully extrapolated to predict human iodide exposure. Therefore, it is likely that the model would also produce a reasonable estimate of predicted ClO_4^- kinetics in the human.

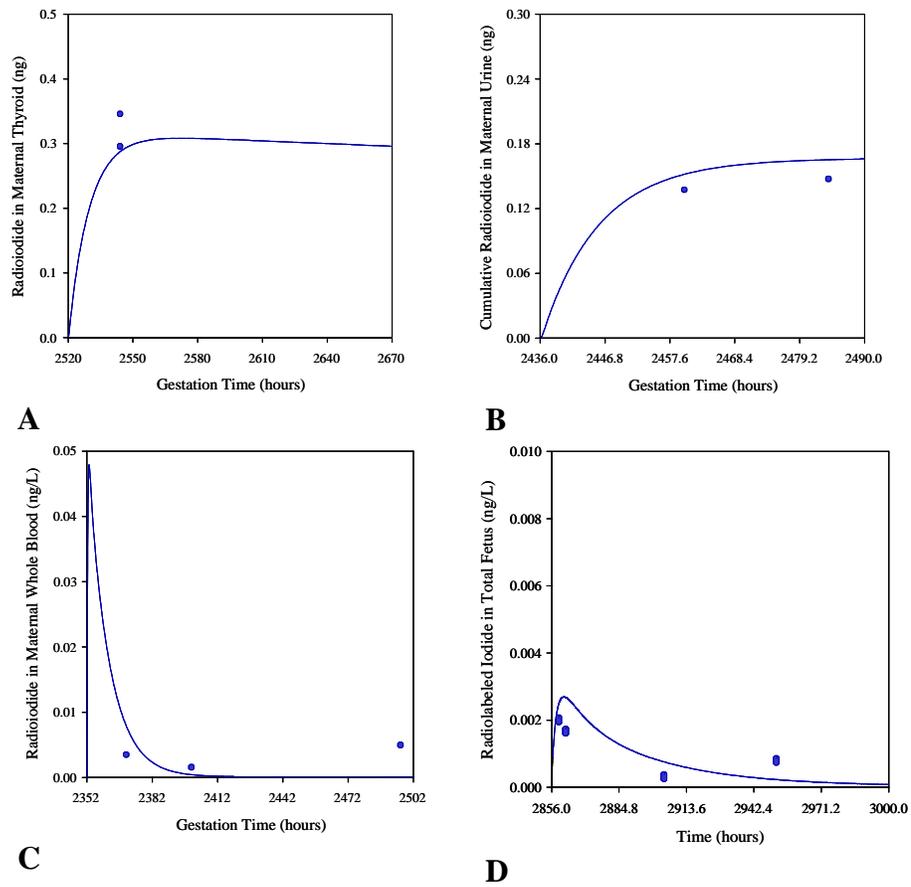


Figure 2. Radiiodide in Human-Maternal Thyroid(A) and Urine (B), Serum (C), and Total Fetus after tracer ^{131}I dose to human mother at gestation weeks 14-15. Solid lines indicate model prediction. Circles indicate data from individual subjects from Aboul-Khair *et al.*, (1966) and Chapman *et al.* (1948).

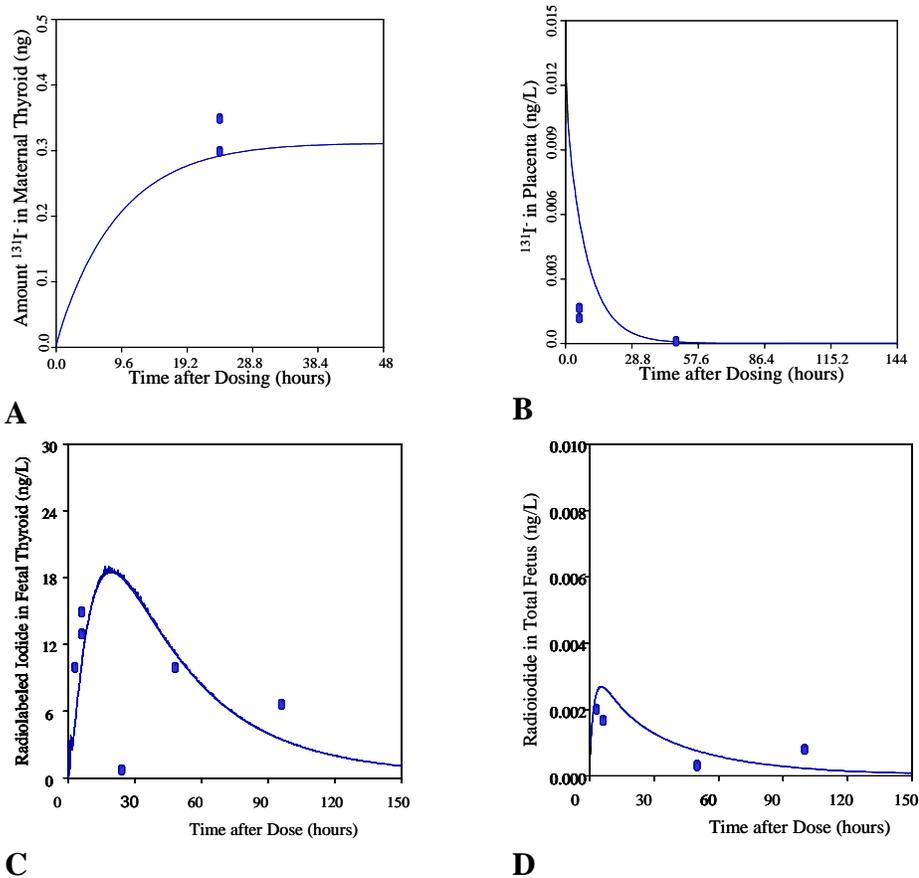


Figure 2. Human gestation model-predicted maternal thyroid (A), placenta (B), fetal thyroid (C) and total fetus (D). Model predictions (solid lines) are shown versus data of Aboul-Khair *et al.* (1966) on gestational weeks 16 and 17 after oral dose of ^{131}I to mother. Circles represent individual subjects.

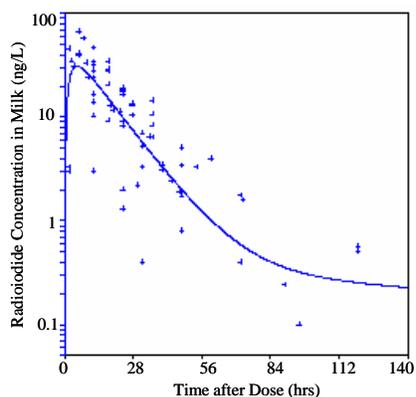


Figure 3. Human lactation model-predicted milk. Data represents several data sets normalized by dose. Model was run at 2 months post-natal. Concentration in milk was not found to vary significantly with change in post-natal time. Data points represent individual subjects from the data of Dydek and Blue (1988), Miller and Weetch (1955), Nurnberger and Lipscomb (1952), Rubow and Klopper (1988) and Weaver *et al.* (1962).

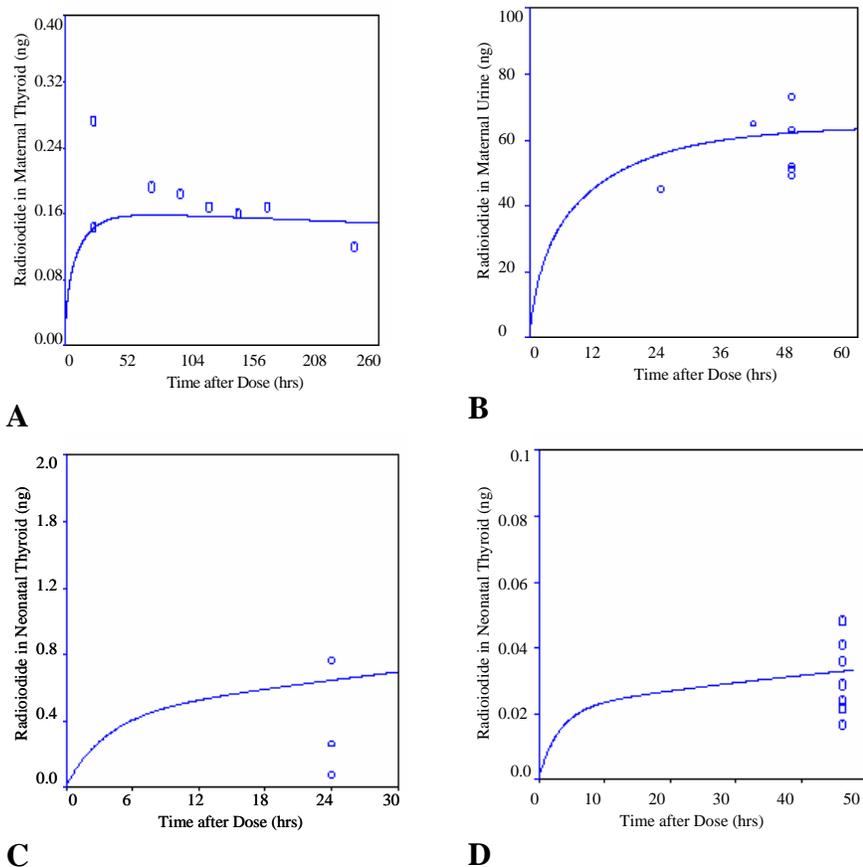


Figure 4. Human lactation model-predicted maternal thyroid (A) and urine (B) at 4 months post-natal; and neonatal thyroid at 5 days (C) and 6 months (D) post-natal. Model simulations (solid lines) are shown versus the data of Nurnberger and Lipscomb (1952), Reilly and Bayer (1952), Ogborn *et al.* (1960) and Quimby *et al.* (1947). Circles represent individual subjects.

At this time, the only tissue in which we were able to test perchlorate kinetics was maternal milk. In a recent study (Gibbs, *personal communication*), the milk of mothers in a city with ~113 ppb ClO_4^- in the drinking water was measured at approximately 2 months post-birth. The model was run with an exposure scenario of maternal ingestion of 2 L of drinking water per day. The model successfully predicted milk concentrations for this population, without any *post-hoc* adjustments to model parameters (Figure 5). In addition to milk, this study collected maternal serum during gestation and cord-blood at birth. We will eventually be able to test the gestation model predictions with these data.

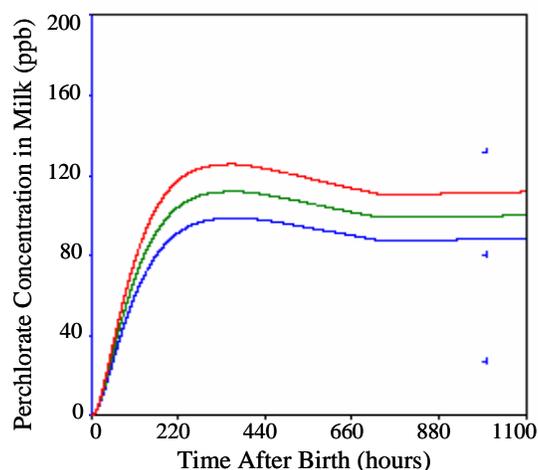


Figure 5. Human lactation model-predicted milk in Chilean women exposed to perchlorate in drinking water. Model simulations (solid lines) were run at mean and plus/minus one SD. Data points represent mean \pm SD of measured values (Gibbs, *personal communication*).

In response to specific concerns regarding neonatal ClO_4^- exposure via maternal milk, the model was run at several doses known to be relevant to the risk assessment. Table 1 shows a comparison of maternal and neonatal dose in terms of drinking water concentration and in daily dose ($\text{mg ClO}_4^-/\text{kg BW}/\text{day}$). The model predicts the milk concentration to be slightly lower than the drinking water concentration at all but the highest dose. However, because the neonate's milk intake is greater than the mother's relative water intake, the actual dose ($\text{mg}/\text{kg BW}$) is five times greater in the neonate. In order to explore the effect of this increased dose, the models were then run at the same concentrations to predict serum ClO_4^- levels for the adult male, lactating woman and nursing infant (Table 2). A comparison across doses shows that despite greater dose, the neonate still has slightly lower serum levels than the mother. Eventually similar comparisons could be performed for the pregnant woman and fetus, using the model described above.

Table 1. Predicted Milk Concentrations Across Doses and Ratio of Neonate:Maternal Dose

Significance	Maternal Dose	Drinking Water Concentration	Milk Concentration	Neonate: Maternal Dose
	$\text{mg}/\text{kg BW}/\text{d}$	ppb	ppb	(per kg BW)
¹ EPA DW Conc.	0.00003	1	1	5
	0.0003	10	9	5
² Chile High Dose	0.003	100	93	5
³ Greer NOAEL	0.007	245	211	5
⁴ EPA PoD	0.01	300	295	5
⁵ Greer High Dose	0.5	17500	3516	1

¹ Proposed Drinking Water Concentration – EPA Draft Proposal 2002, 2003

² High Dose Group from Chile Epidemiological Study – Gibbs *et al.* (*personal communication*)

³ NOAEL based thyroid I- uptake inhibition in adults – Greer *et al.* 2002

⁴ EPA Point of Departure based on maternal thyroid hormone effects – EPA Draft Proposal 2002, 2003

⁵ High Dose Group from Greer *et al.* 2002, 0.5 $\text{mg}/\text{kg}/\text{day}$ for 2 weeks

Table 2. Predicted Serum Concentrations in Adult, Lactating Female and Neonate and Ratio of Neonate:Maternal Serum Level

Significance	Drinking Water Concentration	Male Serum	Maternal Serum	Neonate Serum	Neonate : Maternal
	ppb	ng/L	ng/L	ng/L	
¹ EPA DW Conc.	1	76	194	161	0.8
	10	755	1908	1595	0.8
² Chile High Dose	100	6998	16174	14112	0.9
³ Greer NOAEL	245	14968	31701	28439	0.9
⁴ EPA PoD	300	20320	41129	37236	0.9
⁵ Greer High Dose	17500	676459	997911	281879	0.3

¹ Proposed Drinking Water Concentration – EPA Draft Proposal 2002, 2003

² High Dose Group from Chile Epidemiological Study – Gibbs et al. (*personal communication*)

³ NOAEL based thyroid I- uptake inhibition in adults – Greer et al. 2002

⁴ EPA Point of Departure based on maternal thyroid hormone effects – EPA Draft Proposal 2002, 2003

⁵ High Dose Group from Greer *et al.* 2002, 0.5 mg/kg/day for 2 weeks

DISCUSSION

In order to extend risk characterization of ClO_4^- beyond a simple estimation of its distribution within the body to a quantitative estimate of its biological effect on iodide uptake in the populations of interest, it is necessary to account for dynamic physiological differences, as well as the kinetic changes and interactions of the chemicals in the biological system. Through the use of biologically based models, it is possible to quantitatively account for these differences and develop a reasonable estimate of the resulting internal kinetics. This approach would incorporate all available data sets (rat, human, male, female, young, adult) to quantitatively determine a reasonable estimate of risk to the population of interest, including the human fetus and neonate.

Here, we used the available literature data to determine physiological changes in human gestation and lactation, in conjunction with the kinetic data in the rat and non-pregnant adult human, to build preliminary pharmacokinetic models for human gestation and lactation. The success of these models in predicting the available human data is indicative of the feasibility of this approach. We are now able to provide population-specific predictions of internal dose under a wide variety of relevant human exposure scenarios that can be directly compared with observed health effects to facilitate and improve estimates of risk.

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