OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Public Health Goal

Perchlorate in Drinking Water

February 2015

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Public Health Goal for

PERCHLORATE

in Drinking Water

Prepared by

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PREFACE

This Public Health Goal (PHG) technical support document provides information on health effects from perchlorate in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. The PHG documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. The PHG is a drinking water goal only; therefore, this document does not evaluate the safe levels of perchlorate in foods or other sources.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. 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.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by SWRCB are to consider economic factors and technological feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by SWRCB must be at least as stringent as the federal MCL if one exists.

In July 2014, responsibility for the state's drinking water regulatory program was transferred to SWRCB from the Calfornia Department of Public Health. References in this document to drinking water monitoring and regulation may cite either or both entities as appropriate.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR PERCHLORATE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) is publishing a Public Health Goal (PHG) of 1 part per billion (ppb) (equivalent to 1 microgram per liter [µg/L]) for perchlorate in drinking water. Perchlorate is an oxidizing chemical used in a variety of industrial processes. Perchlorate can occur in the environment either through industrial contamination or from natural sources. Perchlorate exposure in the U.S. is ubiquitous, mostly from ingestion of perchlorate in contaminated food or water. In a survey involving an essentially random sample of people from the U.S., perchlorate was detected in the urine of every one of the 2,820 subjects tested (Blount *et al.,* 2006).

Health Impacts from Perchlorate

For this PHG, OEHHA used decreased uptake of iodide by the thyroid gland as the key biochemical event for assessing the risks due to perchlorate toxicity. The primary action of perchlorate in humans is inhibition of iodide uptake into the thyroid gland. The function of the thyroid gland is the production of thyroid hormone. Iodide is a key component in the structure of thyroid hormone, and by blocking its uptake into the thyroid, perchlorate can potentially cause decreased production of this hormone.

Thyroid hormone is necessary for a variety of basic human physiologic functions, including controlling basal metabolic rates; protein, carbohydrate, and fat metabolism; protein synthesis; proper differentiation and development of cells, including neuronal cells; and the cognitive and physical development of the fetus, infant, and child. Evidence suggests that even small decreases in thyroid hormone levels may be associated with significant adverse effects, including altered cognitive development in children and increased cardiovascular risk factors in adults. Importantly, these changes have been seen at thyroid hormone levels that are within what have been traditionally defined as normal reference ranges, and have occurred in people without any other evidence of overt thyroid disease. These findings suggest that small changes in thyroid hormone levels may be associated with some increased risk of thyroid-related adverse outcomes.

There is a continuum in the possible health effects from perchlorate exposure resulting from its effects on the thyroid, and some susceptible groups or individuals may have perchlorate-related effects at exposures that are lower than those causing similar effects in healthy individuals. For fetuses, infants and children, there is the potential for abnormal growth and development. Of particular concern are effects on brain development. Decreases in thyroid hormone production in infants are related to decreases in IQ. Much of the knowledge on the potential effects of perchlorate comes from extensive data on iodine deficiency in humans.

OEHHA considers effects on thyroid hormone production and subsequent changes to be adverse. Iodide uptake inhibition is the key event that leads to other possible effects from perchlorate exposure. Prevention of iodide uptake inhibition prevents progression to the adverse health effects of perchlorate. The inhibition of iodide uptake by perchlorate has been clearly documented in humans. The National Academy of Sciences (NAS, 2005) deemed "inhibition of iodide uptake by the thyroid as the basis of the perchlorate risk assessment to be the most health-protective and scientifically valid approach." OEHHA agrees with this approach and used it in developing its original 2004 PHG for perchlorate.

The Greer *et al.* (2002) study was used to characterize perchlorate's inhibition of iodide uptake for both the current PHG and the 2004 PHG. It might be argued that the magnitudes of the effects seen in the Greer *et al*. (2002) study were relatively small, and might not be noticeable in otherwise healthy individuals. However, this ignores the impact of these effects on a population basis. Any downward shift in the mean level of the thyroid hormone T4 in a population could increase the number of people who fall into the range of T4 values that are associated with high risks of either subtle or overt thyroid-related disease and toxicity (Miller *et al.,* 2009).

The perchlorate PHG of 1 ppb is intended to help prevent any perchlorate-related decrease in iodide uptake by the thyroid that could lead to decreased thyroid hormone production and that could disrupt the important functions of this hormone.

Estimating the Acceptable Daily Dose for Perchlorate

The PHG was derived by first calculating an Acceptable Daily Dose (ADD). This is consistent with the approach taken by the NAS (2005). The ADD is defined as the estimated maximum daily dose that can be consumed by humans for an entire lifetime without toxic effects, and is similar in definition to the reference dose (RfD) used by the U.S. Environmental Protection Agency (U.S. EPA). The ADD for perchlorate was estimated using data from the human study by Greer *et al*. (2002). This is the same study used in developing OEHHA's 2004 perchlorate PHG, and by the NAS (2005) in developing its perchlorate reference dose.

Health Value from the Greer *et al*. Study

In the Greer *et al.* (2002) study, a daily oral dose of perchlorate was administered to groups of male and female volunteers for 14 days at doses of 0.007, 0.02, 0.1, or 0.5 mg/kg-day. Reductions in iodide uptake by the thyroid gland were seen at all four dose levels, with statistically significant reductions at the highest three doses. These results were plotted and dose-response relationships fitted to estimate the dose of perchlorate likely to cause a five percent decrease in iodide uptake. This dose was defined as the Benchmark Dose (BMD), and its lower 95 percent confidence limit was defined as the BMDL. This is the same method and data set used to establish the 2004 perchlorate PHG, and the BMD of 6.8 µg/kg-day and BMDL of 3.7 µg/kg-day are the same. The units µg/kg-day refer to micrograms (µg) of perchlorate ingested in one day per kilogram (kg) of body weight.

OEHHA used the BMD approach rather than the no-observed-effect level (NOEL) approach for the following reasons. OEHHA, like U.S. EPA, has chosen to use the BMD approach as the standard method in dose calculations, provided there are sufficient data to do so. As the U.S. EPA (2012, p. viii) notes, the BMD approach "involves dose-response modeling to obtain BMDs, i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data, incorporates and conveys more information than the [NOAEL; no-observed-adverse-effect level] or [LOAEL; lowest-observed-adverse-effect level] process traditionally used for noncancer health effects." The NAS (2009, p. 129) in its review of U.S. EPA risk assessment practices similarly has recognized this as a refinement that makes better use of the dose-response evidence available than do calculations based on NOAELs.

The NOEL approach depends heavily on a single chosen dose level used in a study, and how closely it is placed relative to the next highest dose. The NOEL approach treats small and large studies the same, even though small studies, with their limited number of subjects, are less likely than large studies to find health effects at any given dose. In this case, the small group sizes in the Greer *et al*. (2002) study make it more difficult to detect an effect. For these reasons reliability of the NOEL approach can vary depending on the size of the study. In contrast, the BMD approach provides a systematic method for calculating a dose at a low specific effect level. The BMD approach uses all the dose-response evidence in the study, incorporates the shape of the dose-response curve, and takes into account the number of subjects in the study, thus providing a more reliable calculation. The advantages of using the BMD approach for this PHG are particularly important given the limited number of subjects that participated in the Greer *et al*. (2002) study.

Sensitive Populations

In the next step, the ADD of 0.37 µg/kg-day was calculated by dividing the BMDL by an uncertainty factor of 10. The NAS also used a 10-fold uncertainty factor in

developing its perchlorate reference dose (NAS, 2005). This uncertainty factor reflects the varying susceptibility of humans to perchlorate and was used because the Greer *et al*. (2002) study involved only healthy adult volunteers.

A fairly extensive body of evidence suggests that certain population subgroups may be much more susceptible to the effects of perchlorate than healthy adults. In reviewing the literature, OEHHA identified several reasons why infants, especially those that are born prematurely, are likely to have increased susceptibility to perchlorate. We also identified several other groups as likely having increased susceptibility, including fetuses, pregnant women, those with low intakes of iodine, and those exposed to other chemicals in food and water that, like perchlorate, also block iodide uptake into the thyroid.

The major change in the current PHG calculation relates to the updated information on infants as a sensitive population. OEHHA's review and updating of the perchlorate PHG considered the data from several human studies published after the 2004 PHG and the 2005 NAS review. These studies provided qualitative support for recognizing that infants are likely to be significantly more susceptible to perchlorate than healthy adults. Evidence for this includes the following:

1. New data suggest 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). Perchlorate-related toxicity is likely to be greater in infants who are already deficient in iodine.
2. OEHHA's analysis of data from studies conducted in California and elsewhere 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).
3. 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). Because of these low stores, infants may be less able to tolerate transient periods of decreased iodide uptake and decreased thyroid hormone production compared to adults.
4. Human data suggest that perchlorate can interact with other contaminants to produce a greater effect than that caused by perchlorate alone (Blount *et al.*, 2006; Steinmaus *et al*., 2007), and infants are exposed to these same contaminants.

In the 2004 PHG, an uncertainty factor of 10 was applied to address all sensitive groups (pregnant women, lactating women, thyroid compromised adults) except infants, where an uncertainty factor of 3 was used. However, given the evidence discussed above, the extent and ways infants are likely to be more susceptible to

perchlorate than healthy adults, and the fact that the Greer *et al.* (2002) study included only healthy adults, OEHHA has increased the uncertainty factor applied to infants from the factor of 3 used in the 2004 PHG to a factor of 10 in this updated PHG. This was one factor that resulted in the lowering of the updated PHG. The other factor driving the change in the PHG value is the use of updated drinking water intake rates for infants, described below.

Calculating the PHG

The ADD was then used to develop the PHG in the following two steps. First, the ADD was converted into an acceptable drinking water perchlorate concentration (in units of µg of perchlorate per liter (L) of drinking water). This was done by dividing the ADD by a drinking water intake rate expressed in terms of liters of water consumed per day per kilogram of body weight. The second step involves accounting for perchlorate intake from sources other than drinking water.

Water Consumption by Infants

The water consumption rate by infants is one of the factors that influence the final PHG. The recent analysis by OEHHA (2012) of data available from a large survey of food and beverage intake conducted by the U.S. Department of Agriculture shows that drinking water intakes per kilogram of body weight are higher in infants than previously thought. This means that infants are likely to have greater perchlorate exposure per kilogram of body weight for a given concentration of perchlorate in drinking water than was estimated in the 2004 PHG.

OEHHA used its new analysis and chose the 95th percentile drinking water intake rate instead of an average rate in order to protect the infant population, and not just those with average drinking water consumption. Infants who drink more water will have a greater exposure to perchlorate and thus may be at greater risk. In this case the bottle-fed infant will have the greatest intake of water and exposure to water contaminants.

The 95th percentile value of 0.237 L/kg-day for drinking water intake per body weight for infants aged 0-6 months (OEHHA, 2012) was used in the PHG calculations. For comparison, U.S. EPA (2009) typically assumes that infants and children drink 1 liter (L) per day and that they weigh 10 kilograms, that is 0.1 L/kg-day. Using the U.S. EPA value would result in a PHG of 2.7 ppb instead of 1 ppb.

Relative Source Contribution: Addressing Perchlorate Exposure from Other Sources

In the second step of the PHG calculation, an adjustment was made to account for perchlorate intake from sources other than drinking water. Because the ADD is the acceptable daily dose for all sources of perchlorate intake combined (i.e.,

food plus water), estimated intakes from food must be accounted for when developing a PHG for drinking water. This is a standard approach for developing drinking water criteria and is also required under Health and Safety Code Section 116365 (c)(1)(C)(iv). In OEHHA's review, it was determined that food was the only other significant source of perchlorate exposure in the large majority of people. Intake from food is accounted for by multiplying the ADD by the relative source contribution (RSC), defined as the fraction of the ADD (which incorporates perchlorate from food plus water) expected to come from water. Since infants were identified as a susceptible group, the amount of perchlorate expected to come from food was estimated using the median perchlorate levels in powdered infant formula reconstituted with perchlorate-free water (Schier *et al.*, 2010).

Based on these data, OEHHA calculated a RSC of 0.73. These two steps were used to develop an updated health-protective concentration (C) based on the following calculations: C = ADD × RSC ÷ drinking water rate = 0.37 µg/kg-day × 0.73 ÷ 0.237 L/kg-day = 1 µg/L, that is 1 ppb. Thus, the PHG for perchlorate in drinking water is 1 ppb.

Comparison with Other Agencies

In 2004 a PHG of 6 ppb was set for perchlorate, and the California Maximum Contaminant Level (MCL) for perchlorate was subsequently set at 6 ppb (Ting *et al.*, 2006). Currently, there is no federal MCL for perchlorate. The current OEHHA PHG of 1 ppb updates the PHG of 6 ppb set in 2004.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards, that is, the California MCLs. In developing the PHG for perchlorate, OEHHA's approach follows that used by the NAS (2005) to develop its reference dose in several key areas:

1. Both OEHHA and NAS identified the Greer *et al.* (2002) human study as the critical study for evaluating the effects of perchlorate.
2. Both OEHHA and NAS chose iodide uptake inhibition in the Greer *et al*. (2002) study as the key effect on which to base their calculations.
3. Both OEHHA and NAS noted that the subjects in the Greer *et al*. (2002) study were healthy adults and concluded that some people may be more susceptible to perchlorate than these healthy adult subjects. For this reason, both OEHHA and NAS applied an uncertainty factor of 10 to calculate a dose that would address inter-individual variability among humans and be protective of those who are likely to be sensitive to the effects of perchlorate.
4. Both OEHHA and NAS identified the same populations likely to be more sensitive to perchlorate exposure: fetuses, preterm newborns, infants, developing children, pregnant women, people who have compromised

thyroid function resulting from conditions that reduce thyroid hormone production, and people who are iodine-deficient.

There is only one substantive difference between the OEHHA and NAS analyses. The NAS used the no-observed-effect level (NOEL) approach. They determined that the NOEL was 0.007 mg/kg-day, the highest dose in the Greer *et al*. (2002) study that was not associated with a statistically significant response. OEHHA used the Benchmark Dose (BMD) approach and calculated a point of departure of 0.0037 mg/kg-day. As discussed above, the BMD method is a statistical method that is now widely recognized as a better approach because it incorporates more dose-response information from the study than the NOEL method (NAS, 2009; U.S. EPA, 2012).

From the NOEL, the NAS calculated a reference dose of 0.0007 mg/kg-day (the NOEL divided by the uncertainty factor of 10). OEHHA similarly calculated an ADD of 0.00037 mg/kg-day (the BMD-derived point of departure divided by the same10-fold uncertainty factor). Thus the two approaches differ by less than a factor of 2. The NAS did not take the next step to calculate an acceptable drinking water level for perchlorate.

Conclusion

In conclusion, the primary toxic mechanism of perchlorate is a reduction in iodide uptake into the thyroid gland. If severe enough, this can lead to reduced thyroid hormone production. Adequate supplies of thyroid hormone are vital for a variety of physiologic processes, and even small reductions in thyroid hormone have been associated with increased cardiovascular disease risk factors, abnormal fetal brain development, and altered childhood cognition. The purpose of this updated PHG of 1 ppb is to identify a level of perchlorate in drinking water that prevents perchlorate-related reductions in thyroidal iodide uptake and subsequent decreases in thyroid hormone production that may be associated with any of these adverse health effects.

1. INTRODUCTION

Purpose

The purpose of this document is to evaluate current scientific information on perchlorate in order to update the health-protective concentration estimate, or Public Health Goal (PHG), for perchlorate in drinking water. PHGs are based on a comprehensive analysis of information on the toxicology of drinking water contaminants, and are based solely on protection of public health without regard to cost impacts or other factors. PHGs for carcinogens are set at a *de minimis* risk level of one in a million (10^{-6}), assuming a lifetime of exposure to the chemical in the drinking water. PHGs for noncarcinogens are based on levels estimated to be without risk of any adverse effects for exposures up to a lifetime, to the general population as well as any significant identifiable sensitive subpopulations.

Perchlorate is a ubiquitous environmental contaminant. It is apparently formed by sunlight or lightning interacting with oxygen and chlorine in the atmosphere, and falls to the earth in rain (Dasgupta *et al*., 2005; Mohan, 2010). Plants can accumulate perchlorate from the water they take up. Perchlorate is also released to the environment from its use in highway flares, fireworks and other explosives, and rocket fuel. People are primarily exposed to perchlorate through consumption of food and water.

Exposure to perchlorate may cause harmful health effects due to its competition with iodide for uptake into the thyroid gland. Iodide is used by the thyroid gland to make the thyroid hormones thyroxine and triiodothyronine (also known as T4 and T3). Decreased uptake of iodide can decrease production of thyroid hormone and impair normal metabolism and growth. Several other chemicals that people are commonly exposed to, such as nitrate, thiocyanate, and bromide, can also compete with iodide for uptake into the thyroid. Maintenance of normal production of thyroid hormone depends on the availability of iodide, obtained mostly from the diet, as well as the combined effects of the various competitors for iodide uptake.

This document represents an update of an earlier health risk assessment of perchlorate conducted by OEHHA that resulted in the publication of a PHG in 2004. This revision takes into account information which suggests that infants can be especially susceptible to perchlorate, along with pregnant women and their fetuses and those with iodine deficiencies. This revision also incorporates the higher drinking water consumption values described by OEHHA (2012) to be more protective of the entire population.

Chemical Profile

Chemical Identity

Perchlorate (ClO_4^-) is the most oxygenated member of a series of four anions made up of chlorine and oxygen. The anion has a charge of negative one, and can form an acid or a salt in combination with H^+ or another cation such as sodium, potassium or ammonium. Perchlorate salts are ionic, and dissociate completely when dissolved in water. This risk assessment is for the perchlorate anion in water, regardless of the cation.

Physical and Chemical Properties

Ammonium perchlorate (NH_4ClO_4), the salt used as an oxidizer in rocket propellants, is a white, crystalline solid. As ammonium perchlorate is the major source of most of the perchlorate that has been detected in drinking water sources in California and Nevada (U.S. EPA, 1998a), it is used as the model compound to illustrate some of the physical and chemical properties of perchlorate salts (Table 1).

Table 1. Physical and Chemical Properties of Ammonium Perchlorate (from HSDB, 2010)

Property	Value or Information
Molecular Weight	117.49
Color/Physical State	White orthorhombic crystals
Melting Point	130°C, starts to decompose at 439°C
Solubility in water	200 g/L at 25°C
Solubility in organic solvents	Soluble in methanol, slightly soluble in ethanol and acetone, almost insoluble in ethyl acetate, ether
Density	1.95 g/cm^3

Production and Uses

Ammonium perchlorate is used as an oxidizer in solid rocket propellant. Sodium perchlorate is used in slurry explosives, and potassium perchlorate is used in road flares and air bag inflation systems. The manufacture of perchlorate salts begins with the electrolysis of brine (sodium chloride in water) to first form sodium chlorate ($NaClO_3$) and then sodium perchlorate ($NaClO_4$). This is reacted with ammonium chloride to form ammonium perchlorate (NH_4ClO_4) and sodium chloride. The solution is cooled, and the ammonium perchlorate crystals are dried and packaged.

Ammonium perchlorate is mixed with metallic aluminum in a synthetic rubber base to make rocket fuel. This type of fuel is used in the Minuteman missile, which has been deployed in the United States since 1961. Perchlorate salts are also used as a component of air bag inflators, in nuclear reactors and electronic tubes, as additives in lubricating oils, in tanning and finishing leather, as a mordant for fabrics and dyes, and in electroplating, aluminum refining, rubber manufacture, and the production of paints and enamels (U.S. EPA, 2002).

Perchlorate has been used as a growth promoter in leguminous plants (Verteletskaya *et al.*, 1974; as cited in Von Burg, 1995), livestock (sheep and cattle) and poultry (Yakimenko *et al.*, 1981; as cited in Von Burg, 1995). Research from the former Soviet Union indicated that weight gains in livestock of 3 to 31 percent were obtained by addition of ammonium perchlorate to the feed. Feed expenditure was also reduced 7-18 percent. The optimum dose was estimated to be 2-5 mg/kg (Grayson, 1978). Weight gains in livestock may be secondary to hypothyroidism and decreased metabolic rates. This is most likely a non-nutritive effect associated with the inhibition of thyroid hormone production and subsequent hypothyroidism.

Perchlorate has no known nutritional use. In 1952, investigators observed that perchlorate displaces iodide from the rat thyroid (Wyngaarden *et al.*, 1952). Since then perchlorate has been widely used in laboratory studies on the thyroid to block entry of iodide into the thyroid, or to cause discharge of noncovalently bound iodide previously accumulated in the thyroid (Wolff, 1998).

Environmental Occurrence and Human Exposure

Perchlorate can apparently be formed by sunlight or lightning interacting with oxygen and chlorine in the atmosphere (Dasgupta *et al*., 2005; Mohan, 2010). As perchlorate falls to the earth in rain, it can distribute at low levels throughout the environment, in both soil and water. Plants can accumulate perchlorate from the water they take up (U.S. EPA, 2001a; Jackson *et al*., 2005; Sanchez *et al*., 2005 a,b, 2008).

Perchlorate is also released to the environment from its use in highway flares, fireworks and other explosives, and rocket fuel. Perchlorate salts have been widely used as an oxidizer in solid propellants for rockets and missiles since the mid-1940s. Because of its finite shelf life, the propellant containing perchlorate has been periodically washed out of the United States' missile and rocket inventory to be replaced with a fresh supply (U.S. EPA, 1998a). As a consequence of this practice, large volumes of perchlorate have been disposed of since the 1950s. Some of this has leached into soil, and into aquifers used for drinking water. Perchlorate is highly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions (U.S. EPA, 1998a).

Air

Some unreacted perchlorate is occasionally released to the atmosphere during the launch of solid fuel rockets. Releases have also occurred as a consequence of open burning and detonation of old rocket fuel or surplus materials. No data were found on levels of perchlorate in ambient air.

Perchlorate dust can also be suspended in air, and can be inhaled by individuals working in areas where perchlorate is manufactured (Lamm *et al.*, 1999).

Soil

As a result of past disposal practices, including replacement of rocket fuel supplies noted above, soil and groundwater near the facilities that had been engaged in rocket fuel manufacturing and disposal are contaminated (U.S. EPA, 1998a). Releases to soil might also have occurred because of the past open burning and open detonation of perchlorate-containing material. Another way in which soil can become contaminated is by irrigation with perchlorate-contaminated water.

A report by TRC Environmental Corporation (1998) raised the concern that some chemical fertilizers may be contaminated with perchlorate. In the past, some fertilizers derived from Chilean caliche (a natural perchlorate source) were found to be contaminated with perchlorate. Since this discovery, the producer of Chilean caliche has changed its practice and eliminated the perchlorate contamination. U.S. EPA (2001b) tested a variety of fertilizers collected from representative sites around the nation and did not find perchlorate contamination to be a problem.

Water

Drinking water sources have become contaminated with perchlorate as a consequence of soil pollution in areas where solid rocket fuel has been manufactured, used, or disposed of. Perchlorate salts are soluble in water and once dissolved, perchlorate ion can persist in surface and ground waters for several decades (U.S. EPA, 1998a).

Until March 1997, the detection limit for perchlorate in water was rather high, at 400 µg/L (ppb). In March 1997, California Department of Health Services (DHS), now the California Department of Public Health (DPH), developed a more sensitive analytical procedure, using ion chromatography, and achieved a detection limit in the 4-5 ppb range (DHS, 2000). Shortly thereafter, the new technology was adopted by a number of commercial laboratories. U.S. EPA Method 314.0 (Federal Register, 2000) now exists for analysis of perchlorate in water and has a detection limit as low as 0.5 ppb.

Since March 1997, California DPH (and now SWRCB) has reported measurements of perchlorate concentrations in thousands of drinking water sources and wells throughout the state. Between 2010 and 2013, perchlorate concentrations above 4 ppb were reported in 248 drinking water sources in California (Table 2).

Urbansky *et al*. (2000) analyzed samples of eight domestic brands and eight imported brands of bottled water and did not find perchlorate (with a detection limit of 5 ppb) in any of the samples.

Blount *et al.* (2010) measured perchlorate concentrations in tap water samples collected from 3,262 subjects from the 2005-6 United States National Health and Nutrition Examination Survey (NHANES), and reported a geometric mean of 0.714 µg/L (95% confidence interval (CI), 0.551-0.998 µg/L). Using these measurements, and 24-hour recall data on tap water consumption, the authors estimated a median perchlorate intake of 9.11 ng/kg-day from tap water for U.S. men and women over age 12. The relevance of these findings to California, especially to those areas with documented perchlorate contamination, is unknown, and estimated intakes in potentially susceptible groups such as infants were not reported.

Food

Plants take up perchlorate from water, and probably also from fertilizers which contain perchlorate (U.S. EPA, 2001a; Trumpolt *et al.*, 2005). In a greenhouse study, U.S. EPA researchers watered lettuce plants with one of five different concentrations of perchlorate (0.1, 0.5, 1.0, 5.0, and 10.0 µg/mL) for a period of 90 days following planting. They found perchlorate levels rose steadily over the first 50-60 days, and then generally leveled off. The amount of perchlorate detected in the leaves correlated with the water concentration. At about 50 days into the study, the lettuce irrigated with 10.0 µg/mL (ppm) perchlorate had a perchlorate content of about 300 µg/g on a wet weight basis (U.S. EPA, 2002).

Table 2. Reported Perchlorate Detections in California 2010-2013 (from SWRCB, 2014) [a, b]

	Peak ≥ 4 µg/L		Peak ≥ 6 µg/L		
County	**No. of Sources**	**No. of Systems**	**No. of Sources**	**No. of Systems**	**Highest Peak (µg/L)**
Contra Costa	1	1	1	1	7.9
Fresno	1	1	-	-	4.5
Kern	2	2	1	1	14
Los Angeles	98	31	68	21	108
Monterey	1	1	-	-	4.8
Orange	11	7	3	2	9
Riverside	49	8	38	8	68
Sacramento	2	1	2	1	13
San Bernardino	57	19	37	16	94
San Diego	10	2	8	2	9.9
Santa Barbara	1	1	-	-	4.6
Santa Clara	5	4	3	3	10
Sutter	3	3	1	1	10
Tulare	6	3	5	3	20
Ventura	1	1	-	-	5.2
TOTAL	248	85	167	59	-

[a] Data for perchlorate were extracted from the water quality monitoring database (http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/EDTlibrary.shtml).

[b] Where raw and treated samples or other results indicate more than one sampling point for the same source, they are counted as coming from a single source. All detections greater than the detection limit for purposes of reporting (DLR), however, are available here: http://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/Documents/Perchlorate/perchloratedetects2010-2013forweb.xls.

Scientists at Texas Tech University (Lubbock, Texas) developed sensitive methods for assaying perchlorate in biological samples and reported perchlorate accumulation in a variety of crops as well as in animals, trees and aquatic plants (Smith *et al.,* 2001, 2004; Tan *et al*., 2004; Yu *et al*., 2004; Jackson *et al*., 2005). Concentration factors well over 100-fold, compared to the concentration in the water, were reported in some plants. Sanchez *et al*. (2005a,b) showed that perchlorate accumulated in lettuce and other leafy vegetables when grown with

Colorado River water contaminated with perchlorate at low ppb levels. Later studies by the same workers documented uptake in a wider variety of crops (Sanchez *et al*., 2006, 2008).

In 2004-5, the U.S. Food and Drug Administration (U.S. FDA) measured perchlorate concentrations in many different types of foods. Results for 27 foods and beverages collected from areas where perchlorate was known to contaminate drinking water were reported (U.S. FDA, 2009). Multiple assays were conducted on each product, for a total of 775 results. Reported average values ranged from 0.15 ppb (µg/L) in potatoes (set at one-half the limit of detection, since all values were non-detect) to 92.4 ppb in "greens." Shrimp was second-highest, at 19.83 ppb. Cow's milk averaged 5.81 ppb.

Using food consumption estimates from the U.S. Department of Agriculture's Continuing Survey of Food Intake by Individuals (CSFII 1994-96 and 1998 Supplemental Children's Survey), U.S. FDA estimated dietary perchlorate consumption for various population groups. Mean perchlorate intake of persons aged 2 years and above was estimated to be 0.053 µg/kg-day. The estimated mean intakes for children aged 2-5 years, and for females aged 15-45 years, were 0.17 and 0.037 µg/kg-day, respectively. The estimated 90th percentile intakes were 0.12 µg/kg-day for all people aged 2 years and older; 0.34 µg/kg-day for children aged 2-5 years; and 0.074 µg/kg-day for females aged 15-45 years.

More recently, U.S. FDA has included perchlorate analysis in its Total Diet Study, which involves a periodic analysis of 285 foods selected to be representative of the total U.S. diet. As reported by Murray *et al*. (2008), food products were sampled in 2005-2006. Estimates of perchlorate intake were made using the CSFII data as described above. Upper and lower bound consumption estimates for various population groups are shown in Table 3. These perchlorate exposure estimates tend to be higher than the earlier FDA estimates, but they cover more foods.

There are concerns that breast milk may represent an exposure pathway for infants. In a study reported by Yu (2000), groups of female rats were treated with perchlorate in drinking water at 0, 0.01, 0.1, 1, and 10 mg/kg-day throughout gestation and lactation. On postnatal day 10, the rats were milked. Yu found the levels of perchlorate in milk were about twice as high as the corresponding levels in maternal serum across all doses, suggesting that perchlorate is actively sequestered into milk. Clewell *et al.* (2003) reported that perchlorate was indeed transferred to the pup through suckling as perchlorate was detected in milk, as well as in the neonate serum, gastrointestinal contents, and skin.

Human mammary gland during lactation has been shown to express the sodium-iodide symporter (NIS), the protein responsible for moving iodide into the thyroid and, in this case, into breast milk. The mammary gland may have the capability to actively secrete perchlorate into the breast milk (Vayre *et al.*, 1999; Tazebay *et*

al., 2000). Another concern is that iodide in breast milk is necessary for thyroid hormone synthesis by the newborn. Perchlorate inhibits the NIS in the lactating mammary gland and can interfere with the secretion of iodide into breast milk. This reduction in iodide transfer has been seen in cows and goats (Howard *et al*., 1996; Lengemann, 1973; Mountford *et al*., 1987).

Rice *et al*. (2007) analyzed the relationship between perchlorate in feed given to dairy cows and the resulting levels in milk. They reported that significant perchlorate exposures occurred from perchlorate in corn silage, alfalfa, and grass. Sanchez *et al*. (2008) also showed that perchlorate in alfalfa makes a major contribution to perchlorate levels in the milk of dairy cows.

Table 3. Estimated Perchlorate Intakes from U.S. FDA's Total Dietary Survey: Results for 2005–2006 (as reported in U.S. EPA, 2008b)

Population group		Perchlorate intake from food µg/kg-day	
		Lower-bound	Upper-bound
Infants	6-11 mo.	0.26	0.29
Children	2 yr.	0.35	0.39
Children	6 yr.	0.25	0.28
Children	10 yr.	0.17	0.20
Teenage Girls	14-16 yr.	0.09	0.11
Teenage Boys	14-16 yr.	0.12	0.14
Women	25-30 yr.	0.09	0.11
Men	25-30 yr.	0.08	0.11
Women	40-45 yr.	0.09	0.11
Men	40-45 yr.	0.09	0.11
Women	60-65 yr.	0.09	0.10
Men	60-65 yr.	0.09	0.11
Women	70+ yr.	0.09	0.11
Men	70+ yr.	0.11	0.12

Kirk *et al*. (2005) analyzed perchlorate concentrations in 47 different samples of dairy milk from 11 different states and 36 human milk samples from lactating women from 18 different states. Detectable levels of perchlorate were found in all but one of the samples tested. The mean perchlorate concentration in the breast milk samples was 10.5 µg/L. Iodine concentrations in milk were inversely correlated with perchlorate concentrations, but only in the six samples with perchlorate concentrations above 10 µg/L (coefficient of variation (R^2) > 0.9). In a later study, Kirk *et al*. (2007) measured perchlorate levels in 10 lactating women in six breast milk samples per day per woman for three days. The mean

perchlorate concentration was 5.8 µg/L (standard deviation (SD) ± 6.2 µg/L). Considerable variability was seen both among and between individuals.

Pearce *et al*. (2007) measured breast milk perchlorate levels in 49 women from the Boston area and in 17 different infant formulas. Perchlorate was detectable in all 49 breast milk samples tested and in all 17 infant formula samples tested. The median breast milk perchlorate concentration was 9.1 µg/L. This was about 3 times higher than the median perchlorate concentrations in the urine samples of these women. There was no correlation between breast milk iodine and perchlorate concentrations ($R^2 = 0.05$, $p = 0.1$), and no correlation in those women with breast milk perchlorate concentrations above 10 µg/L. The median breast milk iodine concentration was fairly low (median = 155 µg/L; range, 2.7 - 1968) and the authors estimated that 47 percent of the breast milk samples did not contain enough iodine to meet the infant iodine intake recommended by the Institute of Medicine.

Dasgupta *et al*. (2008) measured perchlorate, thiocyanate, and iodine in the urine and breast milk of 13 breastfeeding women. The mean breast milk perchlorate concentration was 9.3 µg/L (SD ± 7.5 µg/L). Selectivity factors were determined for each chemical based on the relative excretion of each in breast milk and urine. Total perchlorate excretion was based on urinary and breast milk excretion only and possible excretion via other pathways was ignored. The median fraction of total excretion in the milk for perchlorate, thiocyanate, and iodine were 0.541, 0.053, and 0.177. The selectivity factors for perchlorate over iodide transport, and thiocyanate over iodide transport, were 3.14 and 0.27, respectively. The authors note that these transport selectivities are an order of magnitude lower than those indicated in *in vitro* studies. The authors did not specifically report a correlation coefficient for the relationship between breast milk iodine and perchlorate concentrations but did note that in their plot of these data (their Figure 3) that there were no subjects in the high iodine-high perchlorate quadrant.

More recently, Valentín-Blasini *et al.* (2011) measured perchlorate in urine samples from 92 infants ages 1-377 days and estimated perchlorate intake doses based on these measurements. The median estimated intake was 0.160 µg/kg-day. Of the 205 individual dose estimates (multiple urine samples were collected from each subject), 9 percent exceeded the NAS (2005) reference dose of 0.7 µg/kg-day. Breast-fed infants had a higher estimated perchlorate exposure dose (geometric mean = 0.220 µg/kg-day) than infants consuming cow milk-based formula (geometric mean = 0.103 µg/kg-day, $p < 0.0001$) or soy-based formula (geometric mean = 0.027 µg/kg-day, $p < 0.0001$).

Kirk *et al.* (2013) used data on daily water intake, daily urine volume, predicted daily milk volume, and perchlorate concentrations in water, urine, and breast milk samples from 13 lactating women in Texas to estimate average daily exposure to perchlorate from food and water. The amount of perchlorate from water was estimated by multiplying the amount of daily water intake from each water source

used by the subject by the perchlorate concentration in samples of that water source and summing the results across all water sources. The amount of perchlorate from non-water sources (presumably mostly food) was then estimated by subtracting the estimate of exposure from water from the total amount of perchlorate excreted in urine and breast milk. The mean urinary perchlorate concentration was 3.6 µg/L, with a mean estimated daily urinary perchlorate excretion of 5.8 (±5.3) µg. Total excretion of perchlorate in breast milk was not provided, although based on perchlorate milk concentrations and typical milk consumption rates in infants the authors estimated that the average daily transfer of perchlorate to infants through breast milk was 12.87 (±8.99) µg. Using urine and breast milk perchlorate excretion rates, the mean maternal daily dose of perchlorate was estimated to be 0.186 µg/kg-day with an estimated 0.173 µg/kg-day (93 percent) coming from non-water sources. Importantly, mean perchlorate water concentrations in this population were low (0.46 µg/L ±1.11) so these data are likely not relevant to subjects who live in areas with greater perchlorate contamination in drinking water.

Huber *et al.* (2011) used urinary concentrations of perchlorate measured in the 2001-2 NHANES and drinking water perchlorate concentrations from the U.S. EPA Unregulated Contaminant Monitoring Rule (UCMR) database to estimate the possible amount of perchlorate exposure due to food and water in U.S. adults and children ages 6 and older. The UCMR database included perchlorate water concentrations in a “near census” of 3,086 large public water systems (those serving >10,000 people) and 797 representative small public water systems, measured between 2000 and 2005. NHANES is a large, essentially nationally representative survey performed in the U.S. by the Centers for Disease Control and Prevention. Residential location for NHANES subjects was only available to the researchers on a county-level basis. Subjects in NHANES with urinary perchlorate measurements were divided into one of three bins. Bin I (n=320) included subjects residing in counties where at least one water system had at least one UCMR water perchlorate measurement above the detection limit of 4 µg/L. Bin III (n=2,059) included subjects residing in counties where a drinking water system had been sampled for perchlorate but all measurements were below the detection limit. Bin II (n=325) included subjects who had no UCMR measurements for a water system in the county in which they resided. Estimates of 24-hour perchlorate intake were calculated for all subjects using single spot urinary perchlorate concentrations with adjustments for urinary creatinine. Estimates in Bin III subjects were assumed to represent typical daily exposure from food, while the difference in estimates between Bins I and III was assumed to represent typical daily exposure from water. Overall, the mean estimates were 0.081 µg/kg-day in Bin III (food only) subjects and 0.101 µg/kg-day in Bin I subjects (food + water), and the authors estimated that about 19.7 percent of perchlorate exposure in subjects from exposed counties came from water. Estimates of intakes from water in specific age groups ranged from 2.1percent for ages 6-11 to 25.6 percent for ages 12-19. Overall, these data suggest that food is typically the predominant source of perchlorate for most people in the U.S. Potential weaknesses included the fact that not all water systems were

measured (i.e., many smaller public water systems and all private wells were not measured), the potential impact of fasting was not accounted for, data on the amount of water consumption subjects had from any particular water source was not incorporated, and no estimates were available for children under age 6. Also, the detection limit for perchlorate at the time the water sources were measured was 4 µg/L, so there is little information in this study on the impacts of lower exposures (i.e., those above zero but below 4 µg/L). In addition, many counties have multiple water systems with highly variable perchlorate levels. As such, it is likely that many people in a county with a single positive drinking water perchlorate measurement will actually be drinking water from a system with very low or no perchlorate. The inclusion of these lower exposure people in Bin I would lead to underestimates of the true intake of perchlorate from drinking water. Because of these weaknesses, the specific contribution of perchlorate from water in people who have elevated levels of perchlorate in their drinking water cannot be accurately estimated using the data from this study.

2. METABOLISM AND PHARMACOKINETICS

Absorption

Test data from human studies indicated that perchlorate is readily absorbed from the gastrointestinal tract and excreted primarily via the urine. Eichen (1929; as cited in Stanbury and Wyngaarden, 1952) orally administered 1-2 g perchlorate to patients and recovered 70 percent of the dose in the urine in 12 hours and 85-90 percent in 24 hours. In a similar experiment, two human subjects each drank a solution of 794 mg of sodium perchlorate dissolved in 100 mL of water (Durand, 1938). Fifty percent of the administered dose was recovered in the urine by five hours and 95 percent in 48 hours. These human data suggest absorption of perchlorate through the oral route is virtually complete.

Besides the thyroid, the sodium-iodide symporter (NIS) appears to be expressed and active in mammary gland, salivary glands, gastric mucosa, and placenta (Vayre *et al.*, 1999; Tazebay *et al.*, 2000; de la Vieja *et al.*, 2000; Mitchell *et al.*, 2001). These transport systems exhibit functional similarities with their thyroid counterpart and may play a role in the absorption of iodide into the body. Because perchlorate is completely ionized in aqueous systems, its permeability through intact skin is expected to be limited (U.S. EPA, 1998a). Inhalation exposure during showers is considered possible but not likely to be an important route of exposure. This is because the droplets produced in showers are generally too large to be inhaled. Exposure to vapors of the chemical via the inhalation route is expected to be negligible because of the low vapor pressure of perchlorate salts at room temperature. However, inhalation of airborne perchlorate particles could be an important exposure route in occupational settings. Lamm *et al.* (1999) studied a group of workers in a perchlorate production plant and reported that there was a correlation between airborne perchlorate dust concentration and the amount of perchlorate excreted in urine.

Distribution

Anbar *et al.* (1959) injected white rats and rabbits intraperitoneally with radiolabeled potassium perchlorate (approximately 3-14 mg per animal) and measured the specific activity per gram of tissue in various organs from 30 minutes to 12 hours post administration. The ratio of the specific activity of perchlorate in thyroids versus the specific activity in blood reached a limiting value of 4.3 ± 0.3 in both rats and rabbits, at about 6 hours after the injection. These data demonstrate that the thyroid of these species concentrates perchlorate ions. There were also indications that perchlorate is retained in the salivary gland and testes.

Chow *et al.* (1969) measured perchlorate uptake using radiolabeled perchlorate in male Sprague-Dawley rats. Rats were injected with 0.1, 0.2, or 5.0 meq/kg of perchlorate (14, 28, or 690 mg/kg, respectively) two hours prior to sacrifice. At

the low and middle doses, radiolabeled perchlorate concentrations in the thyroid were higher than those in the blood. At the high dose, perchlorate concentrations in the thyroid and blood were about the same. In a similar study, rats were exposed to 0.69, 1.4, 2.8, 6.9, or 14 mg/kg of perchlorate. The apparent accumulation of perchlorate in the thyroid, as reflected by the thyroid/blood ratio (which ranged from 31.1 to 2.5), was found to be inversely related to the perchlorate dose (U.S. EPA, 2002).

Chow and Woodbury (1970) also studied perchlorate accumulation by the thyroid. They administered perchlorate by intraperitoneal injection at 0.69, 14, or 280 mg/kg to groups of male Sprague-Dawley rats. The treated rats were sacrificed at 0.033, 0.067, 0.13, 0.2, 0.5, 1.0, 2.0, and 4.0 hours after dosing. The amount of perchlorate accumulation in the thyroid compared to that in the plasma was highest at the lowest dose. At the higher doses (at or above 14 mg/kg), the level of perchlorate in the thyroid was lower than in the plasma.

It has been shown that perchlorate inhibits iodide transport into the thyroid. Thyroid tissues can also concentrate several related monovalent anions. Measurement of the ability to be concentrated by thyroid tissues, or to inhibit iodide transport, has resulted in the following potency series for monovalent anion-based inhibition of iodide transport in thyroid slices: $TcO_4^- \geq ClO_4^- > ReO_4^- > SCN^- > BF_4^- > I^- > NO_3^- > Br^- > Cl^-$ (Wolff, 1964; as cited in Wolff, 1998). These relative potencies are based on *in vitro* data and high anion concentrations. It is not clear whether they also apply to *in vivo* scenarios, real-life human situations, or lower environmentally relevant exposure levels. Anbar *et al.* (1959) showed that the inhibition of iodide transport by perchlorate is a truly competitive process. They intraperitoneally injected ^{36}Cl-labeled perchlorate and iodide ions in various concentrations to groups of rats and found that either iodide or perchlorate could inhibit the accumulation of the other anion by the thyroid (Table 4).

Recently, the apparent accumulation of perchlorate by the thyroid of rodents has been disputed. Citing *in vitro* electrophysiological data, de la Vieja (2000) suggested perchlorate acts as a blocker of NIS, but it is not translocated via NIS into the cell. De la Vieja (2000) theorized that because ^{36}Cl chlorate (ClO_3^-) is a byproduct of the reaction employed to chemically synthesize ^{36}Cl perchlorate for the uptake study, it is possible that ^{36}Cl chlorate, rather than perchlorate, accounts for the measured radioactivity, given that chlorate is readily translocated via NIS into the cell.

Table 4. The Ratio between Concentrations of Iodide and Perchlorate Ions in the Thyroid (from Anbar *et al.*, 1959)

Iodide dose (mmol)	Perchlorate dose (mmol)	Ratio[a] I^-/ClO_4^-		
		60 min	120 min	360 min
0.14	0.028	4.7	6.9	3.3
0.14	0.14	2.1	2.7	2.2
0.028	0.14	0.53	0.58	0.67

[a]Ratio = concentration of iodide in thyroid / concentration of perchlorate in thyroid.

Goldman and Stanbury (1973) administered ^{36}Cl-labeled potassium perchlorate to male Sprague-Dawley rats by intraperitoneal injection (approximately 40 μg stable perchlorate per injection). The rats were maintained on a low iodine diet for 4-5 weeks prior to perchlorate administration. The level of perchlorate in the thyroid peaked at four hours after administration, then declined to approximately five percent of its peak at 96 hours. The decay followed an exponential function with a half-life of 20 hours. When the levels of radioactivity in the serum and the urine are plotted against time, they also followed an exponential function with a half-life of approximately 20 hours. Goldman and Stanbury (1973) also showed that most of the administered perchlorate was excreted in the urine. The retention of the radiolabel in selected tissues 96 hours after the administration of perchlorate is shown in Table 5.

There are also data indicating that perchlorate can pass through the placenta and affect the fetal thyroid. Thyroid enlargement and reduction of thyroidal iodide uptake have been detected in fetuses of laboratory animals exposed to perchlorate (see the discussion in the Developmental and Reproductive Toxicity section.)

Selivanova and Arefaeva (1986) administered a single oral dose of perchlorate to rats and observed a two-phase biological decay curve. The first biological half-life ranged from 1-2 hours and accounted for a calculated 96 percent of the dose. The second-phase half-life, which accounted for only four percent of the administered dose, ranged from 72 to 80 hours. Yu *et al.* (2000) injected perchlorate intravenously at doses of 0.01, 0.1, 1, and 3 mg/kg to male Sprague-Dawley rats and monitored the serum concentration of perchlorate over time. The estimated early- and terminal-phase half-lives of perchlorate were 2-3 hours and 12-26 hours, respectively.

Table 5. Percent Dose of ^{36}Cl/g Tissue 96 Hours after Intraperitoneal Injection of ^{36}Cl-perchlorate (from Goldman and Stanbury, 1973)

Organ	Percent dose/g tissue[a]
Thyroid	0.142 ± 0.1
Kidney	0.125 ± 0.09
Spleen	0.098 ± 0.03
Liver	0.048 ± 0.04
Brain	Background

[a]Mean ± standard deviation; each value represents five animals.

The differences in biological half-lives of perchlorate in rats in the studies above (Goldman and Stanbury, 1973; Selivanova and Arefaeva, 1986; Yu *et al.*, 2000) are partly due to the three different routes of administration. The difference may also be explained by the fact that the rats in the study reported by Goldman and Stanbury (1973) had been maintained on a low iodine diet for 4-5 weeks before the administration of perchlorate.

Metabolism

There are data to suggest that perchlorate is not metabolized in humans (Anbar *et al.*, 1959). Four patients were orally administered 200 mg of radiolabeled perchlorate (5 μCi), double labeled with ^{36}Cl and ^{18}O. The perchlorate was excreted unchanged in the urine with the two labels (^{36}Cl and ^{18}O) remaining associated in the same molecule. The results also showed that there was no reduction of perchlorate *in vivo*, as there was very little radioactivity associated with Cl^- and ClO^{3-} ions in urine.

Excretion

As described above, 95 percent of a dose of sodium perchlorate administered orally to human subjects was eliminated in the urine by 48 hours after administration (Durand, 1938). Lamm *et al.* (1999) monitored urinary perchlorate levels of two workers during three days with measurable occupational perchlorate exposure and during the subsequent three days without known perchlorate exposure. The perchlorate body burden, as measured using urinary perchlorate concentrations, increased over the three days of work exposure, with decreases between the 12-hour work shifts. The elimination of perchlorate after the last exposure period appeared to follow a first-order kinetics pattern. The average perchlorate elimination half-lives measured for the two workers were 7.9 and 8.2 hours.

Greer *et al.* (2002) administered oral doses of perchlorate in water to human volunteers and estimated half-life values ranging from 6.0 to 9.3 hours, with an

average value of 8.1 hours. These are similar to those reported in Lamm *et al.* (1999).

Selivanova and Arefaeva (1986) administered a single oral dose of perchlorate to rats, rabbits, and calves at 2, 20, 200, and 600 mg/kg in a single oral dose. They reported that in all cases, little or no perchlorate could be detected in the blood after 72 hours. A majority of the administered perchlorate was excreted in the urine; the feces excreted $\leq$ 8.5 percent. Yu *et al.* (2000) injected perchlorate intravenously to rats at doses of 0.01, 0.1, 1, or 3 mg/kg and reported that between 72 percent and 97 percent of the administered dose was excreted in the urine over a 24-hour period.

3. ADVERSE EFFECTS OF THYROID HORMONE AND IODINE DEFICIENCY

The most important and early effect of perchlorate exposure is its effect on reducing iodide uptake by the thyroid. Significant reduction in iodide uptake can lead to decreased thyroid hormone production. For this reason, this chapter reviews studies of the adverse health effects of iodine deficiency and decreased levels of thyroid hormone. This discussion is preceeded by a brief review of normal thyroid physiology.

Thyroid Physiology

The principal hormones secreted by the thyroid are thyroxine (T4) and triiodothyronine (T3). Iodide is a key component of both. While T4 is produced only by the thyroid gland, about 80 percent of T3 is formed outside the thyroid by deiodination of T4. T4 and T3 influence the growth and maturation of tissues, cell respiration and total energy expenditure, and the turnover of essentially all substrates (including carbohydrates, cholesterol, and proteins), vitamins, and hormones (including the thyroid hormones themselves).

The major components of thyroid hormone are iodide and tyrosine. Tyrosine is generally not the rate-limiting component. Iodine is a trace element, and its uptake into the thyroid can be rate-limiting in thyroid hormone production. Ingestion is the main route of iodine intake. Once ingested, iodine is reduced to iodide (I^-) in the gastrointestinal tract and is readily absorbed into the bloodstream.

Thyroid tissue has a special ability to selectively concentrate iodide from the blood where the concentration is usually very low. The thyroid can actively transport iodide into the thyroid such that the iodide concentration in the thyroid can be several hundred-fold higher than concentrations outside the thyroid. Such concentrations are presumably required to promote efficient thyroid hormone production and patients lacking the ability to concentrate iodide have goiters and are hypothyroid (Wolff, 1998). The molecule that is responsible for transport of iodide into the thyroid is called the sodium-iodide symporter (NIS). The structure and regulation of NIS have been characterized (de la Vieja *et al*., 2000). Recently mouse NIS has been cloned and transferred into normally non-iodide-transporting cells, and these cells show perchlorate-sensitive iodide uptake capability (Perron *et al*., 2001). These researchers also found evidence to indicate that the NIS is present in tissues other than the thyroid, including the stomach, lactating mammary gland, small intestine, skin, and brain.

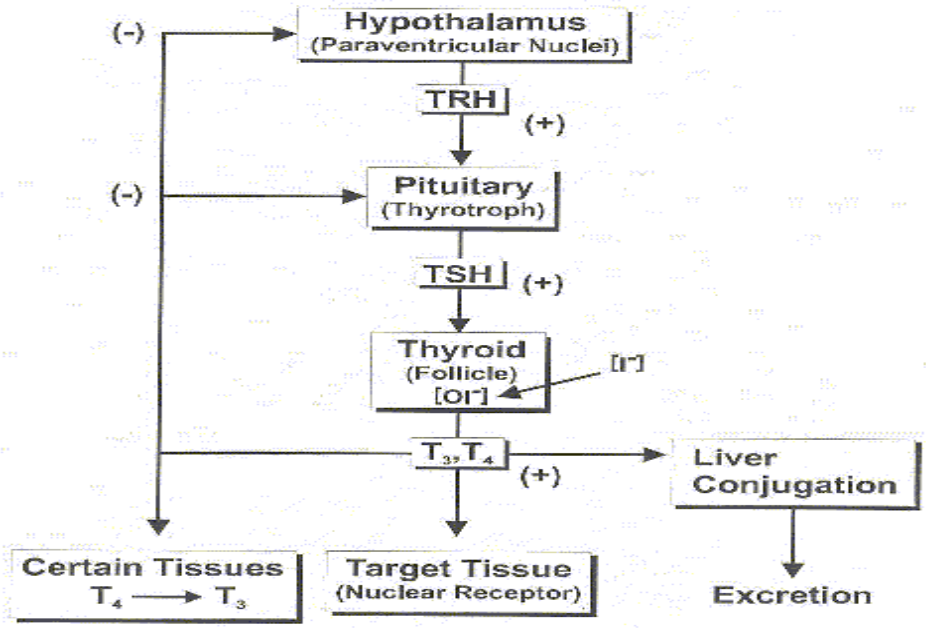
In humans, a majority of T4 and T3 in plasma is bound to proteins. In normal plasma, the T4 protein binding distribution is: 80 percent to thyroxine-binding globulin, 15 percent to transthyretin, and 5 percent to albumin and lipoproteins. For T3, the distribution is 90 percent bound to thyroxine-binding globulin and the

rest to albumin and lipoproteins, with little binding to transthyretin. Very small proportions of T4 and T3 are free (not protein bound) in plasma, 0.03 and 0.3 percent, respectively. Only the free hormone enters cells, exerts its biologic action, and determines thyroid physiologic status (Dillmann, 2000).

Control of T4 and T3 concentrations in blood is mainly regulated by a negative feedback loop involving three organs: the thyroid gland, which produces thyroid hormones; and the pituitary gland and hypothalamus, which respond to and help maintain optimal levels of thyroid hormones (Figure 1). When levels of thyroid hormone decline, the hypothalamus secretes thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce thyroid-stimulating hormone (TSH), which then prompts the thyroid gland to produce T4 and T3. The stimulated thyroid actively transports iodide into the thyroid gland, and then incorporates the iodide into thyroid hormone molecules. T4 and T3 are metabolized in the liver and other tissues. Some thyroid hormone derivatives are excreted in the bile, and some of the iodide in them is reabsorbed. Cells in the hypothalamus and pituitary gland respond to circulating levels of thyroid hormones, i.e., when hormone levels are high, there is a signal to reduce the output of TRH and TSH. Similarly, when thyroid hormone levels are low, the pituitary is prompted to release more TSH, which stimulates the thyroid to increase thyroid hormone output. This negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Circulating T4, T3, and TSH can readily be measured in the serum of experimental animals and humans and serve as biomarkers of exposure and effect of agents that disrupt thyroid-pituitary status (U.S. EPA, 1998a, and 1998b; Hill *et al.*, 1989).

In mammals, when demands for more thyroid hormone are small, existing thyroid follicular cells can meet the demand. With increased need, as a result of certain chemical exposures or chronic iodine deficiency, the thyroid responds by increasing the size (hypertrophy) and number (hyperplasia) of thyroid follicular cells to enhance hormone output. With continued TSH stimulation, there is actual enlargement of the thyroid gland (goiter) and, at least in rodents, neoplasia of the thyroid follicular cells could eventually occur. Since TSH-producing pituitary cells are also stimulated, they too sometimes undergo hyperplasia and neoplasia (U.S. EPA, 1998b).

Figure 1. Hypothalamic-Pituitary-Thyroid Axis (from U.S. EPA, 1998b)

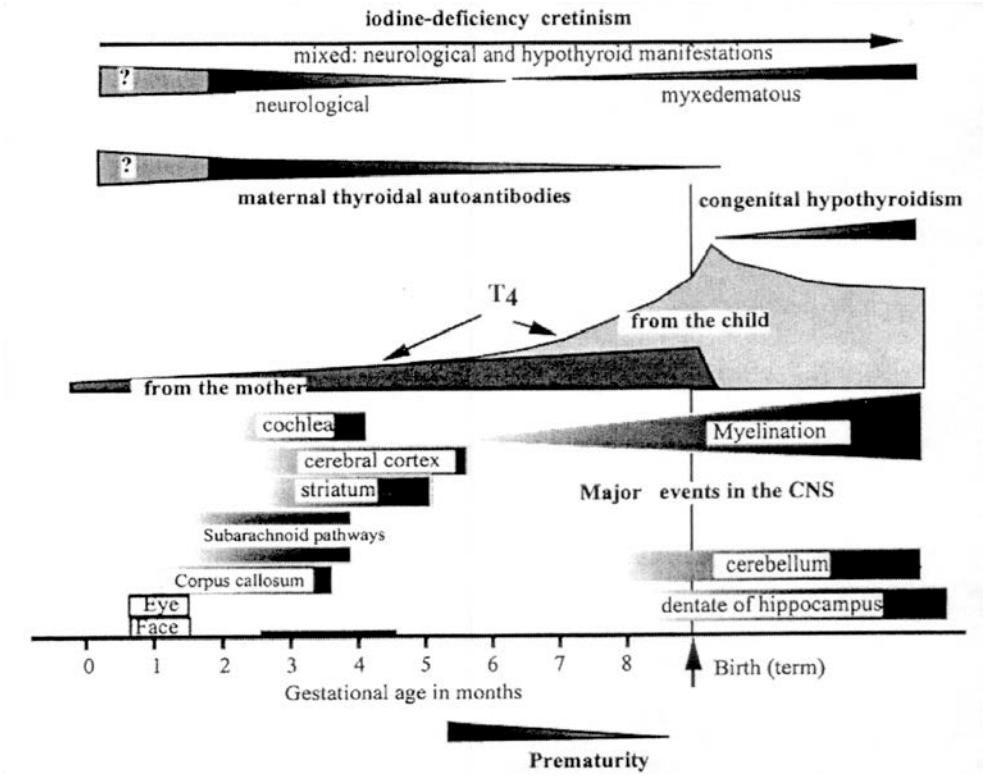


Too much or too little thyroid hormone can lead to illness. Thyrotoxicosis occurs when tissues are exposed to excess amounts of thyroid hormones, resulting in specific metabolic changes and pathophysiologic alterations in organ function. The most frequent cause of thyrotoxicosis is Graves' disease, accounting for 60 to 90 percent of cases (Dillmann, 2000). Graves' disease is an autoimmune disorder with B-lymphocytes producing immunoglobulins, some of which bind to and activate the TSH receptor, stimulating excess thyroid growth and hormone secretion. Hypothyroidism results from decreased secretion of thyroid hormone from the thyroid gland; it can be caused by destruction of thyroid tissues or defects of thyroid hormone production (e.g., congenital enzyme defects, congenital mutations in TSH receptor, iodine deficiency or excess). In some rare occasions, hypothyroidism can also be caused by pituitary or hypothalamic diseases.

The most severe neurological impairment resulting from decreased thyroid hormone production or iodine deficiency is cretinism. Characteristics of cretinism include mental retardation, spastic dysplasia, and problems with gross and fine motor control. In some extreme forms, the affected individuals cannot walk or stand. A number of studies indicate that even less severe iodine deficiency can reduce maternal serum thyroid hormone levels and may subsequently impair the brain development of the offspring (Glorieux *et al*., 1988; Rovet *et al*., 1987; Tillotson *et al*., 1994; Vermiglio *et al*., 1990; Pop *et al*., 1999, 2003; Haddow *et al*., 1999; Bleichrodt and Born, 1994). These studies are reviewed in further detail in the following sections. The nature and severity of the adverse effects are related to the degree of iodine deficiency or the extent of maternal thyroid hormone decrease.

Most data suggest that fetal damage during development is inversely related to maternal serum T4 levels (Pop *et al*., 2003; Kooistra *et al*., 2006). Maternal serum free T4 (fT4) is able to pass through the placenta and is converted to T3 in the fetal brain. The T3 generated in the fetal brain is believed to be necessary for the development of the brain, specifically the cerebral cortex, the extrapyramidal system, and the cochlea (Porterfield, 2000). The availability of a minimum level of maternal fT4 is crucial for proper fetal brain development in the first and second trimesters, as the fetal thyroid is not fully mature and functional during that time period. Figure 2 shows the approximate timing of major insults to the brain resulting from hypothyroxinemia (a low level of serum T4), superimposed on major neurodevelopmental events (Morreale de Escobar *et al.*, 2000).

Figure 2. Approximate Timing of Major Insults to the Brain Resulting from Hypothyroxinemia, Superimposed on Major Neurodevelopmental Events.



Thyroid Problems in Pregnant Women with Low Iodine Intake

A number of human studies have shown that pregnancy stresses the thyroid (Crooks *et al*., 1967; Glinoer *et al*., 1990, 1992, 1995; Smyth *et al*., 1997; Caron *et al*., 1997; Brent, 1999; Kung *et al*., 2000). In areas of iodine deficiency (e.g., intake level <100 µg/day), there is an increased risk of abnormally low serum T3 and T4 levels, and thyroid enlargement and goiter in pregnant women. The nature and severity of changes in thyroid function are related to the severity of

the iodine deficiency. In an epidemiologic survey reported by Delange and Ermans (1991; as cited in Delange, 1994), the investigators found the prevalence of goiter in an area with severe iodine deficiency is influenced by age and sex, with maximal frequency in females during puberty and childbearing age (Figure 3).

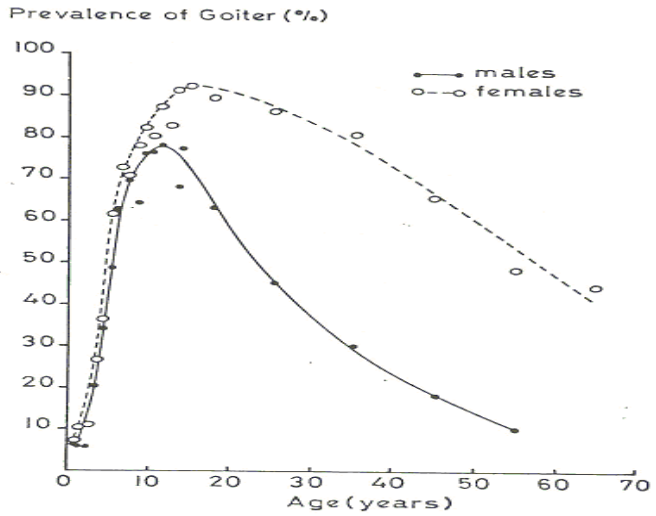


Figure 3. Changes in the Prevalence of Goiter as a Function of Age and Sex in Severe Endemic Goiter (Idjwi Island, Zaire) (Delange, 1994)

Crooks *et al*., 1967. Crooks *et al.* (1967) studied enlargement of the thyroid gland in pregnant and non-pregnant women in Aberdeen, Scotland and Reykjavik, Iceland. In the Scotland study, they found that the thyroid gland was visible and palpable in 70 percent of pregnant women but in only 37 percent of non-pregnant women in the reproductive age group. By contrast, in the Iceland study, the frequency of thyroid enlargement was about the same in pregnant (23 percent) and non-pregnant women (19 percent). The authors (1967) suggested that the results can be explained by the fact that Icelandic diet is based on fish and contains high levels of iodine. This hypothesis is supported by the significantly higher mean plasma inorganic iodine concentration measured in non-pregnant Icelandic women (0.691 µg/dL) compared to the mean of 0.420 µg/dL found in Scottish non-pregnant women ($p<0.001$).

Glinoer *et al*., 1990. Glinoer *et al*. (1990) suggested that in conditions of marginally low iodine intake, pregnancy constituted a goitrogenic stimulus. They followed a group of 606 healthy pregnant women in Brussels, Belgium, an area of marginally low iodine intake (50-70 µg/day), and monitored their T3, T4, TSH, and human chorionic gonadotropin (hCG) levels in serum during the first, second, and third trimesters. All subjects were evaluated clinically and determined to be without detectable thyroid abnormality at the beginning of the study. The authors found that a normal thyroid is faced with a triple challenge during pregnancy. First, there is a significant increase in circulating levels of the major T4 transport protein, thyroglobulin (Tg), in response to high estrogen

levels. As a result, the thyroid has to increase its T4 output in order to maintain a stable T4/thyroglobulin ratio of 37-40 percent.

Second, several thyroidal stimulating factors of placental origin (mainly hCG) are produced in excess. This contributes to a decrease of serum TSH (mainly in the first half of gestation) and an increase in thyroid volume (Table 6). They found that during pregnancy thyroid volume increased by an average of 18 percent. This increase was statistically significant and thyroid size increased in a majority of women (73 percent). Goiter, defined as thyroid volume greater than 23 mL, was found in nine percent of the cohort at delivery.

Table 6. Changes in Mean Thyroid Volume in Healthy Women during Pregnancy (from Glinoer *et al*., 1990)

Stage of Pregnancy	N	Total volume (mL)
First trimester	168	12.1 ± 4.5
Second trimester	172	12.8 ± 4.5
Third trimester	33	13.9 ± 4.8[a]
Delivery	179	15.0 ± 6.8[b]

[a]$p < 0.03$ vs. beginning of pregnancy.
[b]$p < 0.001$ vs. beginning of pregnancy.

Third, pregnancy is accompanied by a decrease in the availability of iodide for the maternal thyroid, due to increased renal clearance (Aboul-Khair *et al*., 1964; as cited in Glinoer *et al*., 1990) and losses to the feto-placental complex during late gestation, resulting in a relative iodide deficiency state.

Glinoer *et al*., 1992. In a related study, Glinoer *et al*. (1992) monitored the thyroid condition of pregnant women in an area without overt iodine deficiency, but with a marginal iodine supply (less than 100 µg/day in 80 percent of women). They found that maternal thyroid function at delivery was characterized by a relative hypothyroxinemia; increased T3/T4 ratios, indicating preferential T3 secretion; slightly increased TSH levels within the normal range in 97 percent of women; increased serum thyroglobulin values, which were above normal in 60 percent of women; and goiter formation in almost 10 percent of women. In the newborns, they found fT4 levels were significantly higher than in the respective mothers. However, mean neonatal TSH and Tg levels were significantly higher than maternal values. Furthermore, these values were highly correlated with maternal data, suggesting the limited availability of iodine was the common link.

In a review paper, Glinoer (2001) again stressed the profound alterations in the thyroid economy associated with pregnancy. In healthy iodine-sufficient pregnant women, this leads to a physiological adaptation of the thyroid and an increased production of thyroid hormones. When gestation takes place in conditions with iodine restriction or deficiency, pregnancy may lead to pathological alterations affecting both thyroid function and the anatomical

integrity of the thyroid gland. The more severe the iodine deficiency, the more obvious, frequent, and profound the potential maternal and fetal repercussions.

Smyth *et al.*, 1997. Supportive results were reported in two other studies, one in Ireland and the other in France. Smyth *et al*. (1997) evaluated ultrasound-measured thyroid volume of 115 pregnant women during one of the three trimesters. These women (Group A) were enrolled based on availability, and each trimester's study group comprised different individuals. Control values for thyroid volume were obtained from 95 pre-menopausal females. All subjects were from Dublin, Ireland, an area of moderately low dietary iodine intake (median urinary iodine was 82 µg/day). All pregnant women studied delivered live-born, normally formed, singleton infants and received no iodine-containing supplements during their pregnancy. The authors reported that the mean thyroid volume of 13.9 ± 0.8 mL, observed in the first trimester, was significantly greater than the control value (11.3 ± 0.5 mL; $p < 0.05$) and reached a maximum of 16.0 ± 0.7 mL, a 47 percent increase ($p<0.01$), in the third trimester.

In a related study, Smyth *et al*. (1997) studied a group of 38 pregnant women (Group B), prospectively. Casual urine samples were collected sequentially during the 3 trimesters of pregnancy and at approximately 6 weeks postpartum. Of those 38 subjects, 20 had thyroid ultrasound scans during each trimester of pregnancy and at 6 weeks postpartum. Thyroid volumes greater than 18.0 mL were defined as enlarged. The number of enlarged thyroids increased from the non-pregnant control value of 6.3 percent, through 19.5 percent in the first trimester, to reach a plateau of approximately 32 percent in the second and the third trimesters, which was maintained up to 40 days postpartum.

Urinary iodine of the women in Group A and Group B were also measured. Urinary iodine measurements collected from 1,063 pre-menopausal women over a one-year period were used for comparison. The researchers found that urinary iodine levels measured throughout the pregnancies of the women in Group A and Group B (Table 7) were higher than in the controls (median 70 µg/L). They suggested that in an area of moderately low dietary iodine intake, urinary iodine loss during pregnancy may result in maternal thyroid enlargement.

Table 7. Median Urinary Iodine Excretion (µg/L) in Pregnancy (Smyth *et al.*, 1997)[a]

	First Trimester	Second Trimester	Third trimester
Group A	135	122	122
Group B	155	122	115

[a]Some of the values were estimated from a graph.

Caron *et al*., 1997. In a prospective study, Caron *et al*. (1997) evaluated the thyroid condition of 347 pregnant women living in the southwest of France (with an estimated urinary iodine excretion value of 50 µg/day). Iodine concentration in urine samples and serum thyroid hormone measurements were taken at initial

presentation (before 12 weeks of gestation), and during the nine months of pregnancy. Mean urinary iodine levels were low during the first trimester (6.9 ± 0.4 µg/dL), as well as during the ninth month of pregnancy (8.6 ± 0.6 µg/dL). A thyroid ultrasound was performed one to five days after delivery in 246 mothers. During pregnancy fT4 and T3 concentrations decreased ($p<0.001$), and TSH and Tg concentrations increased ($p<0.001$). Thyroid hypertrophy (thyroid volume greater than 18 mL) was present in 29 percent of the mothers. The percentage of thyroid hypertrophy at delivery was associated with urinary iodine concentration during the first trimester of gestation: 15.4 percent (urinary iodine < 5 µg/dL), 9.2 percent (urinary iodine 5-10 µg/dL), and 3.5 percent (urinary iodine > 10 µg/dL) (Figure 4). Goiter (thyroid volume greater than 22 mL) was present in 11 percent of the mothers. The researchers concluded that in areas with a marginally low iodine supply, pregnancy constitutes a goitrogenic stimulus.

Kung *et al*., 2000. In another prospective study, Kung *et al*. (2000) studied 230 pregnant women living in a borderline iodine sufficient area (Hong Kong). The median urine iodine concentration in healthy adults was 0.77 µmol/L (9.8 µg/dL) in Hong Kong, which was close to the World Health Organization cut-off value of 0.79 µmol/L (or 10 µg/dL) for iodine sufficiency. When recruited into the study, all pregnant women were in their first trimester; subjects with a history of thyroid dysfunction were excluded. These women were prospectively studied at approximately 12-14 weeks, 20-24 weeks, and 36 weeks of gestation, as well as 6 weeks and 3 months postpartum for thyroid function, thyroid volume by ultrasound examination, and urine iodine concentration. Study results are presented in Table 8. The investigators showed that in an area of borderline low dietary iodine intake, pregnancy was an important stress to the maternal thyroid axis. Pregnancy caused an average 30 percent increase (range, 3 – 230 percent) in thyroid volume, with some subjects having a more than two-fold increase. This thyroid enlargement persisted and failed to revert completely even 3 months after delivery.

The researchers also reported that 14 women with excessive thyroidal stimulation in the second trimester had lower urine iodine concentrations and larger thyroid volumes throughout pregnancy. Furthermore, their neonates had higher cord TSH, Tg, and slightly higher thyroid volumes than the neonates of 216 pregnant women without evidence of thyroid stimulation. Seven neonates (50 percent) born to these women had subnormal fT4 levels at birth.

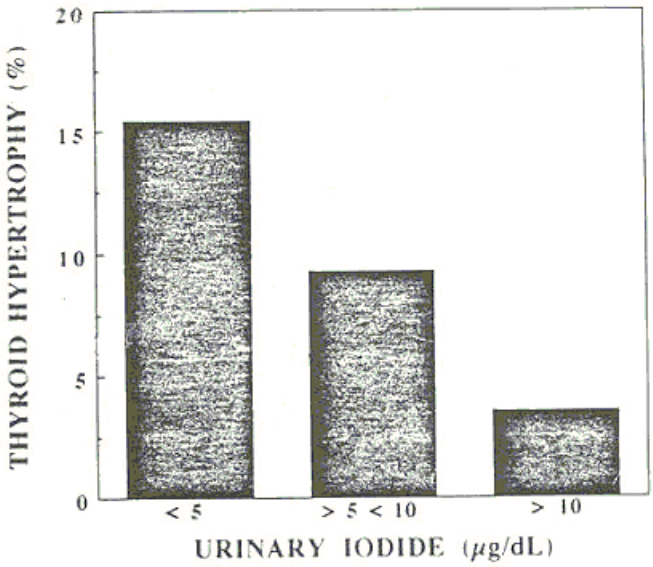


Figure 4. Percentage of Maternal Thyroid Hypertrophy in Relation to Urinary Iodine Concentration during the First Trimester of Pregnancy (Caron *et al*., 1997)

Table 8. Change of Thyroid Function Tests, Thyroidal Volume, and Urinary Iodine Level of Women during and after Pregnancy (Kung *et al*., 2000)

	First trimester	Second trimester	Third trimester	Postpartum 6 weeks	Postpartum 3 months
Total T4 (nmol/L)	154 (132-176)	126 (110-143)[a]	125 (106-142)[a]	89 (81-98)[b]	92 (82-101)[b]
Free T3 (pmol/L)	3.9 (3.6-4.3)	3.4 (3.1-3.7)[a]	3.3 (3.0-3.7)[a]	4.0 (3.7-4.4)	4.3 (4.1-4.6)
Free T4 (pmol/L)	13.4 (12.2-15.0)	11.9 (10.7-13.1)[a]	11.7 (10.1-13.0)[a]	14.5 (13.1-16.0)	14.4 (13.0-15.8)
TSH (mIU/L)	0.49 (0.12-1.00)	0.96 (0.62-1.28)[a]	0.95 (0.60-1.36)[b]	1.15 (0.74-1.58)[b]	1.14 (0.81-1.61)[b]
Urine iodine (µmol/L)	0.84 (0.60-1.09)	0.91 (0.65-1.14)[a]	0.98 (0.72-1.24)[a]	0.83 (0.56-1.08)	0.79 (0.51-1.14)
Thyroid volume (mL)	9.5 (7.2-12.3)	10.3 (7.7-13.6)[a]	11.2 (8.9-13.8)[a]	11.0 (8.3-14.2)[a]	10.6 (8.6-13.7)[a]

Results are medians
[a]$p < 0.05$ vs. first trimester
[b]$p < 0.01$ vs. first trimester

Another source of data supporting the concept that normal pregnancy requires increased thyroid hormone production comes from the observation that women previously diagnosed with hypothyroidism on adequate T4 replacement doses often require an increase in their T4 doses during pregnancy (Table 9).

Table 9. Thyroid Hormone Requirement in Pregnancy (Brent, 1999)

Study	Mean daily dose (µg)	Fraction of women requiring an increased dose	Mean dose increase for those who had an adjustment (µg)
Pekonen *et al*. (1984)	141	7/34 (21%)	62
Mandel *et al*. (1990)	148	9/12 (75%)	46
Tamaki *et al*. (1990)	-	4/4 (100%)	-
Girling and de Swiet (1992)	142	9/32 (28%)	68
Kaplan (1992)	154	27/42 (64%)	42
Pooled data	146	56/124 (45%)	46

Romano *et al*., 1991; Pedersen *et al*., 1993; Glinoer *et al*., 1995. These are three prospective studies showing that in an area with marginal or moderate iodine deficiency, iodine supplementation often can reduce the stress on the thyroid during pregnancy. The first study was carried out in L'Aquila, Italy, an area with moderate iodine deficiency (Romano *et al.*, 1991). There were 35 pregnant women in the study, all of whom had a normal pregnancy and no history of thyroid disease. They had a mean age of 27.1 years (±3.8) and a mean body weight of 61.6 kg (± 4.9) at the first examination during the first trimester. Pregnant women were randomly assigned into group A (n=17) or group B (n=18). Immediately after the first examination, iodide salt equivalent to a daily intake of about 120 to 180 µg iodide was prescribed to all the women in group A. Each trimester all pregnant women in both groups were subjected to three ultrasonographic evaluations of thyroid volume and to measurement of body weight. During each examination, 24-hour urine samples were also taken to determine the iodine urinary excretion. Romano *et al.* (1991) reported that TSH levels of all the subjects were within the normal range and TSH levels measured in group A did not statistically differ from those measured in group B. The effect of iodine supplementation was confirmed by urinary iodine measurements. A significant increase in urinary iodine excretion was found at the second and third examination ($p<0.0001$ and $p<0.01$, respectively, Table 10) only in group A, treated with iodide salt.

Table 10. Iodine Excretion (µg/24 hours) in Both Groups at Each Trimester (mean± standard deviation) (from Romano *et al*., 1991)

	First trimester	Second trimester	Third trimester
Group A	37.0±36.0	154.0±59.0[a]	100.0±39.0[b]
Group B	30.5±42.0	55.0±35.0	50.0±37.0

[a]$p<0.0001$
[b]$p<0.01$ vs. first trimester

Thyroid volume did not change throughout pregnancy in the group treated with iodide salt. However, in the control group (Group B) there was a statistically significant increase in thyroid volume from the first to the third trimester (mean increase = 1.6 ± 0.6 mL; $p<0.0001$). Romano *et al.* (1991) concluded that an adequate dietary iodine intake is necessary to prevent the development of gestational goiter, and iodine deficiency is the main causative factor of thyroid enlargement during pregnancy.

A similar study was also carried out in East Jutland, Denmark, an area with a median daily urinary iodine excretion around 50 µg (Pedersen *et al*., 1993). The researchers selected 54 normal pregnant women and randomly divided them into iodine-treated (28 subjects) and untreated groups (26 subjects). Before iodine supplementation was initiated, the measured variables were nearly identical in the two groups. Treated subjects received 200 µg iodine/day starting from weeks 17-18 of pregnancy until 12 months after delivery. All women were followed at regular intervals during pregnancy. In the control group, serum TSH, serum Tg, and thyroid size showed significant increases during pregnancy. These variations were less in the iodine supplementation group (Figures 5, 6, and 7).

Iodine did not induce significant variations in serum T4, T3 or free T4 in this study. Pedersen *et al.* (1993) concluded that a relatively low iodine intake during pregnancy leads to thyroid stress, with increases in Tg release and thyroid size. It is important to note that even in the iodine-supplement group, there was a significant increase in thyroid volume during pregnancy. Notably, the size of the thyroid returned to initial values one year after delivery independent of iodine supplementation. Pedersen *et al.* (1993) were concerned that thyroidal stress during pregnancy in an area of iodine deficiency can lead to goiter, which is primarily reversible, as was shown in the study. However, at some point iodine deficiency triggers, by an unknown mechanism, irreversible changes in the thyroid with autonomous growth and function and may lead to high incidence of multinodular toxic goiter in elderly subjects. It was suggested that iodine deficiency during pregnancy or even during fetal life could be an important factor for the late development of thyroid autonomy.

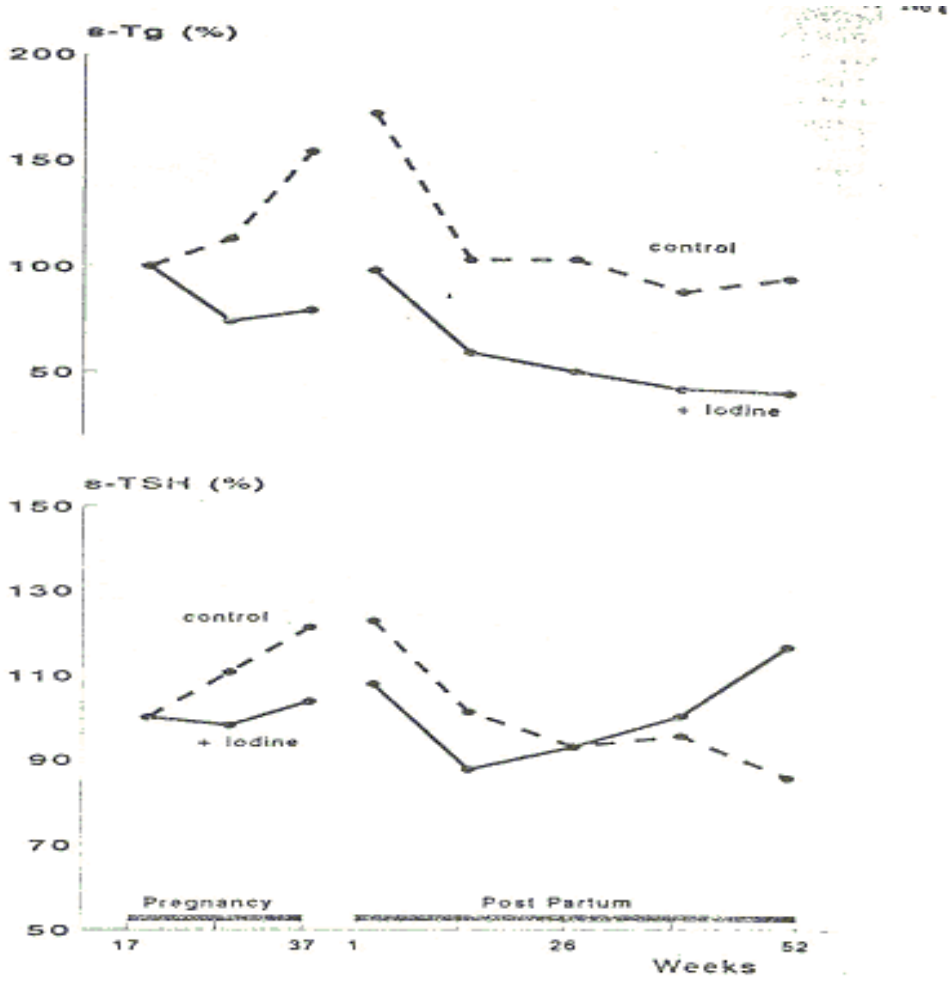


Figure 5. Serum Tg and TSH during Pregnancy and for 52 Weeks Postpartum in Women Receiving Iodine Supplementation and Control Women, as a Percentage of the Initial Values (from Pedersen *et al.*, 1993).
Median values are shown. The increase in serum Tg during pregnancy in the control group was statistically significant ($p<0.01$), but the first value obtained during pregnancy and the values obtained one year after delivery were not different. Tg values from the two groups were significantly different at all periods, except before initiation of iodine supplementation. The increase in serum TSH in the control group during pregnancy was statistically significant ($p<0.01$), whereas no differences between values were found in the iodine supplemented group ($p=0.29$, by Friedman's test). During the postpartum period, no significant TSH differences between the groups were found.

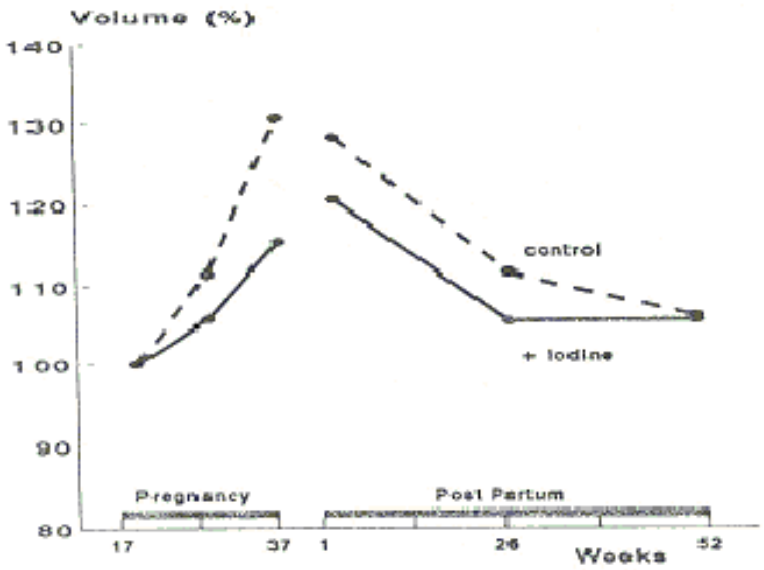


Figure 6. Median Thyroid Volume during Pregnancy and 52 Weeks Postpartum in Women Receiving Iodine Supplementation and Control Women, as Percent of Initial Values (from Pedersen *et al.*, 1993). In both groups, statistically significant increases during pregnancy and decreases during the postpartum period were found ($p<0.05$). The increase during pregnancy in controls was higher than that in the iodine supplemented group ($p<0.05$).

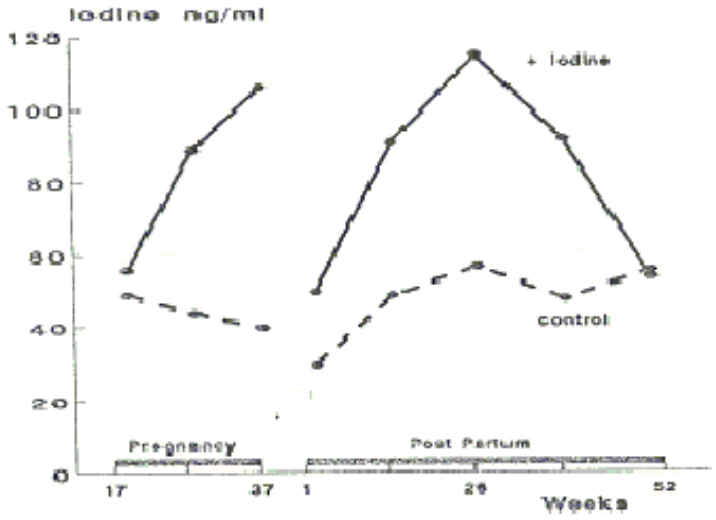


Figure 7. Iodine Concentration in Spot Urine Samples during Pregnancy and for 52 Weeks Postpartum in Women Receiving Iodine Supplementation and Control Women (Pedersen *et al.*, 1993). The last sample was obtained after iodine supplementation was stopped.

Glinoer *et al*. (1995) studied a group of euthyroid pregnant women with mild to moderate iodine deficiency and found pregnancy-related stresses on the thyroid could be prevented by the administration of potassium iodine or potassium iodine plus thyroxine (L-T4). They selected 180 pregnant women at the end of the first trimester on the basis of biochemical criteria of excessive thyroid stimulation, defined as serum thyroglobin > 20 µg/L associated with a low normal fT4 index (<1.23) and/or an increased T3/T4 ratio (>25×10^{-3}). The subjects were randomized in a double-blind protocol into three groups and treated until term with either placebo (Group A), potassium iodine (100 µg/day) (Group B), or potassium iodine (100 µg/day) plus L-T4 (100 µg/day) (Group C). At the beginning of the study, all the subjects were mildly or moderately iodine deficient as indicated by a median urinary iodine concentration of 36 µg/L. Only 10 percent of women had urinary iodine above 80 µg/L. After therapy was instituted, the urinary iodine concentrations in Groups B and C rose to approximately 75-130 µg/L in the second and third trimesters; while urinary iodine of Group A remained low during gestation and at delivery.

Study results showed that total T4 levels of all groups increased during the second and third trimesters compared to those measured during the first trimester. However, the increases observed in Group A (4 percent and 7 percent for the second and third trimesters, respectively) were much smaller than those observed in Group B (9 percent and 11 percent) and Group C (19 percent and 15 percent). Glinoer *et al.* (1995) also reported that in Groups A and B, the ratios of T3/T4 were higher than normal at the start of the therapy and remained elevated during gestation. However, in Group C the ratios decreased rapidly toward normal and were maintained at a level of approximately 22×10^{-3}. These results indicated that thyroid stimulation associated with pregnancy and leading to preferential T3 secretion by the thyroid was suppressed after potassium iodide plus L-T4 administration. Glinoer *et al.* (1995) found an average increase of 30 percent in thyroid volume in Group A. Sixteen percent of the women in this group developed a goiter during gestation, with thyroid volumes up to 34 mL at delivery. The increment in thyroid volume was much less in Group B (mean increase of 15 percent) and in Group C (mean increase of 8 percent). Furthermore, goiter formation in Groups B (10 percent) and C (3 percent) was less frequent than that in Group A. In the same study, Glinoer *et al.* (1995) also evaluated the thyroid status of the newborns, 3-6 days after delivery. They found that the mean thyroid volume of newborns in Group A (1.05 ± 0.05 mL) was significantly larger than those in Groups B (0.76 ± 0.05 mL) and Group C (0.75 ± 0.05 mL). Furthermore, glandular hyperplasia (thyroid volume >1.4 mL) was found in 10 percent of newborns in Group A (range 1.5-2.2 mL) compared to none in Groups B and C ($p=0.01$, by χ^2 test). Glinoer *et al.* (1995) found the study results in agreement with other investigations on goitrogenesis during pregnancy in areas with less than adequate iodine supply.

Rotondi *et al*., 2000. This study found an association between thyroid size and the number of their previous pregnancies in an area with moderate iodine deficiency. The researchers studied the size of thyroids of 208 non-goitrous

healthy females by ultrasound examination. All subjects lived in a region (Naples, Italy) that is known to have moderate iodine deficiency, with usual urinary levels ranging from 40-100 µg/day. All subjects had serum free T3, fT4, and TSH measurements, as well as thyroglobulin antibody and thyroid peroxidase antibody detection. All subjects were clinically and biochemically euthyroid and had no detectable thyroid autoantibodies. The subjects were divided into five groups, according to the number of completed pregnancies (0, 1, 2, 3, 4 or more term pregnancies). The researchers found mean thyroid volume increased progressively among the groups; group 0 (14.8±0.7 mL); group I (16.0±0.9 mL); group II (17.1±0.6 mL); group III (18.2±0.6 mL); group IV (20.3±0.9 mL). The difference in the increases in thyroid volume was statistically significant between group 0 and groups III ($p<0.01$) and IV ($p<0.001$), and also between group I and group IV ($p<0.05$). No independent effect of body weight and age on thyroid volume was seen. Based on the results, Rotondi *et al.* (2000) suggested that, in an area with moderate iodine deficiency, there is a cumulative goitrogenic effect of successive pregnancies and the goitrogenic effect of pregnancy is not fully reversible.

As shown above, several studies have identified associations between pregnancy and increased thyroid size. However, as we review in the following paragraphs, this effect has not been seen in all studies.

Gerghout *et al*., 1994. These researchers studied 10 healthy women before and during a normal pregnancy in an iodine replete area of Amsterdam, the Netherlands. They found no change in thyroid volume during pregnancy (data given before pregnancy and during first, second, and third trimesters, respectively: 10.3 ± 5.1, 10.6 ± 4.4, 9.6 ± 3.8, and 9.4 ± 3.0 mL. Urinary iodine levels or dietary iodine intakes were not reported.

Long *et al*., 1985. These researchers studied a group of pregnant teenagers and found the frequency of goiter in this group was not higher than that in non-pregnant teenagers. They studied 309 consecutive pregnant adolescent girls who were admitted to a medical center in San Diego, California from August 1978 through December 1982. A group of 600 adolescent girls was used as controls to establish the prevalence of goiter in non-pregnant adolescents. The mean gestational age for the first visit was 22 weeks. A thyroid gland was defined as enlarged if it was visible and/or palpable and had a transverse span of $\geq$ 6 cm. Eighteen goiters (6 percent) were identified in the pregnant teenagers versus 27 goiters (5 percent) in the control group. It should be noted that the detection method used in the study is not as sensitive and reliable as the ultrasound detection used in the more recent studies. Long *et al*. (1985) concluded that abnormalities of size and function of the thyroid gland were not more prevalent during the stress of reproduction at a young age.

Levy *et al*., 1980. This study examined the thyroid glands of 49 matched pairs of women in Ohio, one pregnant and one non-pregnant woman per pair. All pregnant women were at least 20 weeks into the pregnancy and had no personal

history of thyroid abnormality. The subjects were paired by race and age (within 5 years) and examined by multiple observers. Observers independently graded each thyroid as “not palpable,” “palpable but not enlarged,” or “enlarged.” They also compared the size of the two glands relative to one another for every pair of subjects. Levy *et al*. (1980) found that in 22 pairs the pregnant woman had the larger thyroid, whereas in 20 pairs the opposite was true. In six pairs the thyroid glands were not palpable, and in one pair the thyroid glands were of equal size. Five pregnant and three nonpregnant women had clinically significant goiters. None of the differences was statistically significant. These results are consistent with the study of Crooks *et al.* (1967) conducted in Reykjavik, Iceland, which showed that pregnancy did not impact the thyroid gland when iodine intake was adequate.

Liberman *et al.*, 1998. This investigation studied the serum T4, TSH, and serum and urinary inorganic iodine levels during the first, second, and third trimesters and after delivery of 16 women. They reported significantly higher levels of mean serum T4 during the pregnancy than after delivery. Similar levels of serum TSH, serum inorganic iodine, and urinary iodine were measured during pregnancy and after delivery. The daily iodine intakes of the subjects were high, as indicated by the relatively high average urinary iodine levels (459 – 786 µg/day). Liberman *et al.* suggested that pregnancy does not have an important influence on serum inorganic iodine or thyroid status in iodine-sufficient regions.

Adverse Neurological Development with In Utero Iodine Deficiency or Low Thyroid Hormone Levels

The changes in thyroid function associated with pregnancy are related to increased hormone requirements. The need can only be met by a proportional increase in hormone production and is dependent upon the availability of iodine in the diet (Glinoer, 2001). The National Academy of Sciences has recommended an Estimated Average Requirement of 160 µg/day and a Recommended Dietary Allowance of 220 µg/day for pregnant women (NAS, 2001). These values are higher than the Estimated Average Requirement of 95 µg/day and the Recommended Dietary Allowance of 150 µg/day for non-pregnant adults (age 19 years and older).

Iodine deficiency disorders range from endemic cretinism to endemic goiter and less severe forms of brain abnormalities. The impact of iodine deficiency differs depending on the age and life stage of the affected individual, as well as the degree of iodine deficiency. The most severe problems caused by iodine deficiency are among fetuses, neonates, and infants because of the irreversible changes that can occur during this period of rapid structural and behavioral development. Cognitive impairment is the most common finding seen with iodine deficiency, and thyroid disorders during pregnancy have been shown to increase the risk of neurologic damage in offspring (Hetzel and Maberly, 1986; as cited in Hollowell and Hannon, 1997). It was considered a paradox that in areas of iodine deficiency, children with cretinism, but with functioning thyroid glands, had

more severe central nervous system damage than some children who were missing a thyroid gland. For prevention of central nervous system damage, iodide has to be supplied before conception or early in the first trimester, a time in development before the fetal thyroid is known to be functional (Hollowell and Hannon, 1997). The finding that maternal T4 reaches the fetus (Vulsma *et al*., 1989) made it understandable that maternal thyroid hormones are necessary for brain development during early fetal development, and severe central nervous system damage can occur as a result of maternal thyroid deficiency.

This theory is supported by the results of a number of animal and human studies.

Animal Studies in Neonates Born to Dams with Iodine Deficiency or Low Thyroid Hormone Levels

Obregon *et al*., 1984 and Woods *et al*. , 1984 and others. Obregon *et al*. (1984) and Woods *et al*. (1984) showed that fetal rat tissues, including brain, contained T4 and T3 before fetal thyroid hormone was produced. Several researchers also reported that nuclear T3 receptors in brain tissues obtained from rat and human fetuses early in gestation (before the development of the fetal thyroid) were relatively saturated with T3 (Bernal and Pekonen, 1984; Perez Castillo *et al*., 1985; Ferreiro *et al*., 1988; as cited in Burrow *et al*., 1994). The presence of occupied T3 nuclear receptors in brain tissues early in fetal development supports a role for maternal thyroid hormones in the maturation of the brain.

Argus Research Laboratories, 1998a and 2001. In two animal developmental studies (discussed in an earlier section), ammonium perchlorate was administered to female Sprague-Dawley rats via drinking water at target doses between 0.01 and 30 mg/kg-day. Morphometric analysis of the pups revealed significant changes in sizes of a number of brain regions (e.g., corpus callosum), although a simple dose-response relationship was not observed for any of the changes (Figure 11).

Potter *et al*., 1982 and Hetzel *et al*., 1987. Severe iodine deficiency has been shown to cause abnormal fetal brain development in a number of animal species. Potter *et al*. (1982) reported that severe iodine deficiency in sheep caused reduction in fetal brain weights and in brain DNA and protein from 70 days of gestation to parturition. They also found unusual morphological changes in both the cerebral hemispheres and the cerebellum of the fetal brains. Hetzel *et al*. (1987) reported that severe iodine deficiency caused abnormal fetal brain development in rat, marmoset, and sheep. The abnormalities included reduced brain weight, change in cell density in the cerebral hemispheres, reduced synaptic counts in the visual cortex, and reductions of brain DNA and brain protein.

Lavado-Autric *et al*., 2003. In this study, the investigators evaluated the effects of a low iodine diet (LID) with (LID-2) or without (LID-1) 1 percent potassium perchlorate in pregnant rats. The potassium perchlorate was used to produce hypothyroxinemia in the pregnant rats. Cell migration and cytoarchitecture in the somatosensory cortex and hippocampus of the 40-day-old offspring were examined (n = 5-7 pups per group). The number of dams per group was not provided. According to the authors, the reproductive performance of the LID-2 animals and the post-natal growth of their pups were normal (although no details are provided). Serum T4 levels were 90 percent lower in both the LID-1 and LID-2 dams than in controls. Levels of serum T3 (the hormonally active form of thyroid hormone) in the LID-1 and control dams were similar. The mean T3 in the LID-2 dams was lower than controls, although the difference was much less than that seen for T4 (mean serum T3 (in ng/mL) in controls: 0.73 ± 0.02 (standard error); in LID-1 animals: 0.65 ± 0.15; in LID-2 animals: 0.37 ± 0.06). Litter size, body weight or postnatal growth measures were not affected.

Using BrdU labeling of cells at specific time points of development, the authors took advantage of the normal migration patterns of cortical neuronal cells to investigate the impacts of thyroid deficiencies on normal structural brain development. Normally, cells born later migrate past cells born earlier and occupy more superficial layers of the cortex. When BrdU was administered on gestation days 14-16, there was a decrease in the proportion of BrdU-labeled cells in the deeper cortical layers and an increase in the subcortical white matter in the LID-1 and -2 offspring compared to controls. The researchers noted that gestation days 14-16 are before the fetal thyroid begins producing thyroid hormone (which usually starts around day 17.5-18). As such, these effects are likely due to deficiencies in maternal thyroid hormones rather than any deficiency of the fetal thyroid. When BrdU was administered on gestation days 17-19, there was a decrease in labeled cells in the superficial layers and an increase in the deeper layers of the cortex. The researchers used single- and double-label immunostaining to find that the BrdU-labeled cells were neurons, not glia.

Overall, these findings provide evidence that the normal pattern of neuronal cell migration in the cortex during fetal development can be disrupted by maternal hypothyroxinemia. In addition, since these effects were assessed in the offspring at postnatal day 40, these findings likely represent permanent rather than temporary alterations to the cortical cytoarchitecture (Zoeller, 2003). This study was different from earlier studies because the level of maternal hypothyroidism introduced was relatively mild and treated pups or dams could not be distinguished from controls by their weight, growth, reproductive performance, or physical appearance. In previous studies, severe hypothyroidism was introduced in the dams of pups by surgical thyroidectomy or strong goitrogens such as high dose methimazole.

The results of this study could potentially provide some mechanistic explanation for the findings in human studies which have linked decreases in maternal thyroid hormone during pregnancy to subsequent altered neurological development in

the offspring (e.g., Pop *et al*., 1999, 2003; Haddow *et al*. 1999). Migration defects in the brain have been associated with neurological deficits in humans (Sun *et al*., 2002). However, it is not currently known how the particular effects seen in this study might impact long-term neurological function.

Auso *et al*., 2004. Normal rat dams were given the goitrogens 2-mercapto-1-methylimidazole on gestation days 12 through 15. Maternal thyroid hormone levels decreased transiently by about 30 percent compared to normal values. There were no clinical signs of hypothyroidism. Mean T4 ($\pm$ SEM) in the treated and control dams was 11.60 $\pm$ 0.67 and 15.90 $\pm$ 1.89, respectively. BrdU was injected from gestation days 14-16 or 17-19 and pups were tested for audiogenic seizure susceptibility 39 days after birth. The cytoarchitecture and radial distribution of the BrdU-labeled neurons on postnatal day 40 were affected in 83 percent of the pups from the treated dams. Infusion of T4 at gestation days 13-15, but not during days 15-18 avoided these alterations. An increase in seizures and wild runs in response to acoustic stimulus was seen in the pups from treated dams versus controls. These data provide evidence that transient and relatively mild decreases in maternal T4 during early pregnancy can lead to permanent architectural changes in the brain.

van Wijk *et al*., 2008. This report describes how a lack of thyroid hormone during early development can result in multiple morphological and functional alterations in the developing brain of Wistar (HsdCpb:WU) rats. The behavioral effects of perinatal and chronic hypothyroidism during development in offspring (male and female) of hypothyroid rats were assessed. Twelve dams (starting 2 weeks prior to mating) and offspring (one litter per dam, eight pups per litter) were fed an iodine-poor diet and drinking water with 0.75 percent sodium perchlorate. This continued either until weaning (perinatal hypothyroidism) or until the day of killing (chronic hypothyroidism). The pups were tested for neuromotor competence, locomotor activity and cognitive function until postnatal day 71, comparing them to age-matched control rats. Early neuromotor competence, as assessed in the grip test and balance beam test, was impaired in both chronic and perinatal hypothyroidism groups. The open field test, assessing locomotor activity, revealed hyperactive locomotor behavioral patterns only in the chronic hypothyroid animals. The Morris water maze test assessed cognitive performance and showed that chronic hypothyroidism affected spatial memory in a negative manner, with perinatal hypothyroidism impairing spatial memory in female rats only. Overall, the effects of chronic hypothyroidism appeared to be more pronounced than the effects of perinatal hypothyroidism. This suggests that the early effects of hypothyroidism on functional alterations of the developing brain depend on the timing of the thyroid hormone deficiency during development and the impacts may be decreased, but not eliminated, if the deficiency is improved.

Gilbert and Sui, 2008. These investigators exposed 106 pregnant Long-Evans rats to 0, 30, 300, or 1,000 ppm perchlorate in drinking water (equivalent to 0, 4.5, 44.2, or 140.3 mg/kg-day) from gestation day 6 until weaning (postnatal day

30). Adult male offspring from an unstated number of litters/group were studied with a series of behavioral tasks. These included motor activity as a general test of neurotoxicity (8-11/group), the Morris maze as a spatial learning test (11-17/group), and fear conditioning as a reflection of the integrity of the hippocampus (number of animals unstated). The authors also utilized neurophysiologic measures of synaptic function in the hippocampus including long-term potentiation (LTP), a well-established model of synaptic plasticity (14-17/group). There was no positive control. In the dams, T4 was reduced relative to controls by 16 percent, 28 percent, and 60 percent in the 30, 300, and 1,000 ppm dose groups, respectively. Little change was seen in T3 across dose groups and TSH levels were only increased in the highest dose group (1,000 ppm). Perchlorate dose was not associated with body weight in the dams or pups, or with pup eye opening, brain or hippocampal weights.

In the pups, small decreases (i.e., 10-20 percent) in serum T3 and T4 were seen in the two higher dose groups compared to controls on postnatal day 21. However, all serum hormone levels returned to control levels in adulthood. Perchlorate exposure did not affect motor activity, spatial learning, or fear conditioning in the male offspring at ages 3-13 months. Significant reductions in baseline synaptic transmission were observed in hippocampal field potentials at all dose levels. This included reductions in synaptic transmission at the perchlorate dose that only marginally reduced (about 16 percent) circulating levels of thyroid hormone in dams (30 ppm, 4.5 mg/kg/day).

The Morris water maze failed to uncover spatial learning deficits in perchlorate-treated animals despite the observations of altered hippocampal synaptic transmission coupled with spatial learning impairments after thyroid hormone disruption induced by propylthiouracil (PTU) or methimazole noted in this report and in contemporary studies in perchlorate treated animals. Perhaps these differences are related to sex. This study examined only male offspring, while in the van Wijk *et al.* (2008) study discussed above, spatial memory effects were only seen in females. It may also be related to differences in the mechanism of toxicity or dosimetry compared to PTU and methimazole.

The lack of behavioral effects in the specific tasks used by Gilbert and Sui (2008) may also be a function of dose, degree of hormonal disruption, or the duration of prenatal exposure. Given the cognitive demands and sensitivity of the behavioral tasks, such outcomes are understandable. The failure of perchlorate to detrimentally impact hippocampal LTP is consistent with a lack of effect on behavioral plasticity and it is possible that the augmentation of PS (population spike) LTP is a reflection of an adaptive or compensatory response in cell physiology that aids in the reversal of learning deficits. Also, many other brain regions are engaged in the performance of simple learning tasks, and significant behavioral compensation may mask underlying behavioral deficits apparent earlier in development or revealed with more demanding cognitive tasks. Despite the lack of behavioral effects in the specific tasks used, the changes seen in synaptic transmission in adult offspring nevertheless provide evidence in

a rodent model that modest degrees of thyroid hormone reduction induced by perchlorate result in persistent alterations in brain function.

Human Studies of Infants Born to Mothers with Iodine Deficiency or Low Thyroid Hormone Levels

Many human studies have been published that demonstrate maternal thyroid deficiency during pregnancy affects neuropsychological development of the child. Some of the studies have shown that these effects may occur at thyroid hormone levels that have been traditionally considered to be within normal ranges.

Man and Jones, 1969. Man and Jones first reported that maternal hypothyroidism was associated with lower intelligence quotient scores (IQs) in 8-month-old infants. Hypothyroidism was defined in this study by two low serum butanol-extractable iodine test values during pregnancy or by one low serum butanol-extractable iodine value with clinical hypothyroidism. They found that 81 percent of 26 infants of women given thyroid replacement therapy after two low serum iodine tests were classified "normal," approximately the same percentage as for infants of euthyroid women. In contrast, only 48 percent of the 56 infants of women with two low serum iodine values who were not given adequate thyroid replacement therapy were "normal."

Glorieux *et al*., 1985. These authors reported that children with significantly retarded skeletal maturation at the time of diagnosis, signifying hypothyroidism *in utero*, obtained lower global IQs than did children whose skeletal maturity was within normal limits.

Glorieux *et al*., 1988. In a later study, Glorieux *et al*. studied 43 infants with congenital hypothyroidism and found that low T4 (<2 µg/dL) and retarded bone surface (<0.05 cm^2) measurements taken before therapy initiation were strongly correlated with mental development at 3, 5, and 7 years of age (Table 11).

Table 11. Mental Outcome in Infants with Congenital Hypothyroidism Relative to Newborn Risk Criteria (Glorieux *et al*., 1988)

Age in years	T4 < 2 µg/dL and bone surface measures < 0.05 cm^2			T4 > 2 µg/dL and/or bone surface measures > 0.05 cm^2		
	n	Mean IQ	IQ distribution	n	Mean IQ	IQ distribution
3	17	91 ± 4[a]	(61 – 120)	40	103 ± 2	(81 – 140)
5	14	88 ± 3[b]	(60 – 109)	30	104 ± 2	(84 – 125)
7	16	86 ± 3[b]	(49 – 98)	27	102 ± 2	(75 – 128)

[a]$p < 0.01$ [b]$p < 0.001$

Rovet *et al*., 1987. Similar findings have been reported by Rovet *et al*., who studied intellectual and behavioral characteristics at 1, 2, 3, 4, and 5 years of age of 23 boys and 57 girls with congenital hypothyroidism. The children were

assigned to two groups based on degree of skeletal maturity at the time of diagnosis. Forty-five children with bone age <36 weeks were assigned to the delayed group; 35 with bone age 37 to term were assigned to the nondelayed group. Both groups were treated for congenital hypothyroidism and the initial starting dosages of L-thyroxine for the delayed and nondelayed were similar, 8.1 mg/kg and 7.8 mg/kg, respectively. Although most children with athyrosis were found in the delayed group, the group did not differ in birth weight, hormone levels, or family background. Hormone levels at diagnosis of both groups are shown in Table 12. Tests showed that although children in the delayed group performed within the normal range, their scores were significantly lower than those of the nondelayed group from age 2 years on. Perceptual-motor, visuospatial, and language areas were most affected.

Table 12. Hormone Levels at Diagnosis in Children with Delayed and Nondelayed Skeletal Maturity (Rovet *et al*., 1987)

	Delayed (n = 45)	**Nondelayed (n = 35)**
	TSH (U/dL)	
Screening	136.1 ± 128.8	130.6 ± 78.6
Confirmation	112.5 ± 119.2	131.9 ± 100.5
	Thyroxine (T4) (µg/dL)	
Confirmation	5.1 ± 4.7	5.5 ± 3.9
1 month	11.0 ± 5.3	10.3 ± 5.7
3 months	12.0 ± 4.5	13.5 ± 3.9
6 months	13.6 ± 2.8	12.6 ± 3.2
9 months	12.4 ± 3.5	14.1 ± 5.3
12 months	12.7 ± 2.7	13.5 ± 2.3

Values represent mean ± standard deviation.

Vermiglio *et al*., 1990. This study demonstrated that normal euthyroid children born to mothers from severe (area A) and less severe (area B) iodine deficiency regions in northeastern Sicily have a defective visual perceptual integrative motor ability. They studied 719 primary schoolchildren (366 males and 353 females) from these areas ranging from ages 6 to 12 years old (conceived and born between 1975 and 1981). A control group consisted of 370 age-matched schoolchildren from an iodine-sufficient area where rates of goiter were lower (area C). The prevalence of goiter in the schoolchildren of these areas and the daily urinary iodine excretion in the general population between 1976 and 1984 are given in Table 13.

Table 13. Prevalence of Goiter in Schoolchildren and Daily Urinary Iodine Excretion in Adults (1976-1984) in the Study Areas (Vermiglio *et al*., 1990)

Study area	Total population	Prevalence of goiter in the schoolchildren (%)	Daily urinary iodine excretion (µg/day)[a]
Area A (with endemic cretinism)	7,432	70.3 (708)	24.3 ± 16.4 (55)
Area B (without endemic cretinism)	10,992	45.9 (763)	31.3 ± 18.7 (150)
Area C (control area)	9,730	8.9 (370)	82.4 ± 43.0 (30)

[a]Mean ± standard deviation; the number of observations is given in parentheses.

Variable degrees of thyroid enlargement were found in 205 of the 719 (28.5 percent) children included in the study from areas A and B (area A: 30.4 percent, visible goiter 15.2 percent; area B: 26.5 percent, visible goiter 16.3 percent). Furthermore, defective visual perceptual integrative motor ability (the Bender Gestalt test) was significantly higher in children from area A (14.4 percent) and area B (13.1 percent) than in those from area C (3.5 percent) (Table 14).

Table 14. Number of Defective, Borderline, and Nondefective Schoolchildren as Assessed by the Bender Gestalt Test (Vermiglio *et al*., 1990)

Performance on Bender[a]	Area A	Area B	Area A+B	Area C
Defective	53 (14.4)	46 (13.1)	99 (13.8)	13 (3.5)
Borderline	57 (15.5)	67 (19.1)	124 (17.2)	14 (3.8)
Nondefective	258 (70.1)	238 (67.8)	496 (69.0)	343 (92.7)
Total	368 (100)	351 (100)	719 (100)	370 (100)

Percent in parentheses.
[a]Performance scores: Defective = below -1 standard deviation from average score of normal children of the same age; Borderline = –1 standard deviation from average score; Nondefective = higher than –1 standard deviation from average score.

The statistical comparisons for the Bender Gestalt Test scores across the different areas were as follows:

Defective:

Area A vs. area B: Chi-square (χ^2) =2.75; p=0.87

Areas A+B vs. area C: χ^2 =36.25; p<0.000001

Borderline:

Area A vs. area B: χ^2 =1.22; p=0.27;

Areas A+B vs. area C: χ^2 =77.55; p<0.000001

Vermiglio *et al*. (1990) also reported higher frequency of neuromuscular and neurosensorial abnormalities among children from areas A and B (a combined overall prevalence of 18.9 percent) compared to those from area C. The Terman Merrill test of general intellectual aptitude was administered to 96 of the 99 "defective" children and 62 of the 124 borderline children from both areas A and B (Table 14). Ninety-one of the 96 "defective" children (94.8 percent) had IQs lower than 90, as did 35 of the 62 borderline (56.4 percent) children (Table 15).

Table 15. Performance on the Terman Merrill Test of General Intellectual Aptitude Administered to Schoolchildren with Defective or Borderline Performance Scores on the Bender Gestalt Test (Vermiglio *et al*., 1990)

Performance on Bender test	Intelligence Quotient Score		
	< 90	**90-95**	**96-100**
Defective (n = 96)	91	5	0
Borderline (n = 62)	35	23	4
Nondefective (n = 12)	0	10	2

Statistical analysis: χ^2 =52.1; p<0.0000005.

Despite the adverse effects described above, the serum T3 and T4 levels of the children from area A and area B were within the normal range. This suggests that serum T3 and T4 are not completely accurate indicators of the neurological damages that may be caused by iodine deficiency.

Tillotson *et al*., 1994. These authors reported the results of a prospective study of psychological outcomes of 361 children with congenital hypothyroidism after five years of treatment and follow-up. They also selected 315 children as controls, matched for school attended, sex, age (within three months), language spoken at home, and social class defined by occupation of the family breadwinner. The severity of the hypothyroidism was assessed using T4 measurements collected at the time of diagnosis (median age 17 days; range 0-114). The study showed that among children with congenital hypothyroidism and given early treatment, those with plasma T4 concentrations of less than 42.8 nmol/L (3.3 µg/dL) at the time of diagnosis had a global deficit in mean IQ of 10 points, while those with higher T4 levels at the time of diagnosis had no deficit.

Bleichrodt and Born, 1994. These authors performed a meta-analysis on 18 studies of iodine deficiency and mental development. Studies included those with information on the general cognitive functioning of children and adults living in iodine-deficient areas and provided the necessary statistical data. Three studies were excluded from the analysis because the composition of the groups studied was different (they were composed exclusively of school children). In the meta-analysis of the effects of iodine deficiency on cognitive development, a large effect size was found with a d-value of 0.90. This means that the mean scores for the two groups (the iodine-deficient group and the non-iodine-deficient group) were 0.90 of a standard deviation (or 13.5 IQ points) apart.

Pop *et al*., 1999. Pop *et al*. (1999) reported that low maternal fT4 concentrations in apparently healthy women during early gestation are associated with an increased risk of impaired neurodevelopment in the infant. They studied a group of 291 pregnant women in an iodine-sufficient area (in and around the city of Veldhoven, Netherlands) between January and November, 1994. No women in the study group were receiving antithyroid drugs or thyroid hormones. Maternal fT4, TSH, and thyroid peroxidase antibodies were assessed at 12 and 32 weeks' gestation, and neurodevelopment of 220 healthy children was assessed at 10 months of age. The authors found that children of women with fT4 levels below the 5th (<9.8 pmol/L, n=11) and 10th (<10.4 pmol/L, n=22) percentiles at 12 weeks' gestation had significantly lower scores on the Bayley Psychomotor Developmental Index (PDI) scale at 10 months of age than children of mothers with higher fT4 values. These findings are shown in Table 16. The mean of PDI scores in all subjects was 100, so the decreases seen here represent about a 7-14 percent decrease. The unadjusted odds ratio for impaired psychomotor development (defined as a one standard deviation decrease from the mean) for fT4 in the lower 10th percentile was 3.6 (95% CI, 1.1-12.1). This rose to 5.8 (95% CI, 1.3-12.6) following adjustment for alcohol use, anti-thyroid antibodies, depression, education and other factors.

Although the mean fT4 value for subjects in the lower 10th percentile of fT4 is not given (only the percentile cut-off points are provided), they can be estimated from Table 1 and Figure 2 of the paper. The mean fT4 for subjects in the lower 10th percentile is approximately 9.8 pmol/L compared to a mean fT4 of a little over 13.1 in the remaining subjects. This represents a difference of about 25 percent. Thus, a 25 percent lower maternal fT4 was associated with about a 7 percent decrease in PDI scores in children.

Evidence of a linear association between maternal fT4 and child PDI scores was seen in those children with maternal fT4 levels in the lower 10th percentile ($r = 0.46$, $p = 0.03$). No correlation was found between maternal thyroid hormone levels at 32 weeks' gestation and PDI scores. All children had normal T4 and TSH values. Six of the 22 women with fT4 values in the lower 10th percentile had high levels of anti-thyroid antibodies.

Table 16. Maternal fT4 Levels at 12 Weeks Gestation and PDI Scores in Children at 10 Months of Age (Pop *et al*., 1999)

Low Maternal fT4 Group[a]	Difference in PDI scores	95% CI
Lower 5th percentile (n = 11)	14.1	5.9-22
Lower 10th percentile (n = 11)	7.4	1.1-13.9

[a] These groups were compared to all the children with higher maternal fT4 levels.

Haddow *et al*., 1999. This study involved measurements of TSH, fT4, and T4 in 25,216 pregnant women in Maine at 17 weeks of gestation. Three subgroups of women were selected from this large cohort: 47 women with TSH levels in the upper 99.7th percentile, 15 women with TSH levels in the upper 98th to 99.6th percentile, and 124 women with TSH levels below the upper 98th percentile (the "controls"). Measurements of thyroid function of the women in the study are shown in Table 17. Notably, the fT4 and T4 values of many of the women in the high TSH groups are within normal reference ranges. The researchers then administered 15 neuropsychological tests to the children of these women at ages seven to nine years old. The tests included assessment of intelligence, attention, language, reading ability, school performance, and visual-motor performance. The staff giving the tests did not know whether the children's mothers were women with hypothyroidism or control women. They found that the children of the 62 women with high serum TSH concentrations (all those above the 98th percentile) performed less well on all 15 tests. Mean IQ scores, as measured by the Wechsler Intelligence Scale for Children, were 4 points lower in the children of women with high TSH levels compared to the children of the control mothers (103 versus 107, $p = 0.06$). Of the 62 women with elevated TSH levels during pregnancy, 48 were not treated for the condition during the pregnancy. The full-scale IQ scores of their children averaged 7 points lower than those of the 124 matched control children (100 versus 107, $p = 0.005$). Results were controlled for education, maternal age, sampling time, sample storage time, and gender. The effect size seen in this study is similar in magnitude to that seen in Pop *et al*. (1999). That is, a difference in maternal T4 or fT4 of about 25 percent was associated with about a 4-7 percent decrease in IQ.

Sixty-four percent of the women with high TSH levels during pregnancy went on to be diagnosed with clinical hypothyroidism over the next 10 years. None of the children were diagnosed as hypothyroid as newborns. Haddow *et al*. (1999) concluded that even mild and probably asymptomatic hypothyroidism in pregnant women can adversely affect their children's subsequent performance on neuropsychological tests.

Table 17. Measurements of Thyroid Function in the Study Women during Pregnancy (Haddow *et al*., 1999)[a]

Variable	Hypothyroidism (n = 62)	Controls (n = 124)
Serum TSH (mU/L)	13.2 ± 0.3[b]	1.4 ± 0.2
Serum T4 (µg/dL)	7.4 ± 0.1[b] (95.2 nmol/L)	10.6 ± 0.1 (136.4 nmol/L)
Serum fT4 level (ng/dL)	0.71 ± 0.1[b] (9.1 pmol/L)	0.97 ± 0.07 (12.5 pmol/L)

[a]Values are geometric means ± the logarithmic standard deviation.
[b]$p<0.001$ for the comparison with the control women.

Pop *et al*., 2003. In another study, Pop *et al.* (2003) reported that a low maternal fT4 during early pregnancy was associated with a delay in infant neurodevelopment. In this study, the researchers followed 115 children and their mothers for two years. Maternal levels of fT4 and TSH were assessed at 12, 24, and 32 weeks of gestation. "Cases" (n = 57) were defined as children of mothers who had fT4 levels in the lower 10th percentile at 12 weeks of gestation and "controls" (n = 58) were defined as children of mothers who had fT4 levels in the upper 50th to 90th percentiles at 12 weeks gestation. Mothers of cases and controls were matched on parity and gravidity. Cases and control families were similar with respect to education, breast feeding, smoking, alcohol use, and income. Mothers with thyroid disease, depression, and TSH levels outside of normal ranges were excluded. Child mental and motor functions were assessed using the Bayley Scales of Infant Development at ages 1 and 2. The results are shown in Table 18. Case children scored 8-10 points lower on the mental and motor scales than control children.

Table 18. Mental and Motor Scale Scores (± Standard Deviation) in Children of Mothers with Low (Cases) and High (Controls) Levels of fT4 at 12 Weeks of Gestation (Pop *et al*., 2003)

Age	Cases	Controls	Difference (95% CI)	p-value
One year				
Mental score	95 ± 15	105 ± 14	10 (4-16)	0.004
Motor score	91 ± 15	99 ± 14	8 (3-12)	0.02
Two years				
Mental score	98 ± 15	106 ± 14	8 (4-12)	0.02
Motor score	92 ± 16	102 ± 16	10 (6-16)	0.005

All children had normal Apgar scores at birth and normal screening results for congenital hypothyroidism on the seventh postpartum day. Pop *et al*. (2003) observed that children of women who had low fT4 levels during early gestation and who exhibited a further decrease of fT4 during gestation had the lowest mental and motor scores. In contrast, children whose mothers had a low fT4 at 12 weeks gestation, but whose fT4 levels increased during later gestation, did not show any delay in development. Maternal fT4 levels at 24 and 32 weeks gestation were not associated with decreases in childhood motor or mental scores.

The mean fT4 levels at 12 weeks of gestation was 11.5 pmol/l in the case mothers and 17.0 pmol/l in the control mothers (estimated from Figure 2 in Pop *et al*., 2003), about a 32 percent difference. Thus, a 32 percent difference in fT4 was associated with an 8-10 point drop in mental and motor scores. This is about the same magnitude of effect as seen in Pop *et al*. (1999) and Haddow *et al*. (1999). Importantly, the cut-off point used to define cases (the 10th percentile)

is above the level traditionally used to define low fT4 levels (the 2.5th or 5th percentiles), thus many of the cases had maternal values of fT4 that would traditionally be defined as normal. Figure 3 of Pop *et al*. (2003) presents a scatter plot of maternal fT4 values and childhood mental and motor scores in the case children at 2 years of age. Evidence of a linear relationship is present for both mental scores ($r^2 = 0.13$, $p = 0.006$) and psychomotor scores ($r^2 = 0.23$, $p = 0.001$). These r^2 values suggest that maternal fT4 accounts for a statistically significant fraction of the total variance in these scores.

Klein *et al*., 2001. In a follow-up investigation, Klein *et al*. (2001) studied serum TSH concentrations of pregnant mothers at a mean of 17 weeks gestation and the standard neuropsychological testing results of their offspring at a mean age of 8 years. They found an inverse correlation between the severity of maternal hypothyroidism and IQs in the offspring. The researchers divided the mothers and their offspring into three groups: group 1, 124 control mothers with TSH concentrations <98th percentile; group 2, 28 mothers with TSH concentrations between the 98th and 99.85th percentile; group 3, 20 mothers with TSH concentrations ≥99.85th percentile. Mothers treated for hypothyroidism during pregnancy were excluded from the study. The mean neuropsychological test score (± standard deviation) for the children of the 124 control mothers was 107 (± 12). Means (and standard deviations) for the children in groups 2 and 3 were 102 (± 15, $p > 0.05$ compared to group 1) and 97 (± 14, $p = 0.003$ compared to group 1), respectively. The incidences of IQs greater than one standard deviation below the control mean were 15 percent, 21 percent, and 50 percent for the children in group 1, group 2, and group 3, respectively. In a related study, the same authors also reported spontaneous abortions and intra-uterine fetal deaths were more than five times as common in the mothers with TSH concentrations above the 98th percentile than in control mothers with TSH concentrations below the 98th percentile.

Kooistra *et al*., 2006. This study involved the children of 108 pregnant women who had fT4 values below the 10th percentile at 12 weeks gestation ("cases") and 96 pregnant women who had fT4 values in the 50th to 90th percentiles at 12 weeks gestation ("controls"). People with clinical disease were excluded. Case and control mothers were matched on parity and gravidity. Newborn development was assessed at 3 weeks of age using the Neonatal Behavioral Assessment Scale. Mean thyroid hormone levels in the cases and controls are shown in Table 19.

Table 19. Mean (± Standard Deviation) Maternal fT4 and TSH, and the Proportion with Anti-thyroid Antibodies at 12 Weeks Gestation (Kooistra *et al*., 2006)

	Cases	Controls	p-value
fT4 (pmol/L)	11.4 ± 1.0	17.0 ± 0.9	< 0.001
TSH (mIU/L)	1.6 ± 1.0	1.1 ± 0.8	< 0.001
Anti-thyroid Antibodies	16.7%	4.2%	< 0.01

A statistically significant difference between case and control children was only seen in the orientation scores. In the linear regression analysis with orientation score as the dependent variable, a statistically significant association was seen for case status ($\beta = 0.173$, $p = 0.02$). The researchers also performed a logistic regression analysis where a "low orientation score" was used as the dependent variable. This was defined as subjects who had scores less than one standard deviation below the mean score. The odds ratio for a low score in controls versus cases was 0.17 (95% CI, 0.05 - 0.51). This was adjusted for maternal smoking, alcohol use, gestational age, depression, gender of the child, and maternal education. No difference was seen between case and control maternal fT4 or TSH during the 2nd or 3rd trimester or in the newborns' heel stick T4. No associations were seen between Neonatal Behavioral Assessment Scale scores and TSH or fT4 later in pregnancy.

This study is different from some of the other studies discussed above because the children were very young at the time their cognitive status was assessed. The authors argue that this is an advantage because it limits the impact of external socioeconomic factors that can affect a child's cognitive development as they age. The authors also note a potential major disadvantage: a test given this early may not be predictive of development later in life.

Vermiglio *et al*., 2004. This was a 10-year prospective study which included 16 healthy women and their offspring from a moderately iodine deficient area (area A, mean urinary iodine levels = 48.1 µg/day) and 11 healthy control women and their offspring from a marginally iodine sufficient area (area B, mean urinary iodine levels = 95.2 µg/day) in Northeastern Sicily. Maternal levels of thyroid hormones were assessed at 8, 13, and 20 weeks of gestation. In the offspring, IQ scores and tests for attention deficit and hyperactivity disorder (ADHD) were done at ages 8-10 years. IQ scores were obtained using the Wechsler Intelligence Scale for Children, 3rd Edition, and tests for ADHD were derived from the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition.

All children were euthyroid at delivery, at ages 18-36 months, and at ages 8-10 years. ADHD was diagnosed in 11 of 16 children from the low iodine area and in none of the 11 children from the moderate iodine area. The mean IQ score in the children from the low iodine area was 18 points lower than the mean score in the

children from the high iodine area (92.1 ± 7.8 versus 110 ± 10, $p < 0.00005$). Maternal fT4, T4, and TSH levels at 8, 13, and 20 weeks were presented graphically and showed that fT4 and T4 levels were roughly 10-20 percent lower for those children from the low iodine area (A) compared to children from the moderate iodine area (B). Eight of the 16 women from the low iodine area had fT4 values that were lower than normal for the gestational week. Seven of these eight gave birth to 8 of the 11 children subsequently diagnosed with ADHD. Only one woman from the control area had a gestational fT4 value outside of normal ranges.

When all children were grouped together, a strong correlation was seen between the child's IQ score and maternal fT4 ($r = 0.56$, $p < 0.005$) and TSH ($r = -0.63$, $p < 0.001$). These results and the graph of these data (Figure 3 in Vermiglio *et al*., 2004) suggest that the relationship between T4 and IQ extends into the normal ranges of T4.

Bath *et al.,* 2013. In this study, mother-child pairs (n=958) in the Avon Longitudinal Study of Parents and Children in southwest England were used to evaluate the relationship between maternal urinary iodine concentrations measured in the first trimester of pregnancy (median 10 weeks, collected in 1991-92) and the IQ (abbreviated form of the Wechsler Intelligence Scale for Children) and reading ability (Neale Analysis of Reading Ability) of the offspring measured at ages 8 and 9 years. The odds ratio for having a verbal IQ score in the lower quartile in those whose mothers had a urinary iodine to creatinine ratio of <150 µg/g, compared to those whose mothers had a urinary iodine to creatinine ratio of ≥150 µg/g, was 1.58 (95% CI, 1.09-2.30, p=0.02). Corresponding odds ratios were 1.69 (95% CI, 1.15-2.49) for reading accuracy and 1.54 (95% CI, 1.06-2.23) for reading comprehension. Evidence of dose-response trends were seen when subjects were divided into three iodine-creatinine ratio groups. The odds ratios for performance IQ (odds ratio = 1.22; 95% CI, 0.86-1.72) and words read per minute (odds ratio = 1.20; 95% CI, 0.83-1.74) were above 1.0, but not statistically significant. Adjustments for over 20 different potential confounding variables including maternal age, parenting score (a seven factor measure of cognitive stimulation at age 6 months), gender, birth weight, breastfeeding, smoking, alcohol consumption, parental education, and housing status had little impact on most results. Strengths include the longitudinal design, the fairly large sample size, and availability of data on a large number of potential confounders. Potential weaknesses include the possibility that despite the large amount of data on potential confounders, it is still possible that some important determinants of child cognition were not accounted for. This seems unlikely given the fact that odds ratios changed only slightly with the many adjustments that were done. Another potential weakness was the use of a single measurement of urinary iodine to represent long-term iodine status, although this would most likely bias results to the null. Overall, these data provide some evidence that relatively low iodine status during pregnancy can impact the child's cognitive development.

Other studies: Not all studies have found associations between fetal hypothyroidism and impaired brain development. Several studies examined children exposed to antithyroid drugs such as carbimazole, propylthiouracil, or thiamazole *in utero* and did not find an association between the treatment and the later intellectual and somatic development of the children (McCarrol *et al*., 1976; Burrow *et al*., 1978; Messer *et al*., 1990). The statistical power of these studies may be limited since they had relatively small sample sizes and the dosage and timing of the treatment were not known in many cases. In the study reported by Burrow *et al*. (1978), most of the treated children were exposed to propylthiouracil *in utero* during the third trimester, and only four were exposed during the first and second trimester. The studies reported by Burrow *et al*. (1978) and Messer *et al*. (1990) were retrospective studies in which maternal T4 levels during the first and second trimesters were not known. It is possible that the treated women had normal T4 levels during the early part of their pregnancies.

Fenzi *et al*. (1990) conducted neuropsychological assessments on a group of 384 school children (aged 6-14 years) residing in an area of known iodine deficiency (Tuscany, Italy). A group of 352 sex- and age-matched schoolchildren from an iodine sufficient area was used as a control group. Goiter prevalences in the endemic and control areas were 51.9 percent and 5.6 percent, respectively. No statistically significant differences in serum total T4, total T3, TSH levels between the endemic and control areas were found. Serum thyroglobulin values were higher in the iodine-deficient area. Global neuropsychological performance and cognitive levels were similar between a group of 50 schoolchildren from the endemic area and another group of 50 schoolchildren from the control area, matched for age, sex and socioeconomic conditions. However, Fenzi *et al.* (1990) also found that some marginal impairment, with particular regard to motor-perceptual functions, was present in areas of moderate iodine deficiency.

The New England Congenital Hypothyroidism Collaborative Program (1981) found that there was no correlation of eventual IQs with the severity of thyroid dysfunction or with the results of biochemical tests at the time treatment was begun, provided it was begun before clinical hypothyroidism appeared. A diagnosis of hypothyroidism was made when an infant's initial blood concentration of T4 was two or more standard deviations below the mean for newborn infants (6 µg/dL or less) and circulating TSH concentrations were elevated on repeated occasions. Three hundred thirty-six thousand newborn infants in Connecticut, Maine, Massachusetts, New Hampshire, and Rhode Island born between January 1, 1976 and June 30, 1978 were screened. Sixty-three infants were diagnosed with hypothyroidism and treated with L-thyroxine in doses sufficient to maintain circulating T4 concentration between 10 and 14 µg/dL during the first year of life and between 8 and 11 µg/dL thereafter. The control group consisted of 57 euthyroid children who had low T4 and normal TSH concentrations on neonatal screening. The revised Stanford-Binet examination was given to all the test subjects at 3 or 4 years of age. The authors reported that the mean IQ for the hypothyroid infants with adequate thyroid treatment was

106±16 and the mean for the controls was 106±15. They also reported that half of the patients with the lowest IQs (more than one standard deviation below the mean) had normal bone maturation. It is important to note that the results of Pop *et al*. (1999) indicated that it is the low maternal T4 level during early gestation (around week 12) that is associated with impaired neurodevelopment in the infant. It is possible that T4 levels at birth are not a perfectly accurate indicator for thyroid related neurodevelopmental deficiencies occurring in early gestation.

Liu *et al*. (1994) examined the IQs of eight children (Group 1) who were born to mothers that were hypothyroid during the first trimester of pregnancy. Maternal free T4 values at the 5th to 10th gestation weeks ranged from 2.3 to 6.3 pmol/L (normal range, 11.6 to 24.5 pmol/L) in six of the eight cases. In the other two cases, maternal total T4 values were 52.8 and 30.9 nmol/L (normal range, 92.7 to 218.8 nmol/L). TSH levels of the eight mothers at that time ranged from 25 to 190 mU/L (normal range < 5 mU/L). Maternal T4 and TSH levels became normal after T4 supplementation by 13 to 28 weeks of gestation. Seven of the eight children had nine siblings who had not been exposed to maternal hypothyroidism throughout gestation (Group 2); they were used as controls. Ages of the children in groups 1 and 2 at the time of IQ examination were 4 to 10 years in group 1 and 4 to 15 years in group 2. The investigators reported that all children in group 1 showed normal IQs. There was no statistically significant difference in the mean IQ between the children in group 1 who had siblings (112±11) and their siblings in group 2 (106±8). The study is limited by the small sample size. The administration of T4 supplement to hypothyroid mothers at 13 weeks of gestation might have averted adverse neurological development in the fetuses.

Summary of Data on Thyroid Hormones and Childhood Cognitive Development

While not all studies have reported clear links between maternal or neonatal levels of thyroid hormone and the subsequent neurological development of the child, at least five studies have. Studies by Haddow *et al*. (1999), Pop *et al*. (1999, 2003), Kooistra *et al*. (2006), and Vermiglio *et al*. (2004) have all reported statistically significant deficits in measures of childhood cognition and development in groups whose maternal gestational levels of T4 or fT4 include values that have traditionally been considered to be within normal reference ranges. For example, in Kooistra *et al*. (2006), the mean 12-week gestational fT4 in the low fT4 group was 11.4 pmol/L. Since the reference range given by the authors was 8.7-19.6 pmol/L, it is likely that the majority these mothers had fT4 values within the reference range. In addition, all of these studies excluded women with overt clinical thyroid disease, so the effects identified are occurring in the children of women who are asymptomatic. The animal studies by Auso *et al*. (2004), Gilbert and Sui (2008), Lavado-Autric *et al*. (2003), and others support the biologic plausibility that relatively mild decreases in maternal thyroid hormone levels in gestation can cause significant and permanent neurological changes in the offspring.

Some of the effects seen in the human studies are difficult to compare from one study to the next since different outcome measures are used in different studies. Despite this, the magnitudes of the effects seen in these studies seem to be markedly consistent across at least several of them. Table 20 shows the effect sizes in four of these studies. In each, a difference in maternal fT4 or T4 of about 11-32 percent is associated with a decrease in the cognitive development score in the offspring of about 7-16 percent.

Table 20. Comparison of Four Studies of Maternal T4 or fT4 in the First Trimester and Subsequent Child Neurologic Development

	Haddow *et al*., 1999	**Pop *et al*., 1999**	**Pop *et al*., 2003**	**Vermiglio *et al*., 2004**	**Averages**
Gestation week	12 weeks	12 weeks	12 weeks	8-13 weeks	
Thyroid measure	T4 (ng/dL)	fT4 (pmol/L)	fT4 (pmol/L)	fT4 (pmol/L)	
Low thyroid group	7.4	9.8[a]	11.5[a]	13.9	
Normal thyroid group	10.6	13.1[a]	17.0[a]	15.7	
Percent difference	30.2%	25.2%	32.4%	11.5%	24.8%
Outcome measure	IQ	PDI	MDI & PDI[b]	IQ	
Percent difference	7%	7%	9%	16%	11%

[a]Approximation based on graphs or other data.
[b]Average of the mean mental developmental index (MDI) and psychomotor developmental index (PDI) scales at ages 1 and 2.

Alterations in Thyroid Hormones and Effects on Serum Lipids and Other Biomarkers of Cardiovascular Disease Risk.

Effects on childhood cognitive development are not the only adverse effects that have been linked with relatively small changes in thyroid hormone levels. The following discussion focuses on the possible cardiovascular effects of relatively minor changes in thyroid hormone levels.

Asvold *et al*., 2007. This was a cross-sectional population-based study of TSH and serum lipid levels in 30,656 subjects from Norway. All subjects had no known thyroid disease and all had TSH levels within normal reference ranges (0.50 – 3.5 mU/l). The researchers found a linear and statistically significant (p for trend < 0.001) increase in total serum cholesterol, LDL cholesterol, non-HDL cholesterol and triglycerides, and a linear decrease in HDL cholesterol (p for trend < 0.001), with increasing TSH. Results were adjusted for age, smoking, and time since last meal. Adjustments for daily medication use, month of serum collection, diabetes mellitus, heart disease and stroke had no substantial impact on results. Although the changes were associated with very low p-values, the magnitudes of the changes were relatively small. For example, the mean LDL in the lowest TSH group (TSH = 0.50-0.99 mU/l) was 4.11 mmol/L while the mean

LDL in the highest TSH group (3.0-3.5 mU/l) was 4.34 mmol/L, about a 5 percent change. Although this level of change might be considered small on an individual basis, a 5 percent shift in cholesterol levels in a large population could represent a large increase in the population risks of diseases associated with cholesterol (e.g., heart disease and stroke). The results of this study are supported by other smaller studies which also reported similar effects for thyroid hormone levels within normal reference ranges (Pallas *et al*., 1991; Bakker *et al*., 2001; Michalopoulou *et al*., 1998).

Canaris *et al*., 2000. This was a cross-sectional study of serum lipids, thyroid hormones, and reported history of thyroid disease in 25,862 self-selected people who attended a statewide health fair in Colorado. Subjects were divided into five groups based on their TSH and T4 levels, and mean levels of serum lipids were determined for each group. The TSH and T4 levels used to define these groups and the mean lipid levels seen in each group are shown in Table 21. Statistically significant trends were seen across groups for total cholesterol (p-trend < 0.001), LDL cholesterol (p-trend < 0.001), and triglycerides (p-trend = 0.02), but not for HDL cholesterol. It is unclear whether these results were adjusted for any other variables although the authors did note that levels of estrogen use were similar across all thyroid hormone categories. Although the presence of a linear trend within the euthyroid group was not specifically evaluated, the overall trend across all of the thyroid hormone groups suggests that a trend is likely to occur over the entire range of TSH values.

Table 21. Mean Lipid Levels in Various Thyroid Groups in Canaris *et al*., 2000

	Group definitions		Cholesterol (mmol/L)			
	TSH (mIU/L)	T4 (nmol/L)	Total	LDL	HDL	TGs
Hypothyroid	> 5.1	< 57.9	6.5	4.4	1.4	2.0
Subclinical hypothyroid	> 5.1	≥ 57.9	5.8	3.8	1.4	1.8
Euthyroid	0.3-5.1	--	5.6	3.6	1.3	1.7
Subclinical hyperthyroid	0.01-<0.3	--	5.4	3.4	1.5	1.6
Hyperthyroid	≤ 0.01	--	5.2	3.4	1.3	1.6

Abbreviations: TGs, triglycerides.

Dullaart *et al*., 2007. This was a cross-sectional study of carotid artery intima media thickness (IMT) in 44 men and 34 women who all had fT4 (11.0-19.5 pmol/L) and TSH (0.5 mU/L -4.0 mU/L) levels within normal reference ranges. IMT is a subclinical measure of potential atherosclerosis. In multiple linear regression analyses adjusted for age, gender, pulse pressure, body mass index,

and HDL cholesterol, IMT was inversely related to fT4 ($\beta = -0.19$, $p = 0.046$), but not to TSH or the presence of anti-thyroid antibodies.

While several previous studies have reported that overt thyroid disease is associated with increased cardiovascular disease risks (Boelaert and Franklyn, 2005; Vanhaelst *et al*., 1967; Becker, 1985), the findings from this study and the studies of Asvold *et al*. (2007) and Canaris *et al*. (2000), all provide evidence that cardiovascular disease risk is also affected by decreases in thyroid hormone levels within normal reference ranges.

4. TOXICOLOGICAL EFFECTS IN HUMANS

The primary action of perchlorate in the human body is that it blocks iodide uptake in the thyroid gland. The function of the thyroid gland is the production of thyroid hormone. Iodide is a key component in the structure of thyroid hormone, and by blocking its uptake into the thyroid gland, perchlorate can potentially cause a decreased production of thyroid hormone.

As perchlorate competitively blocks iodide from entering the thyroid gland, many of the adverse effects of perchlorate exposure in the low dose range are expected to be similar to those of iodine deficiency. For this reason, an overview of some of the adverse health effects of iodine deficiency was provided in the chapter "Adverse Effects of Thyroid Hormone and Iodine Deficiency."

Developmental and Reproductive Toxicity

Studies of the impacts of perchlorate on thyroid hormone levels in newborns or children are discussed in this section. Impacts in adults, including pregnant women, are discussed in the following section.

Several studies have assessed the association between maternal exposure to perchlorate in drinking water during pregnancy and changes in thyroid hormone levels in newborns. Most of these studies used thyroid hormone levels that were collected as part of state-mandated screening programs for congenital hypothyroidism. Although these programs typically involve measuring thyroid hormone levels at any time in the first two weeks after birth, levels collected in the first 1-2 days after birth may be particularly important for several reasons. First, studies have shown that subtle changes in thyroid hormone levels may have greater impacts on brain development if they occur in the fetal period than if they occur later (i.e., in the newborn or young child) (Pop *et al*., 2003; Kooistra *et al*., 2006). Since thyroid hormone levels generally cannot be measured in the fetus during pregnancy in humans, the best practicable way to assess any effect in the fetus caused by perchlorate exposure to the mother during pregnancy would be to measure thyroid hormone levels in the child as soon after birth as possible (e.g., within the first 1-2 days after birth). Second, most of the human studies on newborn thyroid function and maternal perchlorate exposure categorized exposure based on the concentration of perchlorate in the mother's residential drinking water *during* pregnancy, not on the actual perchlorate intake of the newborn *after* birth. This is important since the half-lives of both perchlorate and thyroid hormones in newborns are fairly short (less than 24 hours) (Greer *et al.,* 2002; Van den Hove *et al*., 1999). As such, any effect that the mother's perchlorate exposure during pregnancy might have on the fetal thyroid should be seen within the first 24 hours after birth (e.g., within the thyroid hormone and perchlorate half-lives). But, they may not be seen at a later time if perchlorate exposure changes at birth. For example, the newborn may be fed an

infant formula with a different perchlorate concentration than that of the drinking water used by the mother during pregnancy. Perchlorate exposure may also change after birth in breast-fed infants if the mother uses water from the hospital or bottled water that has a different perchlorate concentration than the residential water used before birth.

The exposure to the infant might also be affected by the different kinetics of transplacental versus breast milk transfer of perchlorate. Since most of these studies based exposure status solely on the water source used by the mother before birth, any change in exposure in the child after birth could lead to a misclassification of exposure that would bias results towards the null and could cause any true effect to appear to diminish relatively soon after birth. Since the half-life of thyroid hormone in the child is short, this bias would most likely begin to occur within 24 hours after birth and become stronger thereafter. Because of this potential bias, our evaluation of these studies adds an additional emphasis on thyroid hormone measurements collected within the first 24 hours after birth.

Clinical Studies of Developmental and Reproductive Toxicity

Crooks and Wayne (1960) administered potassium perchlorate at 600 to 1,000 mg/day to a group of pregnant women that were suffering from hyperthyroidism and observed a very slightly enlarged thyroid in 1 of the 12 infants born to the mothers. They also reported that the enlarged thyroid returned to normal size in six weeks, and no other abnormalities were observed. Several key parameters were not provided in the paper: detailed dosage information, time of perchlorate treatment in relation to the gestation period, thyroid function of the newborns, and the neurological as well as behavioral development of the offspring. Furthermore, interpretation of the result is made difficult by the fact that the women were suffering from thyrotoxicosis (excess quantities of thyroid hormones).

Epidemiologic Studies of Developmental and Reproductive Toxicity

DHS, 1997. A preliminary health review of a potentially perchlorate-exposed area of Rancho Cordova, CA by the California Department of Health Services (DHS, 1997) included an analysis of several state databases for possible perchlorate-related adverse health effects. Analysis of newborn thyroid hormone data for the period 1985 through 1996 did not indicate a positive correlation between residence in potentially perchlorate-exposed areas and neonatal hypothyroidism. The TSH levels of neonates with initially low T4 levels in the potentially exposed areas were found to be statistically lower than those in the control areas, contrary to what was expected.

Kelsh *et al*., 2003. This investigation used the California Newborn Screening database to study the thyroid health of newborns whose mothers resided in the city of Redlands, California during the years 1983 through 1997. Perchlorate at variable levels has been detected in groundwater wells in this city. The

outcomes assessed were neonatal primary congenital hypothyroidism (PCH) and elevated neonatal serum levels of TSH (> 25 µU/mL). PCH is a severe condition, usually caused by a missing or partially missing thyroid gland in the child. It is generally associated with very large increases in TSH (e.g., > 25 µU/mL) and often requires treatment with thyroid hormone to prevent severe neurologic and physical growth deficits. TSH measurements were only collected when T4 levels were low (typically below 9 µg/dL or in the lowest five percent of the remaining daily tray samples). PCH was defined as an elevated TSH plus a physician's confirmatory diagnosis. Newborns of San Bernardino and Riverside counties, excluding newborns from Redlands and other communities where perchlorate has been detected, were used as the comparison group. The Colorado River is one of the water sources of Riverside County, so the water serving some of the "unexposed" comparison group may have been contaminated with perchlorate. Perchlorate exposure in the comparison areas would most likely bias any true associations towards the null. There is little information about the perchlorate levels in the drinking water in this area during the study period since the detection limit of perchlorate in water before 1997 was about 400 ppb. In the 2001 and 2002 Consumer Confidence Reports, the City of Redlands reported that the concentrations of perchlorate in its water system ranged from non-detect to 9 ppb, with an average concentration below 1 ppb. However, the California Department of Public Health reported perchlorate levels in Redlands wells in 1997 ranged from 4 ppb to 130 ppb, although it is unknown how much water from the high exposure wells was used for drinking.

Kelsh *et al*. (2003) found no increase in the prevalence of PCH in Redlands newborns over the 15-year study period, although there were only two cases of PCH reported in Redlands during this time. Because there is a normal transient surge in neonatal TSH levels immediately after birth, measuring TSH levels within the first few hours of birth can lead to a high rate of false positives in screening programs for PCH. Because of this, the researchers did further analyses, which excluded subjects who had TSH measurements collected within the first 18 hours of birth. However, as discussed above, measurements collected in the first day after birth may actually be the most relevant, and as discussed below, the post-natal surge in TSH does not necessarily invalidate associations identified between perchlorate and neonatal thyroid hormone levels during this time.

The odds ratio for an elevated TSH for Redlands compared to San Bernardino/Riverside Counties for all subjects (regardless of the age at measurement), and for only those subjects with TSH measurements collected at ≥ 18 hours of age reported by Kelsh *et al.* (2003) were 1.24 (95% CI, 0.89-1.68) and 0.69 (95% CI, 0.27-1.45), respectively. The prevalence ratio for PCH standardized by ethnicity, sex, birth weight, and birth year for Redlands compared to San Bernardino/Riverside Counties was 0.45 (95% CI, 0.06-1.64). The researchers found that Hispanic ethnicity, low and high birth weight, and female sex were risk factors for PCH.

Kelsh *et al*. (2003) did not calculate the odds ratio for having a low T4 (typically defined in this study as a T4 value below 9.0 µg/dL or part of the lowest 5% of the remaining daily tray samples), although this can be estimated using the data in Table 3 of their study. As discussed above, T4 was measured in all newborns, but TSH was only measured if T4 levels were low. Thus, the number of subjects who had "TSH levels screened" represents the number of neonates with low T4 levels. The odds ratio for having a low T4 in Redlands compared to San Bernardino/Riverside Counties was 1.18 (95% CI, 1.13-1.24; $p < 0.0001$). The data used in this calculation is shown in Table 22. This odds ratio is unadjusted. However, it is unlikely that adjusting for age at collection, ethnicity, sex, birth weight, or birth year would have any major impact on this odds ratio since adjusting for these factors had little impact on the TSH odds ratios provided by the authors.

Table 22. Estimated Odds Ratio Calculation for Low T4 in Redlands and in San Bernardino and Riverside Counties Using Data Provided in Kelsh *et al.,* 2003.

Community	Low T4	Normal T4	Total
Redlands	2,081	13,267	15,348
San Bernardino/Riverside	81,401	614,566	695,967
Total	83,482	627,833	711,315

Odds ratio = (2,081/13,267) / (81,401/614,566) = 1.18 (95% CI, 1.13–1.24)

Kelsh *et al*. (2003) also did not report specific results for neonates who had serum TSH measurements collected *before* 18 hours of age. However, data provided in the tables of Kelsh *et al*. (2003) can be used to estimate the odds ratio for having a high TSH level in subjects who had their TSH levels measured during this time. This odds ratio, comparing Redlands to all of San Bernardino/Riverside Counties, was 1.57 (95% CI, 1.14-2.16; $p < 0.0001$). The data used in these calculations are shown in Table 23.

The major strength of the Kelsh *et al.* (2003) study is its large sample size. However, it is limited by the small number of cases of PCH in Redlands, and like most other ecological studies, it was limited by the lack of detailed information on individual exposure (discussed below).

Table 23. Estimated Odds Ratio Calculation for a High Neonatal TSH Level in Subjects with Blood Collected < 18 Hours of Birth Using Data Provided in Kelsh *et al*., 2003

	TSH Levels		
Area (Perchlorate)	**Elevated[a]**	**Not Elevated**	**Totals**
Redlands (High)	38	4,808	4,846
San Bernardino & Riverside (Low)	1,175	232,990	234,165
Totals	1,213	237,798	239,011

[a]Definitions of "elevated" changed by year, and were between ≥15 and ≥25 µU/mL

Odds ratio = (38/4,808) / (1,175/232,990) = 1.57 (95% CI, 1.14-2.16)

Crump *et al*., 2000. These investigators studied 162 school-age children (ages 6-8 years old) and 9,784 newborns in three cities in northern Chile that had different concentrations of perchlorate in their drinking water: Taltal (perchlorate concentration, 100 to 120 µg/L), Chañaral (5-7 µg/L), and Antofagasta (non-detectable: <4 µg/L). Approximately 25 separate water sources were sampled in each city. Water samples were taken from water faucets at participating schools, homes of students, and public buildings located near the schools.
The unadjusted mean levels of TSH, T4, fT4, and T3 of the school-age children were very similar across the three cities. Among all the school-age children, there was a small, non-significant increased risk of goiter in Chañaral (26.5 percent) and Taltal (23.3 percent) compared with Antofagasta (17 percent), although this was not seen in analyses confined to life-long residents. The reason for the high prevalence of goiter in the unexposed city of Antofagasta is unknown.

Overall, neonatal TSH levels were similar across the three cities. Adjusted for sex and age, linear regression comparisons of the logarithm (log) of TSH of the newborns by city showed that average logTSH in Taltal was significantly lower than the averages of the other two cities. However, as shown in Table 8 of the Crump *et al*. (2000) paper, TSH levels in those neonates with TSH measured on day 1-2 after birth were higher in Taltal (4.2 µU/mL ± 1.2) than in Antofagasta (3.2 µU/mL ± 3.5) or Chañaral (3.2 µU/mL ± 3.5), although the sample size was small (n = 62).

It should be noted that the iodine levels measured in the schoolchildren in all three study cities were very high. Mean urine iodine levels were 766 µg/L, 614 µg/L, and 756 µg/L for Taltal, Chañaral, and Antofagasta, respectively. These levels are much higher than those in the NHANES III database, where a mean urinary iodine level of 305 µg/L was found for 6-11 year old children in the U.S. The high urinary iodine levels and high prevalence of goiter in this study make it difficult to interpret the relevance of its findings to children in California, where

rates of goiter are much less and moderate iodine deficiency (a potential susceptibility factor) is likely much more common.

Crump *et al*. (2000) found that schoolchildren with lifelong residence in Taltal were five times more likely to have a self-reported family history of thyroid disease than schoolchildren with lifelong residence in Antofagasta. These results were adjusted for age, sex, and urinary iodine (Table 24). Chañaral children had no increased prevalence of self-reported family history of thyroid disease. Families of 19 out of 61 (31 percent) children in Taltal were reported to have some history of thyroid disease. Twelve of these families reported having a single relative (usually a mother or grandmother) with goiter, hypothyroidism, or unspecified thyroid disease; and seven reported having two or more relatives with thyroid disease. The reason why there was evidence of perchlorate-related thyroid effects in older relatives, but not in the children themselves, is unknown. The authors note that the reports on familial history of thyroid issues were not verified and recall bias is possible. They also speculated that this finding might be related to the major changes in average iodine intakes that have occurred in Chile over the last several decades. Iodized salt was introduced to this region in the late 1970's, and average urinary iodine levels in Chile have risen dramatically since that time. For example, according to the International Council for Control of Iodine Deficiency Disorders, average urinary iodine levels in Chile rose from 109 µg/g creatinine in 1982 to 1,191 µg/g creatinine in 2001 (ICCIDD, 2009). It is possible that the elevated odds ratio for a family history of thyroid disease reflects effects that occurred several decades ago when intakes of iodine were low and this low iodine caused some people to be especially susceptible to perchlorate. It is also possible now that iodine levels are much higher, these high levels are protective, and the children and other members of this community are now much less susceptible to perchlorate.

Table 24. Odds Ratios for the Association between Self-Reported Family History of Thyroid Disease[a] among Schoolchildren and City of Residence[b] (Crump *et al*., 2000)

	Schoolchildren with less than lifelong residence (n=162)		Schoolchildren with lifelong residence (n=127)	
City	**Odds ratio**	**95% CI**	**Odds ratio**	**95% CI**
Antofagasta	1.00	-	1.00	-
Chañaral	0.89	0.25-3.19	1.04	0.21-5.09
Taltal	3.35	1.19-9.38	4.97	1.29-19.17

[a]Direct relative (parent, sibling, grandparent, great-grandparent, aunt, uncle, or cousin) with history of goiter, hypothyroidism, or subtotal thyroidectomy.
[b]Adjusted for age, sex, and urinary iodine; excluded one child with autoimmune hypothyroidism.

Assuming the level of perchlorate contamination of the city of Taltal has not changed significantly in the last few decades, a LOAEL of 100 µg/L for familial

thyroid problems can be identified from Crump *et al.* (2000). Applying the default values of 2 L/day for drinking water consumption and 70 kg for an adult body weight, the LOAEL is equivalent to an intake of 3 µg/kg-day from drinking water alone.

Lamm and Doemland, 1999. These authors identified six counties in California (Los Angeles, Orange, Riverside, Sacramento, San Bernardino, and San Diego) and one county in Nevada (Clark) that have had detectable levels of perchlorate (4 to 16 µg/L) in at least some of their drinking water sources. They then compared the rates of primary congenital hypothyroidism (PCH) in these seven counties with overall state rates. All infants were screened by their serum T4 levels, and those with a low T4 (i.e., less than the 10th percentile) were further screened for high TSH levels. An infant was considered to be potentially congenitally hypothyroid if the serum TSH was $\geq$ 25 µU/mL. Infants with these high TSH values were then evaluated by a physician to confirm whether or not they had PCH.

County- and ethnicity-specific data for the two states were obtained for the years 1996 and 1997. Within the seven counties, nearly 700,000 newborns were screened. In all, 249 cases of PCH were identified, where 243 were expected based on the state incidence rate, for an overall risk ratio of 1.0 (95% CI, 0.9-1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. Out of the 36,016 newborns screened in Clark County (Nevada) between 1996 and 1997, seven cases were observed and 8.3 cases were expected. The risk ratio was 0.8 (95% CI, 0.34-1.74). Based on these results, Lamm and Doemland (1999) concluded that the study did not indicate an increase in the incidence of PCH in those counties with detectable perchlorate levels. Results were not adjusted for several variables related to thyroid hormone levels such as age, gender, or iodine intake, although there is no evidence these factors were related to perchlorate exposure and caused important confounding. Perhaps more importantly, although Clark County of Nevada obtained nearly all of its drinking water from Lake Mead, which is known to be contaminated with perchlorate, the six California counties obtained their drinking water from multiple sources, many of which were not contaminated with perchlorate. Because of this, there was likely significant misclassification of exposure for the California counties. Misclassification could also occur if there were errors in the county to which subjects were assigned, although there is no evidence that this would result in major bias, and bias from this source seems unlikely given the very large size of most of the exposed counties. Importantly, errors in misclassifying exposure would most likely be non-differential and most likely cause bias towards the null. Finally, PCH is a very serious disease, usually requiring treatment with thyroid hormone, and it is generally associated with large increases in TSH. As discussed in subsequent sections, much smaller changes in thyroid hormone levels may also be associated with significant health outcomes, and these more subtle effects would probably be missed in a study that solely focused on physician-diagnosed cases of PCH.

Li *et al*., 2000a. In a related study, Li *et al*. (2000a) compared serum T4 levels of newborns (collected 1 to 4 days after birth) from the city of Las Vegas, Clark County, Nevada, which has perchlorate in its drinking water, to those from the city of Reno, Nevada, which does not (detection limit, 4 µg/L). A total of 17,308 newborns from Las Vegas and 5,882 newborns from Reno born from April 1998 through June 1999 were included in the study. During the study period, monthly drinking water perchlorate levels for Las Vegas ranged from non-detectable to 15 µg/L. Perchlorate was not detected in the Las Vegas water supply in 8 of the 15 months covered by the study, which the authors suggest may be due to the changing conditions of the water supply to this city. Separate analyses were done to evaluate births in the seven months when perchlorate was detected.

Overall, Li *et al*. (2000a) reported no differences in mean T4 levels (approximately 17 µg/dL) between the two cities ($p = 0.41$), including analyses involving those months where perchlorate was detectable in Las Vegas drinking water (shown in their Figure 1, no p-value given). Specific analyses stratified by age at the time T4 was measured were not presented in detail but are shown in Figure 3 of their paper. Based on this figure it appears that among infants who had their T4 levels collected on day one after birth, the mean T4 level in Las Vegas was about 4 µg/dL (about 22 percent) lower than the mean T4 in Reno.

Li *et al*. (2000a) used the monthly perchlorate measurements in Las Vegas drinking water to estimate the cumulative perchlorate exposure for each newborn during the first three months of pregnancy and for all nine months of pregnancy. Cumulative exposures in Las Vegas ranged from 9 ppb-months to 83 ppb-months; the Reno newborns during this period were presumed to have had no drinking water-related prenatal exposure. In linear regression analyses involving T4 levels collected on all days after birth (not just day one), no association was found between cumulative perchlorate exposure and mean neonatal T4 levels (regression coefficient (β) = -0.0003; $R^2 = 0.002$). Exposure assessment in this study was ecologic and information on whether or not the mothers or infants consumed water from public supplies, or how much they consumed is unknown. As discussed below, this would most likely cause bias towards finding no effect. Misclassification of true long-term thyroid hormone status could also have caused some bias, but again, this would most likely be non-differential and thus most likely cause bias toward finding no effect (also discussed below).

Li *et al*., 2000b. Li *et al*. (2000b) studied neonatal blood TSH levels sampled between December 1998 and October 1999 in Las Vegas (up to 15 ppb perchlorate in drinking water) and Reno (perchlorate below the detection level of 4 ppb). Serum TSH levels were measured in all neonates who had T4 measurements below the 10th percentile in each daily batch of T4 samples (measured in the first 30 days of life). TSH measurements collected on the first day after birth were excluded. (As discussed above, associations between maternal perchlorate exposures and neonatal thyroid hormone levels are probably best evaluated using TSH measurements taken within the first 24 hours after birth.) In addition, only neonates with birth weights of 2.5 – 4.5 kg were

included. The authors found that neonatal TSH levels were not significantly different between Reno and Las Vegas. Mean TSH values in Las Vegas and Reno were 11.5 µIU/mL (± 1.3) and 12.5 µIU/mL (± 1.3), respectively. The TSH regression coefficient adjusted for age and sex comparing Las Vegas to Reno was -0.0004 (95% CI, -0.0241– 0.0233; p=0.973). Several factors could have caused at least some bias towards finding no effect, including the lack of control of birth weight and ethnic origin, the use of broad categories to control for age at TSH collection (2-7 and 8-30 days), the small sample size, and perhaps most importantly, misclassification of exposure and effect and the exclusion of subjects who had TSH measurements at age < 24 hours.

Brechner *et al*., 2000. This study identified an association between low-level perchlorate exposure in maternal drinking water and serum TSH levels in newborns. As in the studies discussed above, T4 was measured in all newborns, but TSH was only measured in newborns who had low T4 levels. The investigators compared serum TSH levels in newborns from Yuma, a city that obtains its public drinking water entirely from the Colorado River below Lake Mead, with TSH levels in newborns from Flagstaff, a city that obtains none of its public drinking water from the Colorado River below Lake Mead. Although Lake Mead was known to have perchlorate contamination, no useful water monitoring data were available for Yuma and Flagstaff during the study period (between 1994 and 1997) because the detection limit of perchlorate in water was 400 ppb during that time. In March 1997, the detection limit of perchlorate was improved to approximately 4 ppb. In August 1999, U.S. EPA reported that perchlorate levels in Yuma were about 6 ppb in both raw water and finished drinking water (Brechner *et al.,* 2000).

In unadjusted analyses, Brechner *et al*. (2000) found that median newborn TSH levels in Yuma were significantly higher than in Flagstaff (19.9 vs. 13.4 mU/L). The odds ratio for having a low T4 (and thus being screened for TSH) were not presented by the authors but can be estimated using the data they present in Figure 1 of their study. This odds ratio, comparing Yuma to Flagstaff, was elevated (1.19; 95% CI, 1.07-1.33; p = 0.006). The data used to estimate this odds ratio are shown in Table 25. It should be noted that this odds ratio is not adjusted for age or ethnicity. Four cases of congenital hypothyroidism were reported in Yuma but none in Flagstaff. Because of a normal surge in TSH that occurs soon after birth, a major factor influencing newborn TSH levels is the time (after birth) at which the TSH blood samples are collected. This time was significantly earlier in Yuma than in Flagstaff, and this may have caused some of the increase in TSH levels seen in Yuma compared to Flagstaff. However, according to the authors, the difference in mean TSH levels between Yuma and Flagstaff remained after adjusting for age in days at measurement, gender, and race/ethnicity (p = 0.009) (Brechner *et al*., 2001). Although the effect size of the adjusted results was not reported, median TSH levels in the two cities stratified by age at measurement are presented. Using the data provided in Table 2 of their article, it can be seen that median TSH levels are greater in Yuma than in Flagstaff on most days of age, with the greatest difference seen on days 0-2 of

age (shown in Table 26). The interpretation of this study is complicated by the fact that TSH levels were only measured in samples with low T4 measurements (discussed below). In addition, this study did not adjust for birth weight or gestational age. The difference in altitude between the two cities has also been cited as a possible bias but it is not clear that this potential confounder would be strong enough to cause the results observed. In fact, several studies suggest the opposite: that high altitudes actually decrease thyroxine levels and have little to no effect on TSH levels (Kotchen *et al.,* 1973; Sawhney and Malhotra, 1991; Richalet *et al.,* 2010).

Table 25. Estimated Odds Ratio for Having Low T4 Using Data Provided in Brechner *et al.,* 2000.

	Low T4		
	Yes	**No**	**Total**
Yuma	1,291	6,308	7,599
Flagstaff	519	3,020	3,539

Odds ratio = (1,291/6,308) / (519/3,020) = 1.19 (95% CI, 1.07-1.33)

Table 26. Median TSH Levels in Yuma and Flagstaff Stratified by Day of Blood Collection (Brechner *et al*., 2000)

Flagstaff				Yuma			
Days	**N**	**%**	**TSH**	**N**	**%**	**TSH**	**Difference[a]**
0	14	3.2%	24.0	121	11.0%	30.4	6.4
1	122	27.5%	22.0	531	48.3%	25.2	3.2
2	25	5.6%	13.4	116	10.6%	16.5	3.1
3	24	5.4%	11.5	21	1.9%	13.8	2.3
4	15	3.4%	12.3	17	1.5%	12.3	-0.1
5	243	54.9%	10.6	293	26.7%	11.4	0.8
Total	443	100.0%	14.5	1099	100.0%	20.8	6.3

[a]Median TSH in Yuma minus median TSH in Flagstaff.

Buffler *et al*., 2006. This study was an ecologic analysis comparing neonatal TSH levels in California communities with perchlorate concentrations above 5 µg/L to those in communities with no known drinking water perchlorate measurements above 5 µg/L. Perchlorate levels in community water sources were obtained for the years 1997 and 1998 from the California Drinking Water Program and used to estimate weighted average perchlorate concentrations in community water. TSH levels among 342,257 California newborns screened in 1998 were obtained from the California Department of Health Services (now the California Department of Public Health). The major outcomes assessed in this study were primary congenital hypothyroidism (PCH) and the elevations in TSH typically used to screen for this disorder (i.e., a TSH > 25 µU/mL collected more

than 24 hours after birth). As discussed for the Kelsh *et al.* (2003) study above, PCH was defined as a TSH level > 25 µU/mL and a physician's confirmatory diagnosis. Subjects who were screened before 24 hours of age were excluded from the analyses by the authors because the normal physiologic post-natal surge of TSH that occurs during this period can increase the rate of false positives when screening for PCH.

Overall, Buffler *et al*. (2006) reported an adjusted odds ratio for having TSH > 25 µU/mL of 0.73 (95% CI, 0.40-1.23) comparing the high to low perchlorate communities. The corresponding odds ratio for PCH was 0.71 (95% CI, 0.40-1.19).

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. The odds ratio for all subjects (those with TSH measured < 24 hours of age combined with those with TSH measurements at ≥ 24 hours of age) were not reported by the authors but can be estimated from the data given in Table 1 of Buffler *et al*. (2006). The unadjusted OR for high TSH comparing communities with perchlorate concentrations above and below 5 µg/L in all subjects regardless of the age of measurement was 1.59 (95% CI, 1.33-1.91). The data used in these calculations are presented in Table 27. Again, this odds ratio was not reported by the study authors, and it is an unadjusted odds ratio.

Table 27. Data for Estimated Unadjusted Odds Ratio Calculations for All Subjects in Buffler *et al*. (2006)

	TSH levels		
Perchlorate	**Elevated**	**Normal**	**Totals**
> 5 µg/L	147	50,179	50,326
< 5 µg/L	537	291,394	291,931
Totals	684	341,573	342,257

Odds ratio = (147/50,179) / (537/291,394) = 1.59 (95% CI, 1.33-1.91)

Given the normal TSH surge that occurs in the first 24 hours after birth, OEHHA evaluated the possibility that this elevated odds ratio could be due to earlier TSH testing in the communities with perchlorate > 5 µg/L. An estimate of the percentage of neonates with TSH measurements before and after 24 hours can be obtained by subtracting the number of TSH levels measured at ≥ 24 hours (given in Buffler *et al*., 2006, Table 4) from the total number of TSH measurements (at any age) (given in Buffler *et al*., 2006, Table 1). These data are shown in Table 28 below. (These are estimates since Buffler *et al*., 2006, Table 1 appears to include all subjects whereas Buffler *et al*., 2006, Table 4

appears to only include subjects who have all of the data on the co-variates used in their adjusted analyses.)

Table 28. Subjects with TSH Measurements before and after 24 Hours of Birth (Buffler *et al*., 2006)

	Perchlorate	
	≤ 5 µg/L	**> 5 µg/L**
Total subjects (Table 1)	291,931	50,326
High TSH (Table 1)	537	147
Total > 24 hours (Table 4)	185,528	29,114
Percent	63.6%	57.9%
High TSH (Table 3)	124	15
Percent with high TSH	0.067%	0.052%
Total < 24 hours (calculated)	106,403	21,212
Percent	36.4%	42.1%
High TSH (Table 3)	413	132
Percent with high TSH	0.39%	0.62%

Based on these numbers, the percentage of all neonates with TSH measurements collected within the first 24 hours of birth was greater in the high perchlorate communities than in the low perchlorate communities (42.1 versus 36.4 percent). Importantly though, when analyses are confined to only those subjects with TSH measurements collected at < 24 hours of age, the unadjusted odds ratio for high TSH comparing communities with and without perchlorate > 5 µg/L remained elevated (OR = 1.60; 95% CI, 1.32-1.94). If age at measurement were an important confounder, the expected odds ratio would be near 1.0 after stratifying by age. It is unlikely that the entire 60 percent increase in risk would go away with an even more detailed stratification or adjustment by age at sample collection.

Steinmaus *et al.,* 2010. Individual data were obtained from the Buffler *et al.* (2006) study, and analysis of these confirmed the elevated odds ratios discussed above. For example, the odds ratio for TSH > 25 µU/mL within the first 24 hours of birth was 1.53 ($p < 0.0001$; 95% CI, 1.24-1.89). For TSH levels measured more than 24 hours after birth, the odds ratio for TSH > 25 µU/mL was similar to that reported in Buffler *et al.* (2006). However, a TSH level of 25 µU/mL was the 99.99th percentile of all TSH levels in this age stratum and there were very few exposed cases (n = 13). Because significant neurologic effects have been seen with smaller changes in thyroid hormones (Pop *et al*., 1999, 2003; Haddow *et al*., 1999; Klein *et al*., 2001; Kooistra *et al*., 2006; Vermiglio *et al*., 2004), lower TSH cut-off points were also used to define “high” TSH in this paper. When this was done, elevated odds ratios for high TSH were seen both in infants in whom TSH measurements were collected before 24 hours of age and in infants in whom TSH measurements were collected after 24 hours of age. For example, the odds

ratio for having a TSH level above the 95th percentile in samples collected after 24 hours of age comparing perchlorate exposed and unexposed communities was 1.27 ($p < 0.0001$; 95% CI, 1.22-1.33). This study only evaluated TSH levels and it is currently unknown whether the effects seen here cause actual impacts on health and development. These analyses adjusted for age of sample collection, gender, mother's age, per capita income, race/ethnicity, birth weight, and feeding type (breast milk vs. formula), none of which had substantial effects on results. For example, the adjusted and unadjusted ORs for having a TSH level of 25 μU/mL or greater for collection ages less than 24 hours were 1.53 and 1.52, respectively.

The authors of the Steinmaus *et al.* (2010) paper considered analyzing TSH concentrations and community perchlorate concentrations as continuous variables. But, because of the extensively overlapping and continually changing water sources in many parts of California, assigning a single perchlorate concentration to each individual would have introduced considerable misclassification. This would have introduced particularly strong bias in those subjects in the upper ranges of community perchlorate concentration. Instead, communities (and the subjects who lived in those communities) were divided into two groups based on whether or not it was likely the sources of their residential drinking water had perchlorate concentrations greater or less than 5 ppb. Some exposure misclassification was still likely with this type of categorization. However, since the misclassification was most likely non-differential, the bias would likely be in the direction of the null, not in the direction of finding false positive associations.

Li *et al*., 2001. This study was an ecologic analysis of various thyroid diseases comparing two counties in Nevada: Clark County, where average water perchlorate levels of around 0-14 ppb were reported, and Washoe County which has not had detectable levels of perchlorate in its major drinking water supplies. The largest city in Washoe County is Reno and the largest city in Clark County is Las Vegas. Relative risks for 6 of the 8 diseases assessed comparing Clark County to Washoe County were above 1.0 (although none were statistically significant) including goiter (RR = 1.24), nodular goiter (RR =1.45), thyroiditis (RR=1.69), and other thyroid conditions (RR=1.89). Relative risks for congenital and acquired hypothyroidism were 0.60 and 1.01 respectively. Disease rates were based on Medicaid records which could be a very insensitive marker of the true rates of these diseases since only a fraction of the people in these counties are on Medicaid rolls.

Chang *et al*., 2003. In a similar study comparing Clark County (known to have elevated perchlorate levels in its drinking water) to the rest of Nevada, no differences were seen in the rates of pediatric neurobehavioral diseases including autism and attention deficit-hyperactivity disorder (ADHD) assessed using Medicaid records. As stated by the authors, "Perchlorate levels in drinking water were measured in Nevada waters following the 1997 detection of perchlorate in the Lower Colorado River with the newly refined perchlorate

assay. The only public water system found to contain perchlorate was that of the Southern Nevada Water Authority (SNWA) that obtained its water from Las Vegas Bay and distributed it to about 96% of Clark County, including the city of Las Vegas. Perchlorate has not been detected in the public water supply of Reno, of Washoe County, or elsewhere in the state of Nevada. The perchlorate content of the raw and finished waters of SNWA have been measured at least monthly, and at times weekly, since July 1997. Perchlorate levels in 149 finished water samples taken between July 1997 and May 2002 had a mean of 10.9 ppb (SD ± 3.9; median = 10.5 ppb; range = nondetect to 23.8 ppb)." Information on the frequency of neurobehavioral diseases in Nevada youths under 18 years old came from the service records of the Nevada Medicaid program for the years 1996–2000. Patients were defined as those under age 18 who were diagnosed with or treated for either ADHD (ICD9 314) or autism (ICD9 299). The "disease incidence" in Clark County and the rest of Nevada was defined as the average annual number of new cases in Medicaid youths seen or treated in each area divided by the number of Medicaid-eligible youths in that area, in the midpoint of 1998. These unadjusted "disease incidences" were then compared, although the results of formal statistical significance testing are not provided. As discussed in many of the other studies reviewed in this section, and reviewed below, results might have been affected by exposure and outcome misclassification or confounding, although too few data are provided to quantitatively evaluate the extent of these issues for this study. Also, no difference was seen in comparisons of 4th grade performance results, although the methods used in this study to assess both exposure and outcome are likely too inaccurate to identify subtle or even moderate effects.

Tèllez Tèllez *et al*., 2005. Neonatal and maternal thyroid function was assessed in subjects from the same three cities in northern Chile used in Crump *et al*. (2000). These cities (and their mean perchlorate levels in drinking water) were Taltal (100-120 µg/L), Chañaral (5-7 µg/L), and Antofagasta (non-detectable: <4 µg/L). No clear difference was seen in maternal thyroid hormone levels between the three cities (discussed in a subsequent section). In addition, no differences were seen across cities in mean neonatal cord blood fT4 or TSH. Interestingly, 72.7 percent of the births in the high exposure city were males (compared to 49 percent in the low exposure city), and 57 percent of the births from the high exposure city were done by cesarean section (compared to 39 percent in the low exposure city). The reasons for the unusually high proportion of males and the unusually high rate of cesarean-sections in Taltal are unknown.

Mean concentrations of perchlorate in breast milk were similar in the high and low exposure cities (95.6 µg/L versus 81.6 µg/L), although these were highly variable within a city and the median levels were markedly different across cities (< 4 in Antofagasta and 104 µg/L in Taltal). No association was seen between breast milk iodine and perchlorate concentrations. Mean urinary iodine levels in the women were greater than 300 µg/L in all three cities, suggesting that very few women had low iodine intakes. As discussed below, relatively high iodine intakes may help prevent the thyroidal effects of perchlorate. The mean number

of cigarettes smoked per week (0.52 for the 62 women in Taltal) suggested that few if any women were moderate or heavy smokers. In addition, differences in urinary levels of perchlorate were not as great as might be expected based on the perchlorate concentrations reported for the drinking water of each city. For example, maternal urine perchlorate concentrations measured during the post-partum visit for the low, medium, and high exposure cities were 22.3, 17.5, and 49.1 µg/L, respectively, although these measurements were only done in a fraction of the women in the study. A graphical display of the urinary perchlorate levels from all three cities shows a marked overlap across cities. Part of this may have been due to the fact that a large percentage of the women (45 percent) from the high exposure city of Taltal went to the low exposure city of Antofagasta to give birth. Taltal (population about 10,000) is fairly remote and is about 200 miles away from the much larger city of Antofagasta (population about 250,000). Part of it may have been due to exposure from foods or some other unknown source in Antofagasta. Regardless of the cause, the lack of a large contrast in exposure between the subjects from each of the cities probably decreased the likelihood that true associations, if present, could be found.

Amatai *et al*., 2007. T4 values were measured in newborns in the neighboring cities of Ramat Hasharon and Hertzlia, Israel, whose mothers resided in areas where drinking water had perchlorate concentrations that were very high (340 µg/L, n = 97), high (42-94 µg/L, n = 216), and low (< 3 µg/L, n = 843). The high perchlorate concentrations were found in wells near a military plant. Heel-stick T4 values were measured in all newborns in these communities as part of the national screening program, and were done at 36-48 hours after birth in >90 percent of all newborns. Mothers also completed a questionnaire asking whether they drank tap water, filtered water, or bottled water during their pregnancy.

The mean T4 values in the very high, high, and low perchlorate areas (in µg/dL) were 13.93 (± 3.8), 13.91 (± 3.4), and 13.98 (± 3.5), showing no differences between the exposure areas. It is unclear if these means were adjusted for other factors associated with neonatal T4 levels including gender or the age of the child when the heel stick was taken. No association was seen with neonatal T4 and maternal age, birth weight, gestational age, or sex. No difference was seen in mean T4 values in the analysis confined to only the children of exposed women who reportedly drank tap water during pregnancy, although this included less than 30 percent (n = 93) of the women from the very high and high exposure areas.

Serum perchlorate levels were reported in this study on a small number of people who lived in the study areas and who were proxy donors (i.e., blood bank donors who were not involved in the actual study). Mean serum perchlorate levels (in µg/L) in the very high, high, and low perchlorate areas in these subjects were 5.99 (± 3.89, n = 4), 1.19 (± 1.37, n = 19), and 0.44 (± 0.55, n =14). As seen in Figure 3 of this paper, and in the reported standard deviations, there was considerable overlap in serum perchlorate among the three exposure categories. Serum iodine levels were also measured in the proxy donors. Serum iodine

levels were higher in proxy subjects from the very high/high exposure areas compared to the low exposure area (3.10 µg/L ± 1.25 versus 2.24 µg/L ± 0.85; p = 0.031).

The strengths of this study are its collection of at least some individual data on exposure (i.e., the source of drinking water during pregnancy) and the presumably large contrast in perchlorate exposure. Urinary perchlorate levels would have likely provided a better indication of true exposure but were not measured. Other weaknesses are the lack of information on adjustments for potential confounders, the lack of individual data on smoking and iodine, and the relatively small number of women from the exposed areas who drank tap water during pregnancy. In addition, the authors reported that >90 percent of infants in these areas had their blood sampling at 36-48 hours after birth, suggesting that very few (probably < 4) of the highly exposed study infants had thyroid hormone measurements within the first 24 hours of birth. As discussed above, since the half-life of perchlorate and thyroid hormones is relatively short, this may have limited the ability of this study to find an association between maternal perchlorate exposure during pregnancy and neonatal thyroid hormone levels.

Blount *et al.,* 2009. Perchlorate, nitrate, thiocyanate, and iodine were measured in maternal urine (n=34), amniotic fluid (n=130), cord blood (n=126), and maternal serum (n=132) in mother-offspring pairs from New Jersey. Only subjects scheduled for elective cesarean section were included, and maternal blood and urine were collected within one hour before the surgery. Correlations between the various exposure parameters, as well as associations between perchlorate concentrations in cord blood and birth weight, length, and head circumference, were assessed. Correlations between perchlorate concentrations in maternal urine and concentrations in maternal blood and cord blood were low (-0.10 and 0.20, respectively). Clear associations between concentrations of cord blood perchlorate and birth weight, length, and head circumference were not seen. Because the sample size was relatively small, only very large changes in birth weight, head circumference, and birth length would be detectable with sufficient statistical power. In addition, these outcomes are indicative of effects that are likely much more severe than would be expected from the fairly low perchlorate exposures measured in this study (median urine perchlorate = 2.76 ug/L; 90th percentile = 4.35 ug/L). Finally, urine, rather than blood, is the most common metric used to assess perchlorate exposure, but urine perchlorate was only measured in 34 pregnant women and analyses of associations between maternal urinary perchlorate and fetal birth weight, head circumference, and birth length were not presented.

Cao *et al.*, 2010. Urinary concentrations of perchlorate, nitrate, iodine, thiocyanate, T4 and TSH were measured in 92 full term infants from Pennsylvania. In analyses adjusted for age, sex, and body mass index, increasing urinary perchlorate concentrations were associated with increasing urinary TSH concentrations, but only in children with low urinary iodine. The adjusted regression coefficient between the logarithm of urinary perchlorate and

logarithm of urinary TSH was 0.10 (95% CI, 0.01- 0.19) in children with urinary iodine levels < 100 µg/L and –0.04 (95% CI, –0.12-0.04) in children with higher iodine levels. Increasing urinary concentrations of perchlorate, nitrate, and thiocyanate were also associated with higher urinary T4. Both urinary levels of thyroid hormones and urinary levels of perchlorate (and other analytes) were "adjusted" for urine dilution by dividing their values by the subject's urinary creatinine concentrations. The use of urinary creatinine on both sides of the mixed model analyses (i.e., as part of the dependent variable and as part of the independent variable) may have led to the positive correlations identified in this study, making these results difficult to interpret.

Summary of Studies of Perchlorate and Infant Thyroid Hormone Levels

Table 29 summarizes data from the most relevant studies of perchlorate exposure and newborn thyroid hormone levels. In several studies, the authors did not specifically present results for the early newborn period, but provided data that OEHHA used to perform its own evaluation of possible associations for this period. These are clearly marked in Table 29 under the heading "Source of data and results." It should be noted that the data points calculated by OEHHA are not based on the original study authors' conclusions and most represent unadjusted estimates. Any conclusions drawn from this table should be interpreted in light of these facts. Table 30 lists the studies that were excluded from Table 29, with reasons for their exclusion. The Li *et al.* (2000) and Amatai *et al.* (2007) studies are excluded from this table because they did not include a substantial portion of subjects who had thyroid hormone levels measured within the first 24-36 hours after birth. As discussed above, this period is likely the most relevant time frame for assessing the effects related to maternal exposures during pregnancy. Tèllez Tèllez *et al*. (2005) is excluded from the table because 45 percent of the newborns from the exposed city were born in the unexposed city and therefore were probably not exposed at the time of birth. The Tèllez Tèllez *et al*. (2005) study is also inconsistent with the other studies in Table 29 because the very high iodine levels in this population may have protected the infants in this study from the effects of perchlorate and, more importantly, because a large fraction of women from the highly exposed town gave birth in the low exposure city. The Buffler *et al.* (2006) study was not included because it used the same data as Steinmaus *et al.* (2010) but did not focus on newborns.

As seen in Table 29, at least some evidence of an association was seen with perchlorate in each of the five data sources that included information on newborn thyroid hormone levels. Several aspects of these findings provide evidence that they represent real effects. First, despite major differences in study populations, study designs, time periods, funding sources, and research groups, these results are markedly consistent across studies. This type of consistency across different studies is one of the major tenets of causal inference (Hill, 1965; Rothman and Greenland, 1998b). Second, several of these results have very low p-values, which means that they are probably not due to chance. Third, these findings are consistent with the known biologic mechanism of perchlorate. That is, these

results show that perchlorate may decrease T4 and increase TSH, both of which are effects that are in the direction expected based on the known mechanism of action of perchlorate.

Several additional issues potentially affect the interpretation of these results and these are discussed in the following sections.

Ecologic assessment of perchlorate exposure: Most of these studies used average perchlorate concentrations in large community drinking water supplies as a surrogate for exposure to the individuals in that community. In all of these studies, the reported perchlorate concentrations were based on a relatively small number of samples. Average perchlorate concentrations were then assigned to individuals without knowledge of whether or not they drank the tap water, how much they drank, or for how long they drank it. In addition, drinking water is not the only source of perchlorate, and some exposure will come from food. Lack of data on other sources of perchlorate would likely cause further misclassification of the study subjects' true perchlorate exposure. Importantly though, study subjects were classified in terms of their perchlorate exposure independently of their thyroid hormone status. Thus, any misclassification of exposure is likely to be independent of thyroid hormone status (i.e., non-differential). This type of non-differential misclassification of exposure will likely bias results towards the null. That is, if an association truly exists, non-differential exposure misclassification will most likely cause the magnitude of the observed association to be less than the magnitude of the true association. It will not likely cause a false association or strengthen an association that is truly weak. There are some rare exceptions to this rule, but these exceptions are not likely applicable to the studies in Table 29 (Rothman and Greenland, 1998a).

Despite this misclassification bias, which was likely present to some degree in every one of the data sources evaluated, OEHHA found evidence of an association in each of the data sources presented in Table 29. If non-differential misclassification of exposure could somehow be corrected for in these studies, the effects found would likely be even greater than those reported.

Table 29. Information on Maternal Perchlorate Exposure during Pregnancy and Thyroid Hormone Levels in Early Newborns

Location and source of data	Exposed (exposure level in water)	Unexposed	Results: Exposed group compared to the unexposed group		Source of data and results	Adjustments, stratifications or exclusions	Summary notes regarding confounding[a] and other related findings	
			T4	TSH				
California	Redlands (9 ppb)	Rest of San Bernardino & Riverside Counties	No data	OR for high TSH = 1.57 (95% CI, 1.14-2.16; p < 0.0001) (Table 23 and page 63)	Calculated by OEHHA using raw data provided in Kelsh *et al.,* 2003	Unadjusted results	Adjustments for birth weight, ethnicity, sex, birth year, age, and multiple births had little effect on the authors' original results.	OR for low T4 = 1.18 (95% CI, 1.13-1.24; p < 0.0001) for all ages (Table 22 and pages 62-63)
California	Exposed communities (> 5 ppb)	Unexposed communities (< 5 ppb)	No data	OR for high TSH = 1.53 (95% CI, 1.24-1.89; p < 0.0001)	Steinmaus *et al.,* 2010	Age, gender, race/ ethnicity, feeding type, birth weight, outliers, household income.	None of these factors except formula feeding had major impacts on results.	
Arizona	Yuma (4-6 ppb)	Flagstaff	No data	Mean TSH = 27% higher (Table 26 for day 0)	Calculated by OEHHA using data and results provided in Brechner *et al.,* 2000	T4 findings based on unadjusted analyses. TSH findings in analyses stratified by age and race/ethnicity.	In follow-up analyses, TSH remained elevated in the exposed town after entering age, race/ethnicity, and sex into the statistical models.	OR for low T4 = 1.19 (95% CI, 1.05-1.33; p = 0.006) for all ages (Table 25 and pages 67-68)

Table 29 (continued). Information on Maternal Perchlorate Exposure during Pregnancy and Thyroid Hormone Levels in Early Newborns

Nevada	Las Vegas (0-9 ppb)	Reno	Mean T4 ≈ 22% lower	No data	Estimated by OEHHA using the data presented in Figure 3 of Li *et al*., 2000a	Unadjusted result	In the analyses done by the authors, adjustments for sex, birth weight, and age appear to show no major confounding effect.	
Chile	Taltal (100 ppb)	Antofagasta and Chañaral (0-5 ppb)	No data	Mean TSH ≈ 45% higher	Based on the results presented in Table 8 of Crump *et al.,* 2000	Results stratified by age	Median age greater in Taltal than other cities (6.6 vs. 4.1 days). Correcting for this would increase TSH levels in Taltal.	OR for a family history of thyroid disease in all lifelong residents was 4.97 (95% CI, 1.29-19.17)

[a]Further details on the assessments of confounding are provided in the next section.

Table 30. Excluded Studies

Excluded studies	Reason for exclusion
Tèllez Tèllez *et al.,* 2005	45% of women from the exposed city delivered in the unexposed city.
Li *et al.,* 2000b	TSH measurements collected on first day after birth were excluded.
Amatai *et al.,* 2007	< 10% of newborns had thyroid hormones measured in first 36 hr after birth.
Cao *et al.,* 2010	Adjustments for urinary creatinine could have created false associations. No data in neonates.
Buffler *et al.,* 2006	Used the same data as Steinmaus *et al.* (2010), but did not focus on newborns.

Misclassification of thyroid hormone status: The studies discussed above assessed thyroid hormone status using a single measurement of thyroid hormone. Thyroid hormone levels can vary in individuals from day to day and within a day. This variability means that a single assessment of serum thyroid hormone concentration could lead to misclassification of true long-term thyroid hormone status in some individuals. Importantly though, any errors in misclassifying outcome are likely to be the same as those associated with misclassifying exposure: non-differential misclassification that will likely bias results to the null. As with exposure misclassification, if these errors could be corrected, the effects reported in the positive studies listed above would likely be even greater than those reported.

Confounding:

a. General concepts

Several general concepts were considered in evaluating confounding in these studies. The first is that a factor must be associated with both the exposure (perchlorate) and the outcome (thyroid hormones) of interest to cause confounding. A factor may be a strong determinant of thyroid hormone levels, but if it is not associated with perchlorate exposure then it is unlikely to cause important confounding (Axelson, 1978). For many of the factors potentially related to thyroid hormone levels such as menopause, premenarche, physical activity, c-reactive protein, thyroid antibodies, thyroid diseases, use of certain medications, and many others there is no evidence or plausible reason to support that they are also associated with perchlorate exposure. As such, there is no evidence or logical reason to believe that they are important confounders in the studies presented in this section.

The second general concept is that in order for a factor to cause important confounding, it not only needs to be associated with the exposure and the outcome of interest, but these associations must be fairly strong (Axelson, 1978). A factor that is only weakly associated with either the exposure or the outcome may still cause some confounding, but the impact of this confounding on the study result will usually be minor and likely unimportant.

An example of this is given below for thiocyanate. In this example, and in the other examples provided in this section, the focus is on those factors which would most likely cause confounding and bias, including those factors commonly referred to in the current perchlorate literature. Here, the potential effect of thiocyanate as a confounder on studies assessing T4 is assessed using the methods presented by Axelson (1978) (Table 31). In this example, the mean serum T4 concentrations for various levels of thiocyanate exposure were obtained using data from NHANES 2001-2. The thiocyanate categories used in this example are the tertiles of thiocyanate in women from NHANES 2001-2 (Steinmaus *et al.,* 2007). These categories were selected because they were the ones in which perchlorate-T4 associations were found to differ in Steinmaus *et al.* (2007). As shown in these analyses (Table 31), mean T4 decreases as thiocyanate increases from the lowest (< 750 µg/L) to the highest tertile (> 1800 µg/L), but this decrease is small. Because the effect of thiocyanate on T4 is small, even if the proportion of women with high thiocyanate levels is twice as high in a study's higher perchlorate-exposed group (e.g., 66.7 percent with high thiocyanate, last row) as in the study's lower perchlorate-exposed group (e.g., 33.3 percent with high thiocyanate, shaded row) this would have only a very small effect (1.4 percent) on the mean difference in T4 between the perchlorate higher and lower exposed groups. This example shows that even if thiocyanate exposure is associated with both thyroid hormone levels and with perchlorate exposure, these associations are likely too weak to cause the effects seen in Table 29. The same is likely true for many other factors such as race, age, sex, obesity, socioeconomic status, nitrate, and iodine. In other words, while it is possible that all of these factors could be associated with both thyroid hormone levels and perchlorate exposure, the magnitude of these associations are, like thiocyanate, probably too weak to cause important confounding. Thus, while they may cause some confounding (e.g., they could change an unadjusted odds ratio of 1.52 to an adjusted odds ratio of 1.53 as in Steinmaus *et al.,* 2010), the magnitude of the confounding effect will likely be too small to affect the overall study conclusions.

Table 31. Analysis of the Potential Magnitude of Confounding by Thiocyanate Using the Methods of Axelson, 1978

Thiocyanate (µg/L)	<750	750-1800	>1800	M_o	Mean Diff	Mean Diff
Mean T4 (µg/dL):	8.632	8.496	8.272	(µg/dL)	(µg/dL)	%
Lower perchlorate exposure	33.3%	33.3%	33.3%	8.47	Ref	Ref
Higher perchlorate exposure	31.7%	33.3%	35.0%	8.46	0.01	0.1%
	26.7%	33.3%	40.0%	8.44	0.02	0.3%
	21.7%	33.3%	45.0%	8.42	0.04	0.5%
	16.7%	33.3%	50.0%	8.41	0.06	0.7%
	0.0%	33.3%	66.7%	8.35	0.12	1.4%

M_o, mean T4 expected in a group of subjects with this distribution of thiocyanate; Mean Diff, mean difference in T4 in the lower perchlorate-exposed group minus the higher perchlorate-exposed groups; Ref, lower perchlorate-exposed reference group

A third general concept of confounding is that factors that are very rare are unlikely to cause significant confounding in large population-based studies. These factors include rare thyroid diseases, certain genetic conditions, and the use of certain medications known to impact thyroid hormone levels.

Fourth, it is important to note that each of the five positive studies listed in Table 29 involved different study populations, different time periods, different study methods, and different research groups. Despite all of these differences, the effects identified across all of these studies were similar and consistent. This consistency decreases the likelihood that confounding is responsible for all of the effects identified. It is possible that the same confounder affected each of these studies. However, some of these studies adjusted or stratified for several of the factors known to be among the most important population-wide determinants of thyroid hormone levels (age, sex, and race) and found these adjustments made very little difference. It is possible that those studies which did adjust or stratify for confounders were not impacted by confounding while those studies or analyses which were unadjusted were affected by confounding. However, there is no evidence or plausible reason to support that this is the case, and again, the consistency of the findings across the different studies argues against this. It is also possible that each of the five studies or analyses in Table 29 was affected by five entirely different confounders. However, it seems highly unlikely that five different confounders would all lead to essentially the same effect in five different studies and in five different study populations. All told, the consistency of the effects seen in the different studies in Table 29, combined with the fact that adjusted results showed essentially no evidence of confounding (see below), argues against the idea that confounding played a major role in these results.

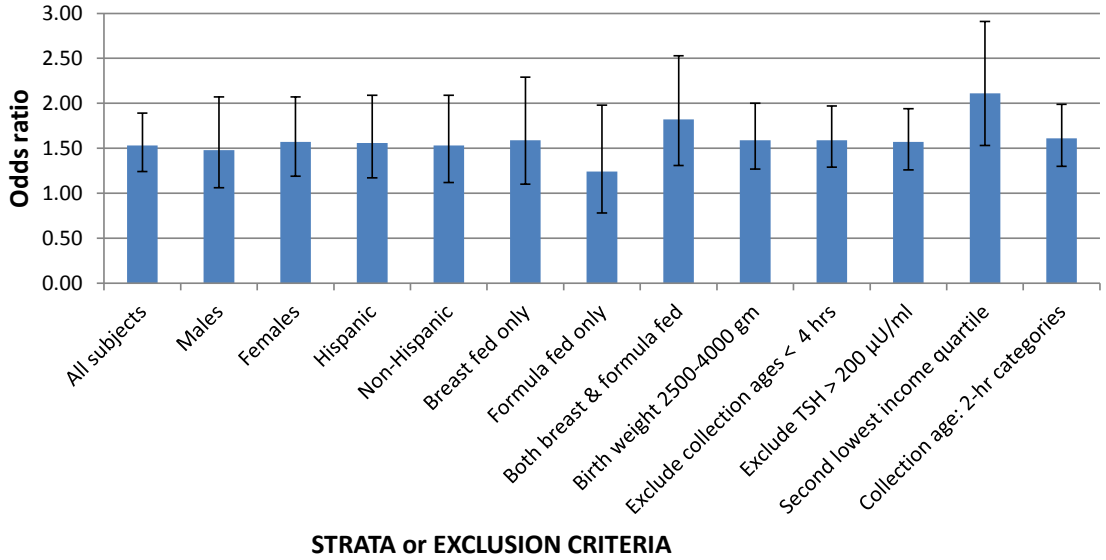
Finally, many of the factors related to thyroid hormone might not cause important confounding for the reasons given above, but they may still act either cumulatively or synergistically with perchlorate to decrease thyroid function. Certain factors such as nitrate and thiocyanate act by the same mechanism as perchlorate, and as discussed in the following sections, some evidence exists that people exposed to one or more of these agents may be particularly susceptible to perchlorate. For example, some perchlorate-thyroxine findings from NHANES suggest that thiocyanate, perchlorate, and low iodine intake could act cumulatively or synergistically (Blount *et al.,* 2006; Steinmaus *et al.* 2007; Steinmaus *et al.,* 2013). Other factors, such as polychlorinated biphenyls (PCBs) or anti-thyroid antibodies likely affect thyroid function by different mechanisms but might also act cumulatively or synergistically with perchlorate to adversely impact thyroid function. Importantly, this type of effect is not confounding, and is usually not evaluated or "controlled" in the same way as confounding. Instead, these types of effects would create groups of people who may be especially susceptible to perchlorate.

b. Evidence for or against confounding in each study

Steinmaus *et al.,* 2010: As discussed above, this study adjusted for many different potential confounding variables including age, gender, race/ethnicity, type of feeding, birth weight, and community average household income, and these had little impact on study results and no impact on study conclusions. For example, in the analysis of whether TSH levels were greater than 25 µU/mL in the first 24 hours after birth, the unadjusted odds ratio for having elevated TSH, comparing perchlorate-exposed and unexposed communities, was 1.52. After adjusting for age, gender, race/ethnicity, type of feeding, birth weight, and community average household income, the odds ratio was 1.53. Thus, the excess odds ratio changed by less than 2% with adjustment. This very small 2 percent change is strong evidence that none of these factors caused major confounding. It also provides evidence that residual confounding from incomplete adjustment of any of these factors is likely to cause effects that are even smaller than 2 percent.

In addition to statistical adjustments, the use of stratified analyses and exclusion criteria are two other ways of evaluating the role of confounding. Figure 8 shows the odds ratios for having a TSH level above 25 µU/mL comparing neonates from perchlorate-exposed and unexposed communities in analyses using a variety of stratification and exclusion criteria. As shown, statistically significant elevated odds ratios are seen in all analyses except the analysis of formula fed infants. The lower odds ratio in formula fed infants is consistent with the exposure misclassification soon after birth discussed on page 60 above. The fact that the elevated odds ratios remained in all the other analyses is evidence that none of these factors had important effects on results, or more importantly, on the conclusion that perchlorate exposure is associated with increased neonatal TSH levels in this dataset.

Figure 8. Odds Ratios for TSH > 25 µU/mL within 24 Hours of Birth Comparing Perchlorate-exposed (> 5 µg/L) and Unexposed (≤ 5 µg/L) Communities in Analyses Stratified by Age, Gender, Race/Ethnicity, Feeding Type, Birth Weight, or TSH Outliers (Steinmaus *et al.,* 2010) [The vertical lines are 95% confidence intervals]



Kelsh *et al.,* 2003: Using the data provided in the tables of this article, OEHHA estimated an odds ratio of 1.18 (95% CI, 1.13-1.24; $p < 0.0001$) for having a low T4 level and an odds ratio of 1.57 (95% CI, 1.14-2.16; $p < 0.0001$) for having an elevated TSH level. While it is possible these elevated odds ratios are due to some unknown confounding, several pieces of evidence argue against this. First, in the analyses done by Kelsh *et al*., adjustments for birth weight, ethnicity, sex, birth year, age, and multiple births had little impact on the odds ratios they calculated. For example, comparing Redlands (exposed) to the rest of San Bernardino and Riverside Counties (unexposed), the odds ratio for having an elevated TSH level changed from 1.28 to 1.24 after adjustments for all of these factors. This small change suggests that none of these factors had important confounding effects in this study. Therefore, despite the fact that a few of these factors were shown to be related to neonatal TSH levels in this study (e.g., ethnicity and birth weight), they were not different enough between perchlorate-exposed and unexposed areas to cause major confounding. This provides strong evidence that the unadjusted odds ratios calculated by OEHHA are also not due to confounding by these factors since the OEHHA analyses involved the exact same perchlorate-exposed and unexposed areas used in the original authors' own calculations.

A second piece of evidence that the unadjusted odds ratio calculated by OEHHA is not due to confounding is the fact that this unadjusted odds ratio is remarkably close to the adjusted odds ratio in the corresponding analysis in Steinmaus *et al.* (2010). That is, for subjects with TSH collection ages within 18 to 24 hours after birth, the odds ratio for having TSH > 25 µU/mL was 1.57 using unadjusted data

from Kelsh *et al.* (2003) and 1.53 in the Steinmaus *et al.* (2010) adjusted analyses. The similarity of these two odds ratios, combined with the fact that adjustments or stratifications for age, gender, race/ethnicity, feeding type, birth weight, and community household income had little effect on the Steinmaus *et al.* (2010) results provides additional evidence that the elevated unadjusted odds ratio calculated by OEHHA using the Kelsh *et al.* (2003) data is not due to confounding by the factors adjusted for in Steinmaus *et al.* (2010).

Brechner *et al.*, 2000: An odds ratio of 1.18 (95% CI, 1.05-1.33; p=0.006) for decreased T4 and a 27 percent higher TSH level was found comparing perchlorate-exposed Yuma to unexposed Flagstaff. The T4 odds ratio was based on unadjusted data provided in the article while the TSH results were based on analyses stratified by age and race/ethnicity. In a subsequent Letter to the Editor, the authors state that the difference in TSH levels remained higher in Yuma than Flagstaff when sex, age, and race/ethnicity were included in the analysis of variance (p= 0.009), suggesting that these three variables were not strongly related to perchlorate exposure and were not responsible for the relationship between perchlorate exposure and TSH identified in this study. Because these three variables caused little confounding in the analysis by Brechner *et al*. (2000), and because the unadjusted odds ratio of 1.18 was calculated using the exact same perchlorate exposed and unexposed areas (Yuma and Flagstaff, respectively) as defined by the authors of this study, it is unlikely these factors confounded and are responsible for the elevated unadjusted odds ratio of 1.18 calculated by OEHHA. Lamm (2003) suggests that the differences in altitude between the two cities might be responsible for the effect identified. However, Lamm provides no evidence for this, and as discussed above, several studies show that altitude causes effects that are directly opposite to those proposed by Lamm (2003). The likelihood that other potential confounders such as socioeconomic status, iodine, or other environmental chemicals had major effects is discussed below.

Li *et al.*, 2000a: Based on data provided in Figure 3 of this article, newborn T4 levels on day 1 after birth appear to be about 22 percent lower in perchlorate-exposed Las Vegas than in unexposed Reno. On almost all other days after birth except day 1, T4 levels appear to be similar between the two cities. This pattern (effects seen on day 1 after birth but not later) is consistent with the likely impacts of exposure misclassification on maternal perchlorate-neonatal TSH relationships that occurs several days after birth (see pages 59-60). In the authors' analyses, adjustments for gender, birth weight, and age appear to have very little effect on results. For example, in the unadjusted analyses, the difference in mean T4 levels between Las Vegas and Reno was close to zero (mean difference = 0.01; means of 17.11 and 17.12 µg/dL, respectively; p = 0.901). In the adjusted analysis, this difference was also about zero (mean difference = 0.069 µg/dL, p = 0.407). As in the preceding studies, the very small difference between the adjusted and unadjusted analyses provides evidence that confounding had very little impact on these results and that age, birth weight, and

sex were not strongly enough related to both perchlorate exposure or thyroid hormone levels to cause substantial confounding.

Crump *et al.,* 2000: In an unadjusted analysis of TSH measurements collected on days 1-2 after birth, the median TSH level in the high exposure city of Taltal was 45% higher than that in the low exposure city of Antofagasta, although the sample sizes were small. Several factors suggest that this result is not due to confounding. First, TSH measurements were, in general, collected later in the exposed city of Taltal than they were in the unexposed city of Antofagasta. Since TSH levels tend to decrease with time after birth, based on this factor alone, one would expect that TSH levels would be lower in Taltal than Antofagasta. Given this, adjusting for age at the time of TSH collection would most likely increase, not decrease the difference seen between Taltal and Antofagasta. Second, in addition to the TSH findings, this study also reported elevated odds ratios (ORs) for a family history of thyroid disease in Taltal compared to Antofagasta (OR = 3.35, 95% CI, 1.19-9.38 in all subjects and OR = 4.97, 95% CI, 1.29-19.17 in lifelong residents). These odds ratios were adjusted for age, sex, and urinary iodine. The unadjusted odds ratio for a family history of thyroid disease in all subjects, which could be calculated using the data provided in their Table 3, was 2.85 (95% CI, 1.07-7.42). The fact that the unadjusted odds ratio is only a little lower than the adjusted odds ratio (2.85 versus 3.35) suggests that while age, sex, and urinary iodine had some confounding effects, the magnitude of this confounding was not strong enough to mask an association. The fact that age, sex, and urinary iodine did not cause significant confounding in the analysis of family history of thyroid disease provides that these factors are not likely to cause major confounding in the analysis of neonatal TSH levels. The notion that iodine is an unlikely confounder in this study is also supported by the fact that the mean urinary iodine levels reported in children in the two cities is very similar: 75.6 ± 40.4 µg/dL in Antofagasta and 76.6 ±47.4 µg/dL in Taltal. This similarity provides strong evidence that iodine was not related to this study's main metric of perchlorate exposure (city of residence) and thus was not likely a major confounder. Ethnic differences are unlikely to have caused the effects seen since there are no major ethnic differences between these cities. Finally, nitrate levels in these cities are below U.S. recommended levels, so nitrate was an unlikely cause of the positive effects seen.

c. Other data on confounding

In this section we evaluate the likelihood that various individual uncontrolled factors might have caused confounding in the studies discussed above. Since many different things can affect thyroid hormone levels, one could hypothesize that many different things could potentially cause confounding. However, since few are strongly related to perchlorate exposure, few are likely to cause confounding. Because of this, OEHHA chose not to evaluate each of the hundreds of different factors that can affect thyroid hormone levels, but rather chose those factors that seem to be discussed most often in the current literature on perchlorate. These factors include iodine, nitrate, other environmental

chemicals like polychlorinated biphenyls (PCBs), and thyroid antibodies. Other factors such as race/ethnicity and thiocyanate were discussed above. The age of neonatal sample collection is not discussed here since all of the studies in Table 29 either adjusted for or stratified by this variable.

Iodine and nitrate: Data from Blount *et al.* (2006) and Steinmaus *et al.* (2007) suggest that iodine is more likely to be an effect modifier than a confounding variable. In other words, findings from these studies suggest that low iodine intake (and high thiocyanate levels) are more likely to produce additive or synergistic effects on thyroid hormone levels with perchlorate than cause false associations between perchlorate and thyroid hormone levels. In both Blount *et al.* (2006) and Steinmaus *et al.* (2007), adjusting for thiocyanate and nitrate had little effect on the perchlorate-T4 regression coefficient in women with low iodine levels. While some studies suggest that nitrate intake may affect thyroid hormone levels, thyroid volume, or goiter, questions regarding actual intake levels, control for iodine intake, and blinding of outcome assessments raise concern about the validity of their results (Tajtakova *et al.,* 2006; Gatseva *et al.,* 2008). Also, common nitrate exposures in the U.S. may not be high enough to affect thyroid function. In a clinical trial, a nitrate dose of 15 mg/kg-day for 28 days did not decrease thyroidal iodide uptake or impact thyroid hormone levels in 10 healthy volunteers (Hunault *et al.,* 2007). This intake level is 5-20 times, or more, higher than average nitrate intakes reported in several populations in Europe and the U.S. (OEHHA, 2000). These findings suggest that nitrate is an unlikely cause of the effects identified in the positive studies discussed above. Although iodine intake has been shown to affect thyroid hormone levels, these effects may only occur at extreme values of iodine, not at the levels most commonly seen in the U.S. For example, Soldin *et al.* (2005) found little evidence of an association between urinary iodine concentrations and serum levels of T4 or TSH in NHANES. Since the potential for confounding is directly related to the strength with which the potential confounder is associated with the outcome of interest, these findings suggest that iodine is very unlikely to cause significant confounding unless studies are done in areas where a very large fraction of the population has very low iodine intakes (and this low intake is associated with perchlorate exposure).

Other environmental chemicals: Some studies have found associations between certain chemicals such as PCBs and thyroid hormone levels (Chevrier *et al*., 2007), but these effects are generally small and there is little evidence that the relationship between PCB exposure and perchlorate exposure is strong enough that PCBs are likely to cause important confounding in studies of perchlorate and thyroid hormones. To further evaluate this, correlation coefficients between urinary perchlorate levels and serum PCB levels were calculated using data from NHANES 2001-2 (the same data used in Blount *et al.,* 2006 and Steinmaus *et al.,* 2007). As shown in Table 32 these correlations are very low and therefore too small to cause any significant impacts on the study results reported above.

Table 32. Spearman Correlation Coefficients (R) between Serum PCBs and Urinary Perchlorate, NHANES 2001-2

PCB	R	p-value	N
52	-0.04	0.09	1474
99*	0.04	0.09	2192
101	-0.01	0.64	2217
118	0.06	0.009	2217
153	0.05	0.01	2216
156	0.06	0.005	2206
180	0.05	0.01	2212
183*	0.01	0.38	2216
184	0.04	0.05	2217
194*	0.05	0.02	2191
199*	0.05	0.01	2202

*The congeners with correlation coefficients > 0.10 between the PCBs and neonatal TSH levels in Chevrier *et al*. (2007).

Thyroid autoantibodies: In its recent report on perchlorate, the American Thyroid Association (2006) calls thyroid autoantibodies "an important confounder in thyroid physiology." In order to evaluate the possibility that these might be important confounders, OEHHA used data from NHANES 2007-8 to evaluate the magnitude of the association between thyroid autoantibodies and serum T4 using linear regression analyses adjusted for age and sex. As shown in Table 33, the magnitudes of these relationships were very small suggesting that these antibodies are unlikely to be important confounders in general population-based studies.

Table 33. Age and Sex Adjusted Associations between Total Thyroxine (T4) (μg/dL) and Thyroid Autoantibodies in NHANES 2007-8.

	Coefficient	SE	p-value
Thyroid peroxidase antibody (IU/mL)	-0.000233	0.00121	0.85
Thyroglobulin antibody (IU/mL)	-0.000221	0.000208	0.31

Study size: Several of these studies involved a very large number of study subjects [Kelsh *et al.* (2003), Brechner *et al.* (2000), and Buffler *et al.* (2006)], and the p-values are less than 0.001. These very low p-values provide evidence that the elevated odds ratios identified in these studies are unlikely due to chance. In Brechner *et al.* (2000), although the odds ratio for low T4 was somewhat small (OR = 1.18; 95% CI, 1.05-1.33), the p-value was 0.006, which again, suggests that the excess odds are unlikely due to chance.

A lack of statistical power may have affected some of the smaller studies. If relative risks are expected to be fairly low (e.g., less than 2.0), large sample sizes are needed to detect statistically significant associations. The small sample sizes and relatively low prevalence rates of the outcomes of interest of some studies might have limited their ability to identify true effects.

Subject selection: There is a concern about the way the neonate blood samples were selected for TSH determination in the studies reported by Brechner *et al*. (2000), Kelsh *et al*. (2003), and Li *et al*. (2000b). In these studies, TSH levels were only measured in newborns who had low T4 levels. The TSH findings of these studies are still important, however, because they indicate that the risk of having *both* a low T4 level and a corresponding high TSH level is greater in newborns from perchlorate-exposed areas than in newborns from unexposed areas. This is important since people with both low T4 and high TSH are more likely to have real thyroid effects than people with just a high TSH level. For example, a high TSH reading in a person who does not have a correspondingly low T4 level could just reflect normal intra- or inter-individual variability, or laboratory or collection error. Regardless, in each of these studies, T4 was measured in all subjects and at least some evidence was seen in each study that perchlorate was associated with a decreased T4. Given the known relationship between T4 and TSH, these decreases in T4 levels are biologically consistent with the reciprocal increases in TSH that were reported. This consistency provides evidence that the associations identified between perchlorate and increased TSH are real effects and are not solely due to bias from selective TSH sampling.

Other susceptibility factors not accounted for: Another important issue in these studies is the overall lack of data on co-variates that might interact with perchlorate to impact thyroid function. None of these studies specifically investigated potentially susceptible subpopulations such as people who have low iodine intakes, smokers, people with anti-thyroid antibodies, or people with high intakes of nitrate or thiocyanate from foods. Risks of thyroid-related effects may be greater in these groups than in the general population samples that were used in the studies in Table 29.

The TSH surge: For the reasons discussed above, the first 24 hours after birth may be the most relevant period for assessing associations between maternal perchlorate exposure during pregnancy and newborn thyroid hormone levels. One potential complicating factor of measurements collected during this time is the normal physiologic surge in TSH that occurs soon after birth. The exact causes of this surge are unknown, but mechanisms related to cold exposure, the shock of birth, or an acute drop in maternal T4 have been proposed. As shown in Figure 9, TSH levels surge right after birth, typically peak at 30 minutes, then gradually fall to normal levels in the next 24-48 hours (Fisher and Klein, 1981). Several authors have warned against measuring TSH levels during this period because it can lead to an increased rate of false positives when screening for congenital hypothyroidism. In other words, many children who have TSH

readings above 25 µU/mL (the traditional cut-off point for this diagnosis) during the first 24 hours may have normal TSH readings a few days later and not require treatment for congenital hypothyroidism.

However, a clinical diagnosis of congenital hypothyroidism is not the only outcome that should be assessed when looking at the possible impacts of perchlorate. This diagnosis is generally associated with very large changes in thyroid hormone levels and severe effects if untreated. As discussed above, much more subtle changes in thyroid hormones have been associated with cognitive effects in children in several studies (Pop *et al*., 1999, 2003; Haddow *et al*., 1999; Klein *et al*., 2001; Kooistra *et al*., 2006; Vermiglio *et al*., 2004). That is, small changes in thyroid hormone levels that are within normal reference ranges have been associated with significant decreases in IQ in children that were not clinically hypothyroid and showed no other evidence of thyroid problems. If researchers only focus on the more severe effect levels that are associated with most cases of congenital hypothyroidism, these more subtle effects will be missed. For this reason, researchers should not only evaluate whether perchlorate is associated with clinically treatable congenital hypothyroidism (and the large TSH changes associated with this diagnosis), but should also investigate whether perchlorate is associated with even smaller changes in thyroid hormone levels. In this regard, while the studies in Table 29 provide little evidence that perchlorate increases the rate of clinical congenital hypothyroidism, they do provide evidence that perchlorate is associated with more subtle changes in newborn thyroid hormone levels.

<u>Health consequences are unknown:</u> The long-term health consequences of the effects seen in Table 29 are unknown. As discussed below, subtle changes in maternal thyroid hormone levels during pregnancy have been linked to cognitive effects in the offspring. This suggests that the fetus is highly sensitive to any changes in thyroid hormone levels during pregnancy. It is unknown whether the neonate is similarly sensitive.

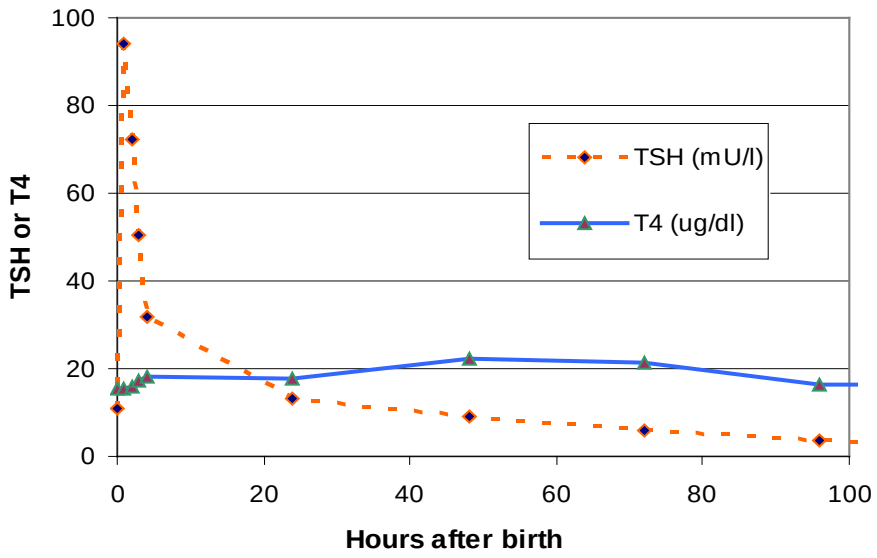
It is possible that the effects seen in Table 29 are also occurring in the fetus. This possibility is consistent with the well-established mechanism of perchlorate, by which a long-term continuous exposure would be expected to cause continuous effects on the thyroid. That is, if the mother is consuming drinking water contaminated with perchlorate during pregnancy, it would be expected that the fetus would be exposed to perchlorate during pregnancy and that some perchlorate would be present in the newborn immediately after birth. If this perchlorate exposure can lead to altered thyroid hormone levels in the newborn (as shown in Table 29), it seems likely that it is also doing so in the fetus. If perchlorate does impact thyroid hormone levels in the fetus, it may cause the same cognitive developmental impacts seen in the studies of maternal thyroid hormone changes during pregnancy (Pop *et al*., 1999, 2003; Haddow *et al*., 1999; Klein *et al*., 2001; Kooistra *et al*., 2006; Vermiglio *et al*., 2004). Whether or not this is the case is currently unknown, and further research is needed to

determine if the effects seen in Table 29 are truly associated with long-term health outcomes.

It is possible that the effects reported in Table 29 are simply temporary effects that occur in the day-old newborn and do not occur in the fetus or older child. For example, it is possible that perchlorate somehow only affects the TSH surge, and either does not affect the thyroid at all (i.e., an extra-thyroidal mechanism), or does not affect the thyroid before or after the surge. There are several reasons to believe that this is not the case. First, the data in Table 29 are not consistent with an extra-thyroidal mechanism. 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. Second, TSH peaks about 30 minutes after birth (Figure 9), and the effects reported in Table 29 mostly involve measurements taken after the major part of this peak. For example, in Buffler *et al.* (2006), of all the newborns who had TSH levels measured in the first 24 hours after birth, less than one percent had measurements within the first three hours of birth. This small fraction suggests that any measurements collected during the highest points of the TSH peak had little impact on the Table 29 results. Finally, as discussed above, the hypothesis that the results seen in Table 29 simply represent a short-term temporary effect that occurs only in the day-old newborn (and not in the fetus) is inconsistent with the well-established mechanism in which a continuous dose of perchlorate is known to cause a continuous suppression of thyroidal iodide uptake and a continuous suppression of thyroid hormone production. Given the abundance of data supporting this mechanism, it seems somewhat implausible that some new and completely unknown mechanism, with no evidence to support it, would be causing these effects.

In summary, the data in Table 29 provide a consistent body of evidence linking perchlorate exposure during pregnancy with changes in thyroid hormone levels in the newborn. Currently, the long-term health implications of these effects are unknown. However, given the known mechanism of perchlorate, these effects may represent effects that are also occurring during the fetal period, which is a critical period of thyroid-hormone-dependent brain development.

Figure 9. Changes in Serum Neonatal T4 and TSH Levels by Hour after Birth (Abuid *et al*., 1973; Cavallo *et al*., 1980; Fisher and Odell 1969; Sack *et al*., 1976) [Data are compiled by OEHHA from averaged individual thyroid hormone measurements from the cited studies from infants with blood collected at different times].



Studies of Perchlorate's Effects on Iodide Uptake and Thyroid Hormone

Clinical Dosing Studies

Stanbury and Wyngaarden, 1952. These investigators studied the effect of perchlorate on the discharge and uptake of iodide by the thyroid in Graves' disease patients. To study the effect of perchlorate on the discharge of accumulated iodide, they gave 30 mg of 1-methyl-2-mercaptoimidazole orally to eight patients. A dose of 200 mg of propylthiouracil was given to a ninth patient. One hour later, a tracer of ^{131}I was given. The accumulation of this isotope in the neck was recorded at frequent intervals until it leveled off. At this point, potassium perchlorate doses varying from 3 to 500 mg were given orally in small volumes of water. In each patient except the one treated with propylthiouracil there was a sharp fall in the counting rate within a few minutes after the ingestion. This always occurred within 30 minutes. With smaller doses the discharge of the ^{131}I was incomplete, but doses of 100 mg caused a fall in counting rates nearly equal to the counting rates recorded from the thigh (background). The investigators also reported that a single oral dose of 10 mg perchlorate caused about a 50 percent release of accumulated iodide. Potassium perchlorate doses as low as 3 mg (equivalent to 2.2 mg perchlorate) caused detectable, but incomplete, release of iodide from the thyroid. Assuming an adult body weight of 70 kg, this is equivalent to an oral dose of 31 μg/kg.

A LOAEL is not identified for this experiment because: (a) the number of patients per dose group is not known; (b) it is an acute exposure; (c) the patients suffered from a thyroid disease which might have affected iodide uptake; and (d) the patients were pretreated with drugs (either 1-methyl-2-mercaptoimidazole or propylthiouracil) that may enhance the release of iodide in the thyroid gland by preventing the oxidation of iodide ion to iodine and thyroid hormone synthesis.

To study the effect of perchlorate on the uptake of iodide in the unblocked gland, Stanbury and Wyngaarden (1952) gave 100 mg of potassium perchlorate to three patients and an hour later, tracers of ^{131}I. No thyroid hormone-disrupting drugs were given. Several days later each patient received a control tracer without previous perchlorate. In two cases, the studies were continued for 48 hours, but in the third, an observation period of only five hours was possible after the tracer. For the two patients with the long observation time, the control uptake was about 70 percent of the administered dose at 24 and 48 hours. When the patients were pretreated with potassium perchlorate, the uptakes were approximately 12 percent and 21 percent of the administered tracer dose at 24 and 48 hours following the administration of the tracer.

The duration of the inhibition of iodide uptake after the oral administration of 100 mg of potassium perchlorate (71.8 mg of perchlorate) appeared to be about six hours. Beyond six hours, accumulation of ^{131}I commenced. Durand (1938; as cited in Stanbury and Wyngaarden, 1952) found that at this time approximately half the administered dose of perchlorate has been excreted in the urine.

A LOAEL is not identified for this experiment because: (a) there were only two subjects that completed the experiment; (b) it is an acute exposure; and (c) the patients suffered from a thyroid disease which might have affected iodide uptake by the thyroid.

Godley and Stanbury, 1954. This study reported using potassium perchlorate to treat 24 patients with Graves' disease. Patients were treated with 600 to 1,200 mg/day for at least 11 weeks with a few as long as 52 weeks. Thirteen patients had determinations of the uptake of radioactive iodide by the thyroid both before beginning perchlorate therapy and within two weeks after medication had begun. The mean control uptake was 77.5 percent, with a range from 60.7 to 108 percent. The mean uptake during perchlorate therapy was 15.9 percent, with a range from 3.4 to 38.8 percent.

Bürgi *et al*., 1974. This investigation studied the effects of perchlorate treatment on the release of endogenous iodide from the thyroid glands of five normal healthy volunteers (three females and two males). The volunteers were given ^{125}I-labeled iodide and ^{131}I-labeled T4 for seventeen days, followed by 3 x 200 mg/day (9.7 mg/kg-day) perchlorate for eight days. Analysis for the two tracers in the urine and serum of the subjects showed that this dose was sufficient to totally block iodide uptake by the thyroid. Additionally, the perchlorate treatment

caused an increase in excretion of non-thyroxine iodine of 65 percent above background.

Brabant *et al*., 1992. In this study five healthy male volunteers were pretreated with 200 μg/day iodine for four weeks before perchlorate exposure. Iodine exposure was discontinued, and the volunteers were given 3 x 300 mg/day of perchlorate for another four weeks. Serum levels of T3 and T4 were measured at the end of the four-week perchlorate-dosing period. Perchlorate treatment had no effect on serum T3 or T4 levels or on thyroid gland volume. However, serum fT4 and TSH levels were significantly diminished by treatment, and thyroglobulin serum levels were almost doubled, indicating the stress of the treatment on the thyroid hormone balance. The perchlorate treatment also reduced intrathyroidal iodine levels.

In a follow-up study, Brabant *et al.* (1994, as cited in U.S. EPA, 2002) repeated the earlier studies with perchlorate treatment lasting longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects, although TSH levels did not increase.

Lawrence *et al*., 2000. The investigators administered perchlorate to nine healthy male volunteers and monitored the impact on thyroid function. Each subject ingested 10 mg of perchlorate (as potassium perchlorate) dissolved in a liter of spring water for 14 days. Baseline serum TSH, total T4, total T3, 24-hour thyroid ^{123}I uptakes, serum and 24-hour urine perchlorate, and 24-hour urine iodine were determined. All blood and urine tests were repeated on days 7 and 14 of perchlorate administration, and 24-hour thyroid ^{123}I uptakes on day 14 of perchlorate administration. All tests were repeated 14 days after perchlorate exposure was discontinued. No effect of perchlorate was observed on serum T4, T3 and TSH (Table 34). It should be noted that the dietary iodine intake levels of the subjects were rather high, as indicated by the high urine iodine values (Table 35). Because iodide and perchlorate compete for the same receptor site on the sodium-iodide symporter (Wolff, 1998), a high dietary iodine intake may reduce the impact of perchlorate on the thyroid. There was no statistically significant difference in serum perchlorate levels after 7 days and 14 days of exposure, indicating no apparent accumulation of perchlorate in the systemic circulation over that period.

Table 34. The Effect of Perchlorate Administration (10 mg/day, about 0.14 mg/kg-day) for 7 and 14 Days on Thyroid Function Tests (Lawrence *et al*., 2000)

Time	T4 (µg/dL)	T3 (ng/dL)	TSH (µU/mL)
Baseline	6.6 ± 0.4	136 ± 6	1.05 ± 0.14
Day 7 of perchlorate	6.7 ± 0.4	140 ± 8	1.00 ± 0.17
Day 14 of perchlorate	6.6 ± 0.5	151 ± 6	0.96 ± 0.12
14 days after perchlorate	6.5 ± 0.5	157 ± 9	1.23 ± 0.17

Values are mean ± standard error.

Table 35. Urine and Serum Perchlorate and Iodine Values before, during, and after Ingestion of 10 mg Perchlorate (about 0.14 mg/kg-day) for 14 Days (Lawrence *et al*., 2000)

Time	Urine perchlorate (mg/24 hr)	Serum perchlorate (µg/mL)	Urine iodine (µg/24 hr)	Serum iodine (µg/dL)
Baseline	<0.5	0	254 ± 69	6.5 ± 0.42
Day 7 of perchlorate	7.7 ± 0.8	0.61 ± 0.02	233 ± 49	6.2 ± 0.34
Day 14 of perchlorate	7.5 ± 1.0	0.59 ± 0.02	385 ± 123	6.4 ± 0.37
14 days after perchlorate	<0.5	0	208 ± 42	6.3 ± 0.57

Values are mean ± standard error.

Lawrence *et al*. (2000) also reported that during perchlorate ingestion, there was a highly significant decrease in the thyroid ^{123}I uptakes at all three time points. In each instance, 150 µCi ^{123}I was administered to a subject and thyroid iodide uptake was measured at 4, 8, and 24 hours. The average decrease below baseline values over all three time points was 38 percent. Two weeks after perchlorate was discontinued, the 24-hour thyroid ^{123}I uptakes were significantly higher than baseline at 4, 8, and 24 hours (Table 36).

Table 36. Thyroid ^{123}I Uptakes before, during, and after Ingestion of 10 mg Perchlorate (about 0.14 mg/kg-day) Daily for 14 Days (Lawrence *et al*., 2000)

Time	Thyroid ^{123}I uptake– baseline (% of dose)	Thyroid ^{123}I uptake 14 days on perchlorate (% of dose)	Thyroid ^{123}I uptake 14 days after perchlorate was discontinued (% of dose)
4 hours	12.5 ± 1.3	8.2 ± 0.7[a]	16.6 ± 2.4[b]
8 hours	17.3 ± 1.9	10.6 ± 1.0[a]	21.9 ± 2.8[b]
24 hours	23.6 ± 2.6	14.0 ± 1.6[a]	27.1 ± 3.3[c]

[a]$p < 0.01$ vs. baseline and after perchlorate treatment was discontinued
[b]$p < 0.01$ vs. baseline
[c]$p < 0.05$ vs. baseline
Values are mean ± standard error

Lawrence *et al*., 2001. In a follow-up study, Lawrence *et al.* (2001) administered a daily oral dose of 3 mg of perchlorate to a group of eight healthy male volunteers for 14 days. They reported that the mean 8-hour thyroid radioactive iodide uptake decreased from 13.1 percent to 11.8 percent during perchlorate ingestion. Similarly, the 24-hour thyroid radioactive iodide uptake decreased from 16.1 percent to 14.5 percent, a 10 percent decrease that was not statistically significant. Assuming a default body weight of 70 kg, the dose used in this study is estimated to be 0.043 mg/kg-day.

Greer *et al*., 2002. Daily oral doses of perchlorate (ClO_4^-) dissolved in 400 mL of water were given to groups of euthyroid human volunteers for 14 days. Subjects (4 male and 4 female; 18-57 years old) of each dose group were exposed to a daily dose of 0.02, 0.1, or 0.5 mg/kg of perchlorate (approximately 1.4, 7, or 35 mg, assuming 70 kg body weight). In a follow-up study, one additional subject of each sex received perchlorate at 0.02, 0.1, or 0.5 mg/kg-day, while six women and one man received a dose of 0.007 mg/kg-day. Subjects drank 100 mL of the perchlorate solution at 4 set times throughout each day. Measurement of 8- and 24-hour ^{123}I thyroid uptakes was performed prior to perchlorate exposure (baseline), on exposure days 2 and 14, and on post-exposure day 15. There was a strong correlation between the 8- and 24-hr uptakes over all dose groups and measurement days. There was no difference between exposure days 2 and 14 in the inhibition of uptake produced by a given perchlorate dose. There was no sex difference. Uptakes measured on post-exposure day 15 were not significantly different from baseline. Table 37 provides the 24-hour thyroid radioiodide uptake data by dose. The researchers measured total T4, fT4, total T3, and TSH in blood sampled throughout the study, and found them to be in the normal range for all subjects. One woman in the lowest dose group had abnormally high TSH on both the screening visit and on exposure day 14. Because of the limited data in the follow-up study, analysis of treatment effects on hormonal levels was confined to the 24 volunteers in the first study. Greer *et al*. (2002) reported no association between perchlorate dose and thyroid

hormone levels except a marginally statistically significant association with a decreased TSH in the 0.5 mg/kg-day dose group.

Table 37. Descriptive Statistics for the 24-Hour Thyroid Radioiodide Uptake Data from Greer *et al*., 2002

Dose group	Time	Number of subjects in dose group	24-hr uptake (mean ± standard error)	
			Raw (%)	% of baseline
0.007 mg/kg-day	Baseline visit	7	18.1 ± 3.1	
0.007 mg/kg-day	Exposure day 14	7	16.5 ± 1.6	98.2 ± 8.3
0.02 mg/kg-day	Baseline visit	10	18.4 ± 1.2	
0.02 mg/kg-day	Exposure day 14	10	15.2 ± 1.1	83.6 ± 4.1
0.1 mg/kg-day	Baseline visit	10	19.9 ± 2.1	
0.1 mg/kg-day	Exposure day 14	10	11.0 ± 1.6	55.3 ± 3.9
0.5 mg/kg-day	Baseline visit	10	21.6 ± 2.0	
0.5 mg/kg-day	Exposure day 14	10	6.9 ± 0.9	32.9 ± 3.8

Braverman *et al*., 2006. This investigation was a double blinded, randomized clinical dosing study in which 14 healthy volunteers received either placebo, or 0.5 mg, 1 mg, or 3 mg of potassium perchlorate once per day for six months. Nine of the subjects were women. Serum thyroid hormone and perchlorate levels, and urine perchlorate, iodine, and creatinine levels were measured monthly. Twenty-four hour radioactive iodide uptake (RAIU) was measured at baseline, and at 3 and 6 months of perchlorate ingestion, and one month after dosing was discontinued.

The mean urinary perchlorate level in the five subjects who received 0.5 mg/day was 332.7 µg (± 66.1) per 24 hours. The corresponding amount in the four subjects who received 3 mg/day of perchlorate was 2,079 µg (± 430) per 24 hours. Only one subject receiving the 1 mg dose completed the study and data on this subject was not reported. There were no significant changes in RAIU, T4, free T4 index (FTI), or TSH during or after the dosing period. The mean urinary iodine level in the 3 mg group was very high before the dosing started (322 µg/g creatinine; SD ± 357). This dropped to 192.8 µg/g (SD ± 110.1) one month after the dosing period. The large standard deviation suggests that this could be due to one outlying value, although the individual data on iodine were not shown.

The reason why this study did not find impacts on RAIU similar to other studies is unknown, although the authors note that this could be due to the small number of subjects, differences in dosing regimens (once daily versus semi-continuous), or the possibility that the NIS may be up-regulated as an adaptive response to long-term exposure. Given the fact that various metabolic and physiologic functions

have been shown to be less well developed and less effective in young children than in adults (Ginsberg *et al.,* 2002; Ginsberg *et al*., 2004), if chronic perchlorate exposure does cause the NIS to up-regulate, this might be less effective in young children than in adults.

Occupational Studies

Gibbs *et al*., 1998. This study monitored triiodothyronine resin uptake (T3U), total serum T4, FTI, and TSH levels in 18 workers occupationally exposed to ammonium perchlorate in air before and after a work shift. They also similarly monitored 83 workers who were not exposed. Based on the thyroid function test results collected, the authors concluded that exposure to a mean of 36 μg/kg-day ammonium perchlorate (ranging from 0.2 to 436 μg/kg-day) was not a significant predictor of the cross-shift change in any of the thyroid parameters. Given the relatively long serum half-life of T4 in adult humans (5-9 days) (U.S. EPA, 1998b), it would be very unlikely that serum T4 levels would exhibit a change over a single work shift.

Gibbs *et al*. (1998) also evaluated the thyroid function test results of workers exposed to ammonium perchlorate based on their working-lifetime dose estimates. They reviewed personnel records and employees were interviewed to determine the number of years worked in each of the seven exposure groups. An average of 2,000 hours worked yearly was assumed. Each subject's working-lifetime cumulative dose was then estimated as:

$$\sum \text{[mean group exposure]} \times \text{[years in exposure group]} \times 2{,}000$$

No significant correlations with estimated lifetime cumulative perchlorate dose were detected with any of the thyroid function measures (T3U, T4, FTI, and TSH levels). However, the tenure of the workers ranged from 1 to 27 years, while thyroid hormone levels are most likely to be affected by relatively recent perchlorate exposures (probably in the range of 1-3 months). Because of this, cumulative dose over a long period of time may not be the best metric for characterizing the effect of perchlorate exposure on thyroid hormone levels.

Lamm *et al*., 1999. This is a cross-sectional study of two similar worker populations from the same industrial complex: ammonium perchlorate production workers and sodium azide production workers. A total of 37 workers were exposed to airborne ammonium perchlorate, 35 males and two females. Twenty-one workers from the azide production plant served as the control group. Perchlorate exposure was measured using full-shift breathing zone personal air samplers for total as well as respirable perchlorate particles. Urinary perchlorate concentration was assessed at the beginning and end of the 12-hour shift in which the perchlorate exposure was measured. Post shift serum samples were collected for measurements of T4, T3, TSH, and anti-thyroid antibodies. The authors reported that there were no differences in thyroid function tests between workers in the azide and perchlorate plants or between the azide workers and

any of the three perchlorate-exposure groups (Table 38). Based on these data, a NOAEL of 0.48 mg/kg-day (33.6 mg/day divided by 70 kg) can be estimated. However, this data set has several limitations: (a) small sample size, (b) high dietary iodine intake among the workers, and (c) given the short biological half-life of perchlorate (approximately 8 hour), the exposed workers might recover from the effects of perchlorate during off-shift hours. Using the medical examination and questionnaire findings, Lamm *et al.* (1999) reported that worker exposures to perchlorate in the plant were not found to be associated with thyroid abnormalities.

Table 38. Perchlorate Exposures and Thyroid-Function Parameters, by Plant and Exposure Groups (Adapted from Lamm *et al*., 1999)

Group	Total airborne perchlorate (mg/day)	Respirable airborne perchlorate (mg/day)	Absorbed dose (mg/shift)[a]	T4 (µg/dL)	T3 (ng/dL)	TSH (µU/mL)
Normal range				5 to 11	87 to 178	0.45 to 4.5
Azide worker	0.014±0.012 (n=4)	0.021±0.014 (n=6)	0.88±1.17 (n=21)	6.73±1.48 (n=21)	142.5±17.5 (n=21)	3.14±1.87 (n=21)
Perchlorate worker A	0.337±0.187 (n=6)	0.091±0.095 (n=11)	3.98±2.69 (n=14)	7.13±1.58 (n=13)	148.4±25.2 (n=13)	2.68±1.14 (n=12)
Perchlorate worker B	6.57±7.14 (n=2)	0.601±0.671 (n=7)	10.9±8.7 (n=8)	7.34±1.12 (n=8)	152.1±23.2 (n=8)	2.41±1.27 (n=8)
Perchlorate worker C	59.4±53.6 (n=12)	8.59±9.39 (n=14)	33.6±14.5 (n=14)	7.03±1.30 (n=15)	152.1±20.4 (n=15)	3.33±2.34 (n=15)

[a]Derived from urinary perchlorate concentration
Values are mean ± standard deviation

Braverman *et al*., 2005. This was an investigation of RAIU and thyroid hormone levels in 29 workers employed in the same perchlorate production facility used in Lamm *et al*. (1999) and in 12 volunteers who did not work at the plant. All subjects were Caucasian males, and eight workers and two controls were smokers. The normal schedule for employees at the plant was to work three 12 hours shifts on three consecutive days and then have three days off work. Serum levels of perchlorate, thiocyanate, nitrite, T3, T4, FTI, and TSH and urine concentrations of perchlorate and iodine were measured just before (pre-shift) and just after (post-shift) the three day shift. Mean serum perchlorate levels in the workers increased from 2 µg/L to 838 µg/L from pre-shift to post-shift. RAIU decreased from 21.5 percent pre-shift to 13.5 percent post-shift (p-value for the difference < 0.01). The authors estimated perchlorate intakes based on differences in pre- and post-shift serum levels and used these estimates to plot dose-response relationships with RAIU. Figure 4 of their paper shows that perchlorate and RAIU relationship in this study was similar to that reported in Greer *et al*. (2002) and Lawrence *et al*. (2000, 2001). Interestingly, the RAIU

levels in the non-worker control subjects (14.4 percent) was significantly lower than the pre-shift level of the workers (21.5 percent, $p < 0.01$ compared to controls) and very similar to that of the post-shift level of the workers (13.5 percent, $p = 0.64$ compared to controls). The reason for this is unknown, although the authors note that this might be consistent with an apparent rebound increase in RAIU that has been noted in other studies. Post-shift workers had a slight but statistically significant increase in T3, T4, and FTI. The reason for this is unknown, although the authors hypothesize it may be due to a decreased iodine concentration in the thyroid enhancing the thyroid's response to TSH.

Although serum levels of thiocyanate and nitrate and urinary levels of iodine were measured, no data were presented on possible interactions between these variables and perchlorate on RAIU or thyroid hormone levels. The authors reported that exposure caused no statistically significant change in serum thiocyanate and nitrate levels but was associated with a statistically significant increase in urinary iodine:creatinine ratio. The pre-shift vs. just-after-shift means were 148 µg/g and 230 µg/g, respectively ($p = 0.02$). The mean urinary iodine:creatinine ratio in controls was 296 µg/g. The authors hypothesize that the increase in urinary iodine might have been a result of less dietary iodine being concentrated in the thyroid with perchlorate exposure.

Environmental Studies

Tèllez Tèllez *et al*., 2005. Neonatal and maternal thyroid function were assessed in subjects from the following three cities (mean perchlorate levels in drinking water) in northern Chile: Taltal (100-120 µg/L), Chañaral (5-7 µg/L), and Antofagasta (non-detectable: <4 µg/L). The neonatal results are discussed in a preceding section. Serum fT4 and TSH measurements were collected from 184 women at two prenatal visits and one post-partum visit. No difference in mean fT4 or TSH was seen across the three cities at any of the three visits. For example, on the first prenatal visit (a mean of about 16 weeks gestation), the mean fT4 (in ng/dL) in the low, medium, and high perchlorate cities were 0.97 (SD ± 0.15), 0.95 (± 0.13), and 0.99 (± 0.13) (Kruskal-Wallis $p = 0.19$). Regression analyses showed no association between urinary perchlorate excretion and levels of fT4, TSH, and T3, although details of this analysis, such as whether urine concentrations were adjusted for urine dilution, or whether perchlorate concentrations were log transformed, were not reported. Maternal goiter was seen in all cities and increased from the first prenatal visit to the post-partum visit in both Taltal and Antofagasta, although the increase was greater in Taltal (from 9.4 percent to 22.5 percent) than in Antofagasta (from 8.7 percent to 11.1 percent).

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. As discussed below, iodine, thiocyanate, and smoking may be important susceptibility factors in perchlorate-exposed women.

Gibbs and Van Landingham, 2008. This study involved a re-analysis of the data collected in the Tèllez Tèllez *et al*. (2005) study. In the previous paper, a regression analysis was done but very few results and details were provided. Most of the results presented in the previous paper were comparisons of mean thyroid hormone levels in each of the three cities. This may have diminished the ability of the study to identify a true effect since there was substantial overlap in urinary perchlorate concentrations across the cities. In this 2008 Letter to the Editor, Gibbs and Van Landingham used individual data on urine perchlorate, urine iodine, serum fT4 and TSH, and an interaction term for iodine and perchlorate in a linear regression analysis involving 150 women from the previous Tèllez Tèllez *et al*. (2005) study. Serum free T4 was entered into the model as fT4 unchanged or as $1/fT4^{½}$. The latter was used in order to achieve a normal distribution of the residuals, which is an assumption of the linear regression model. No associations were identified between urine perchlorate and fT4 or TSH (coefficients were only provided for statistically significant results). In addition, no associations were found in analyses restricted to subjects with urinary iodine levels below 100 µg/L, although this included only 16 subjects. Interestingly, in the analysis using the normalized fT4 variable (i.e., $1/fT4^{½}$), a statistically significant positive interaction was seen for iodide and perchlorate (regression coefficient (β) for the urinary iodide-perchlorate interaction = 6.26×10^{-7}; $p < 0.0001$). Given the apparent complexity of these analyses, and the lack of detail in this Letter to the Editor, the clinical meaning of this coefficient is difficult to interpret. The authors note that the coefficient for perchlorate itself was not significant in this analysis; however, the inclusion of an interaction term which includes perchlorate invalidates the use of the perchlorate regression coefficient for assessing any association with the dependent variable (Greenland, 1998). If these findings truly represent a perchlorate-iodide interaction, they would support the findings seen in Blount *et al.* (2006) described in the next section.

Blount *et al*., 2006 and Steinmaus *et al.,* 2007. These are two studies which used data from the same cross-sectional investigation of urinary perchlorate levels and serum levels of thyroid hormones in 2,299 men and women ≥ age 12 years who took part in the 2001-2002 NHANES. NHANES is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) and is designed to assess the health and nutrition status of the non-institutionalized population of the U.S. This survey involves a complex multistage sampling design with some over-sampling in certain areas and among certain subgroups, but is designed to provide results that are nationally

representative. Information that is collected as part of this survey includes questionnaire data on demographic information, smoking, health history, and medication use. A single serum measurement of T4 and TSH and a single measurement of urinary perchlorate and iodine concentration were also collected. Other information collected included urinary creatinine, thiocyanate, and nitrate; and serum levels of albumin, cotinine, and c-reactive protein. Blount *et al.* (2006) assessed the relationship between serum thyroid hormone levels and urine perchlorate concentrations using a linear regression analysis adjusted for potential confounding variables and co-variates including age, urinary creatinine, estrogen use, c-reactive protein, cotinine, ethnicity, menopause, premenarche, pregnancy, fasting time, body mass index, and kilocalorie intake. Several factors including urinary perchlorate and creatinine and serum TSH were log-transformed to normalize their distributions. Exclusions included subjects with missing data on co-variates, a history of thyroid disease, current use of thyroid medications, extreme values of T4 or TSH (n = 3), and subjects missing perchlorate measurements. No association was found between T4 or TSH and perchlorate in men. In women, separate analyses were done for women with urinary iodine levels above and below 100 µg/L. This level was chosen since it is used by the World Health Organization to define iodine deficiency in a population. Thirty-seven percent of the women in this study had urinary iodine levels below 100 µg/L. The results of the analyses in women are shown in Table 39. A statistically significant association was seen between increasing TSH and increasing perchlorate in women with iodine levels above and below 100 µg/L. A statistically significant association was also seen between decreasing T4 and increasing perchlorate in women with urinary iodine levels below 100 µg/L, but not in women with iodine levels above 100 µg/L. The magnitude of this association ($\beta = -0.8917$) suggests that a ten-fold increase in urinary perchlorate (roughly the difference between the 10th and 90th percentiles) is associated with about an 11 percent decrease in T4. In pregnant women, this level of decrease is a little less than half of the decrease in T4 or fT4 associated with an average seven percent decrease in cognitive function in the offspring (see Table 20).

Table 39. Associations between Thyroid Hormone Levels and the Logarithm of Urinary Perchlorate in Women with High and Low Levels of Urinary Iodine (Blount *et al*., 2006)

	Number of Subjects	Regression Coefficient (β)	Standard Error of β	p-value
Urine iodine < 100 µg/L				
T4	348	-0.8917	0.1811	<0.0001
Logarithm of TSH	356	0.1230	0.0373	0.0010
Urine iodine ≥ 100 µg/L				
T4	724	0.2203	0.3687	0.5503
Logarithm of TSH	697	0.1137	0.0506	0.0249

Differences in the numbers of subjects with an iodine category are due to differences in the number of subjects missing data on co-variates in each analysis. Except for perchlorate and creatinine, only co-variates with p-values < 0.10 were retained in each model.

Steinmaus *et al*., 2007 used the same NHANES database and assessed whether other NIS inhibitors such as thiocyanate and nitrate might interact with perchlorate and iodine to affect thyroid hormone levels. Associations assessed by linear regression between thyroid hormones and urinary perchlorate were stratified by categories of nitrate, thiocyanate, smoking, and cotinine. Smoking and cotinine were evaluated since smoking is a major source of thiocyanate in many people and serum cotinine has been used in past studies as a biomarker for smoking intensity. The results of these analyses are shown in Table 40. In analyses of women with urinary iodine levels below 100 µg/L, regression coefficients between decreasing T4 and increasing urinary perchlorate were greater in smokers, those with high cotinine, and those with high thiocyanate levels than in non-smokers, those with low cotinine, and those with low thiocyanate, respectively. The magnitude of this association (β = -1.66) suggests that a ten-fold increase in urinary perchlorate (roughly the difference between the 10th and 90th percentiles) is associated with about a 20 percent decrease in T4. In pregnant women, this level of decrease is only a little lower than the decrease in T4 or fT4 associated with an average seven percent decrease in cognitive function in the offspring (see Table 20). These findings provide evidence that thiocyanate interacts with perchlorate and low iodine levels to decrease T4 production. The fact that similar effects are seen with all three methods used to categorize thiocyanate exposure (urine thiocyanate, serum cotinine, and smoking history) provides strong evidence that these findings are not due to chance. Interactions with TSH were seen in some analyses but not all and were not as clear as those seen for T4.

Table 40. Association between the Logarithm of Urinary Perchlorate (µg/L) and Serum T4 (µg/dL) and the Logarithm of TSH (µg/dL) in Women with Urinary Iodine < 100 µg/L, 2001-2002 NHANES (Steinmaus *et al*., 2007)

	T4[a]				logTSH[b]			
	N	β	SE	p	N	β	SE	p
All	362	-0.73	0.22	0.004	369	0.13	0.05	0.02
Smoking[c]								
Current	63	-1.66	0.37	0.0005	62	0.13	0.11	0.23
Non-smoker	245	-0.54	0.23	0.04	245	0.11	0.03	0.009
Cotinine (serum)								
High (>10 ng/mL)	64	-1.47	0.30	0.0002	68	0.15	0.08	0.09
Medium[d]	185	-0.57	0.25	0.03	192	0.10	0.06	0.09
Low (ND)	101	-0.16	0.29	0.59	106	0.11	0.05	0.04
Thiocyanate (urine)[e]								
High (>1800 µg/L)	78	-1.67	0.40	0.0009	82	0.13	0.09	0.19
Medium	107	-0.68	0.37	0.09	108	0.20	0.04	0.0003
Low (<751 µg/L)	176	-0.49	0.30	0.11	178	0.10	0.05	0.06

Abbreviations: β, regression slope; ND, non-detectable; SE, standard error.
[a]T4 models were adjusted for fasting time, kcal, body mass index, c-reactive protein, nitrate, race, estrogen use, and pregnancy. T4 model with cotinine was also adjusted for menopause status.
[b]LogTSH models adjusted for age, fasting time, body mass index, race, premenarche, and lactation. LogTSH model with smoking status also adjusted for menopause status.
[c]Smoking data not available on all women and recent former smokers are excluded.
[d]Medium category includes all subjects with serum cotinine levels between 10 ng/mL and non-detectable.
[e]Thiocyanate categories based on tertiles in all women age 12 or older.

Analysis of Blount *et al*. (2006) and Steinmaus *et al*. (2007)

Blount *et al.* (2006) and Steinmaus *et al*. (2007) 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. These studies have several strengths. First, they are based on individual rather than ecological data on perchlorate exposure and thyroid hormone levels. Second, information on a variety of potential confounders was collected and could be controlled for. Third, the studies involved a fairly large sample size, so the researchers could assess certain important susceptibility factors, like iodine status, sex, and thiocyanate intake that were not assessed in other studies. 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. Fourth, the p-values for the associations identified were well below 0.05,

signifying that the probability these findings were due to chance is fairly low. Finally, the findings are biologically plausible in that they are consistent with the mechanism and direction by which perchlorate, iodine, and thiocyanate are all known to affect T4 and TSH levels.

There are also several potential concerns regarding these data. First, the studies are based on cross-sectional data and single measurements of urinary perchlorate, urinary iodine, and serum thyroid hormones. This could lead to some misclassification of true long-term exposure and thyroid hormone status. However, the half-life of perchlorate is short (about 8 hours in Greer *et al.,* 2002), and perchlorate seems to affect the thyroid fairly rapidly (less than one day in rats in Yu *et al.,* 2000). Because of this, shorter-term measures of perchlorate and thyroid status are probably better for assessing true associations than long-term measures. Also, since specimens were collected similarly in all subjects, any error resulting from not using the most relevant measure of perchlorate and thyroid hormone status would most likely cause a non-differential misclassification and likely bias towards the null, not towards the positive effects identified. The use of total T4 rather than free T4 (the physiologically available form) may also cause some misclassification, but again this would be expected to be non-differential and towards the null.

Urinary iodine levels for an individual can vary throughout the day and from day to day. Because of this fluctuation, it has been argued that measuring a single spot urinary iodine level is a poor reflection of an individual's overall, long-term iodine status. However, studies show that moderate to fairly strong correlations exist between single spot fasting urinary iodine concentrations and 24-hour urinary iodine concentrations, the recommended method for evaluating iodine deficiency in an individual (see Table 41). The fact that these correlations are not near zero suggests that while spot urinary iodine measurements may be associated with some misclassification, they still do provide at least some indication of true long-term iodine status in most people.

Table 41. Correlation Coefficients (R) of 24-Hour Urinary Iodine Levels with Single Spot Urinary Iodine Concentrations (µg/L) and Urine Iodine/Creatinine (I/Cr) Ratios

Author/Year	Location	N	R (for µg/L)	R (for I/Cr)
Konno *et al*., 1993	Japan	22	0.83 ($p < 0.001$) (unknown)	0.699
Thomson *et al*., 1997	New Zealand	333	0.49 ($p < 0.0001$) (log converted)	0.587
Knudsen *et al*., 2000	Denmark	31	0.37[a] (Pearson)	0.61[a]
Rassmussen *et al*., 1999	Denmark	21	0.61 ($p = 0.004$) (Spearman)	0.70

[a]Pearson correlation coefficient involves an assumption that the data are normally distributed; distortions of the true correlation can occur if they are not.

Urinary creatinine levels are commonly used to adjust urinary levels of other chemicals for urine dilution. Lamm *et al*. (2007) analyzed the NHANES 2001-2 data using the iodine:creatinine (I/Cr) ratio rather than iodine concentration and found no association between T4 and perchlorate in women with low I/Cr values. However, there are some concerns about the validity of using I/Cr ratios. The first is whether or not dividing a urinary analyte concentration by urine creatinine concentration actually increases the accuracy of classifying true long-term exposure to that analyte, either in an individual or in a group. Increasing evidence suggests that it does not (at least for some chemicals). As seen in Table 41, several studies show that correlations between I/Cr ratios and 24-hour iodine levels are no better than those seen with unadjusted urine iodine concentrations. Studies of other chemicals in urine have also shown that creatinine adjustment does not improve correlations with 24-hour urine levels or with biomarkers of effect (Hinwood *et al*., 2002; Biggs *et al*., 1997). One study presented in Table 41 did find an increased correlation coefficient after creatinine adjustment (Knudsen *et al*., 2000). However, unlike the other studies in this table, this one presented Pearson correlation coefficients. These assume that variables are normally distributed (which many times urinary iodine levels are not) and can give highly distorted results if this assumption is not met.

As discussed above, the most likely bias from misclassifying true long-term iodine status would be bias towards finding no effect. 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. One possible reason for this is that the concentration of creatinine in urine depends on many factors other than urine dilution. Dividing urine iodine concentration by urine creatinine concentration creates a variable with two components: iodine and creatinine. Between-person differences in I/Cr will depend on both the variability in iodine and the variability in creatinine. Thus, I/Cr is not only dependent on iodine excretion and iodine status, but is also dependent on creatinine excretion and all of its determinants. Studies have shown that while urine dilution may be a major determinant of urinary creatinine levels *within* an individual, factors such as age, sex, genetics, physical activity, muscle mass, and diet are major determinants of differences in creatinine excretion *between* individuals and may play an even greater role than urine dilution in determining inter-individual variation in urine creatinine levels (Barr *et al*., 2005). People who have very high or very low levels of urine creatinine because of factors other than urine dilution will have their true iodine status misclassified if I/Cr ratios are used. Because urine creatinine concentration is dependent on all of these factors, using it to adjust for urine dilution may introduce a degree of misclassification of true iodine status which could overwhelm any improved accuracy that results from correcting for urine dilution. Based on these factors, several authors have concluded that I/Cr ratios should not be used for assessing iodine status (Bourdoux, 1993; Furnee *et al*., 1994; Thomson *et al*., 1996, 1997; Rasmussen *et al*., 1999).

In summary, most evidence suggests that the findings in Blount *et al*. (2006) are not due to misclassification of exposure or outcome, and are not due to the cross-sectional nature of the study design. In fact, given that the most likely direction of the bias caused by these factors is towards finding no effect, if any misclassification of perchlorate, iodine, T4, and TSH could be corrected, the associations identified in these studies are likely to be even stronger than those reported.

Another potential problem with cross-sectional data is that one cannot be assured of the appropriate temporality; that is, that the exposure came before and caused the outcome, rather than the outcome coming before and causing the exposure. However, given the abundance of data showing that perchlorate can lead to a decrease in thyroid hormone levels, and no evidence for the opposite effect, it seems highly unlikely that the Blount *et al.* (2006) and Steinmaus *et al.* (2007) results represent an effect of thyroid hormones on urinary perchlorate levels.

Another potential concern in Blount *et al.* (2006) and Steinmaus *et al*. (2007) is the possibility of confounding. Although analyses included a number of potential confounding variables, some factors associated with thyroid hormone levels were not adjusted for. Importantly though, in order for a variable to cause confounding it must be associated with the exposure and the outcome of interest. If it is not associated with both, it cannot be a confounder. In addition, confounding should not be viewed as a qualitative issue, but rather as a quantitative problem. As stated in Rothman and Greenland (1998a), "It is the amount of confounding rather than the mere presence or absence that is important.…" In order for a variable to cause important confounding, it must be strongly (not weakly) related to the exposure and outcome. If a variable is only moderately or weakly associated with the exposure and outcome it will likely cause little confounding and have only minimal impacts on results and overall conclusions (Axelson, 1978). It would be wrong to suggest that a variable that is unassociated with, or only weakly associated with, T4 or urinary perchlorate likely caused the relatively strong association between T4 and perchlorate identified by Blount *et al*. (2006).

In both the Blount *et al*. (2006) and Steinmaus *et al*. (2007) studies, the fully adjusted regression coefficients between T4 and perchlorate were very similar to the unadjusted coefficients. This suggests that although the potential confounders included in the models may have been related to the exposure, or to the outcome, or to both, none were related strongly enough to cause significant confounding. The impact of these variables at causing confounding can be assessed by looking at the effect of each one individually. Table 42 presents the results of the perchlorate-T4 linear regression analysis before and after each potential confounder is removed from either the unadjusted or fully adjusted model. As can be seen, removing any of the individual co-variates had less than a 20 percent effect on the magnitude of the perchlorate-T4 regression coefficient.

Table 42. Impact of Each Potential Confounding Variable on the Perchlorate-T4 Regression Coefficient (β) in Steinmaus *et al.* (2007)[a]

	Remove one variable at a time from the fully adjusted model			Add a single variable to the unadjusted model[b]		
	B	**p-value**	**% change**	**B**	**p-value**	**% change**
None	0.81	0.001	0%	0.78	0.002	0%
Creatinine	0.81	0.002	0%	0.63[b]	0.02	-19%
Age	0.85	0.0003	5%	0.80	0.002	3%
Fasting time	0.81	0.001	0%	0.79	0.001	1%
Albumin	0.83	0.001	2%	0.84	0.0006	8%
Kcals	0.80	0.0004	-1%	0.77	0.002	-1%
BMI	0.88	0.001	9%	0.71	0.004	-9%
C-reactive	0.83	0.001	2%	0.80	0.0008	3%
Nitrate	0.66	0.001	-19%	0.83	0.002	6%
Race	0.81	0.002	0%	0.78	0.0008	0%
Estrogen	0.78	0.002	-4%	0.77	0.002	-1%
Beta-blockers	0.84	0.001	4%	0.76	0.002	-3%
Menopause	0.79	0.0006	-2%	0.81	0.001	4%
Pregnant	0.80	0.0009	-1%	0.78	0.002	0%
Premenarche	0.80	0.001	-1%	0.74	0.003	-5%
Lactate	0.80	0.001	-1%	0.80	0.002	3%

BMI, body mass index
[a]All of the available potential confounders were entered into the model presented here. In Steinmaus *et al*. (2007) only those with p-values < 0.20 were added to and retained in the model. All analyses include the NHANES sample weights.
[b]The independent variables in the unadjusted model are the logarithm of perchlorate and the logarithm of creatinine. This is the coefficient with creatinine removed.

Some factors that might be related to thyroid hormone levels were not adjusted for in the NHANES studies (e.g., anti-thyroid hormone antibodies, PCBs, physical activity, menstruation disturbances). However, OEHHA is not aware of any evidence that these factors are associated strongly enough with perchlorate exposure that they would cause important confounding.

Nitrate has also been shown to be an NIS inhibitor. However, analyses of the NHANES 2001-2 urinary concentration data showed no evidence of an interaction of nitrate with perchlorate and iodine. The reasons for this are unknown, although some possible explanations are:

- Urine nitrate may not be an adequate reflection of serum nitrate or the concentration of nitrate reaching the thyroid gland and NIS.

- Variability in nitrate levels may be greater than variability in perchlorate and thiocyanate. Increased variability would decrease the statistical power of the study to find true associations.
- The *in vitro* studies in human cell lines which have assessed the relative potencies of iodine, perchlorate, thiocyanate, and nitrate may not be relevant to *in vivo* exposures.
- The nitrate exposures in NHANES may not have been high enough to affect thyroid function. In a clinical trial, a nitrate dose of 15 mg/kg-day for 28 days did not decrease thyroidal iodide uptake or impact thyroid hormone levels in 10 healthy volunteers (Hunault *et al.,* 2007). This intake level is 5-20 times or more higher than average nitrate intakes reported in several populations in Europe and the U.S. (OEHHA, 2000).

As a whole, 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 due to chance, confounding, or other bias.

Pearce *et al*., 2010. Urinary perchlorate and iodine concentrations and serum thyroid hormone levels were measured during the first trimester of pregnancy in 480 euthyroid women from Cardiff, Wales and 526 euthyroid women from Turin, Italy. Median urinary iodine levels were 117 µg/L in Cardiff and 50 µg/L in Turin. Median perchlorate levels were 2.6 µg/L (range, 0.3-49) in Cardiff and 5.2 µg/L (range, 0.2-168) in Turin. No correlation was found between urinary perchlorate concentrations and maternal T4 or TSH in either city. Analyses restricted to the large subset of women with urinary iodine levels below 100 µg/L gave similar results. 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).

Pearce *et al.,* 2012. In this cross-sectional study, urinary concentrations of perchlorate, iodine, and thiocyanate, and serum concentrations of TSH, free thyroxine (fT4), free triiodothyronine (fT3), and thyroperoxidase (TPO) antibodies were measured in 134 pregnant women from Athens, Greece, at a mean gestational age of 10.9 weeks (SD ± 2.3 weeks). Mean (± SD) concentrations were 6.5 µg/L (±11.2 µg/L) for perchlorate, 440 µg/L (± 318 µg/L) for thiocyanate, and 135 µg/L (± 77 µg/L) for iodine. In unadjusted analyses, a negative correlation was found between urine perchlorate and serum fT4 in all women (Spearman correlation coefficient (R) = -0.19; p=0.03) and in the subgroup of 50 women with iodine levels below 100 µg/L (R= - 0.23; p=0.09). However, in

multivariable regression modeling, with adjustments for urinary thiocyanate, urinary iodide, TPO titer, gestational age, and maternal age, a clear relationship between the logarithm of urinary perchlorate (µg/L) and the logarithm of serum fT4 (pmol/L) was not seen (regression coefficient (β) = -0.007; p=0.8). Gestational age was found to be inversely associated with fT4 in multivariable regression analyses (β =-0.003; p=0.002 for the association between gestational age in days and the logarithm of serum fT4), and positively correlated with urinary perchlorate concentration (R = 0.18; p=0.04). Based on these results, the authors hypothesized that gestational age appeared to be a confounder in the apparent correlation between urine perchlorate and serum fT4. The reason why gestational age was associated with urine perchlorate is unknown. In addition, unadjusted regression results were not presented, so regression analyses with and without adjustment for gestational age could not be compared. Mean thiocyanate concentrations were below those where the strongest associations between increasing urinary perchlorate and decreasing serum total thyroxine (TT4) were seen in NHANES (Steinmaus *et al.,* 2007). Associations between urinary perchlorate and TSH were not seen in correlation or regression analyses.

Leung *et al*., 2012. Perchlorate, thiocyanate, and iodine in breast milk, and in maternal and breastfed infant urine (aged 1-3 months), and serum thyroid hormone concentrations in infants were measured in 64 infant-mother pairs from the Boston area. Median (range) perchlorate concentrations were 4.4 µg/L (0.5-29.5 µg/L) in breast milk, 3.1 µg/L (0.2-22.4 µg/L) in maternal urine, and 4.7 µg/L (0.3-25.3 µg/L) in infant urine. Urine iodine concentrations were 101.9 µg/L (27-570 µg/L) in mothers and 197.5 µg/L (40-785 µg/L) in infants. In multivariable regression analyses which included breast milk, maternal urine, and infant urine thiocyanate concentrations, no evidence of an association was seen between breast milk, maternal urine, and infant urine perchlorate concentration and infant serum fT4 (logarithm transformed) or TSH (logarithm transformed). For example, the regression coefficient between infant urinary perchlorate concentrations and the logarithm of infant serum fT4 concentrations was 0.004 (p=0.12) (units not provided). The authors noted that they were not able to achieve their targeted enrollment of 275 mother-infant pairs, and state that, “The recruited study population was underpowered to determine the statistical significance of perchlorate and thiocyanate exposures on serum infant thyroid function.”

Mendez and Eftim, 2012 and Steinmaus *et al.,* 2013. Both of these studies used data from the 2007-8 NHANES to investigate associations between urinary perchlorate concentrations and serum concentrations of thyroid hormones. Compared to the original 2001-2 NHANES discussed above (Blount *et al.,* 2006; and Steinmaus *et al.,* 2007), serum thyroid hormone and urinary perchlorate concentrations were collected from a substantially larger number of subjects (5,921 versus 2,268) in NHANES 2007-8. In generalized additive mixed models, Mendez and Eftim (2012) reported associations between increasing creatinine-adjusted urinary perchlorate concentrations and decreasing serum TT4 and decreasing fT3 concentrations in the entire cohort and in separate analyses of males and females. For example, regression coefficients between perchlorate

(µg/L) and TT4 (µg/dL) in males and females were -0.0195 (p=0.01) and -0.0192 (p=0.03), respectively. Both perchlorate and TT4 levels were log-transformed. Similar associations were seen for fT4 in males but not in females. Additional co-variates in the models included age, ethnicity, tobacco smoking, medication use, body mass index, serum cotinine, and urinary iodine, thiocyanate, and phthalate ester metabolites. The authors stated that the models predicted reductions in serum TT4 of 0.46 µg/dL for males and 0.48 µg/dL for females (roughly a 6 percent reduction) for an increase in urinary perchlorate from the 5th to 95th percentiles. The Mendez and Eftim (2012) analyses were limited to only those 1,877 subjects who had data on urinary perchlorate, serum thyroid hormones, and urine phthalate ester metabolites. The authors noted that a previous study involving an overlapping subset of the NHANES subjects had identified a correlation between thyroid hormone levels and urinary phthalate ester and bisphenol A concentrations, "thus controlling for phthalate ester excretion was necessary to estimate the independent impact of perchlorate on hormone levels." Strong evidence of this type of confounding was not reported however.

In Steinmaus *et al.* (2013), subjects in NHANES 2007-8 were categorized into exposure groups based on their urinary perchlorate, iodine, and thiocyanate concentrations, and mean serum thyroxine concentrations were compared between groups. Analyses were adjusted for age, sex, and urine specific gravity. Subjects with urine perchlorate concentrations in the upper tertile (n=1,939) had TT4 concentrations that were 5 percent lower (mean difference=0.40 µg/dL, 95% CI, 0.14-0.65) than subjects in the lower tertile (n=2,084). Subjects with urine perchlorate in the upper tertile, urine thiocyanate in the upper tertile, and urine iodine concentrations below 100 µg/L combined (n=62) had TT4 concentrations 12.9 percent lower (mean difference=1.07µg/dL, 95% CI, 0.55-1.59) than subjects with lower perchlorate, lower thiocyanate, and adequate iodine concentrations (n=376). Similar findings were seen for fT4. Adjustments for other potential confounders including smoking, thyroid antibodies, fasting time, and medication use, or exclusions based on pregnancy, lactation, thyroid disease, or thyroid medication use had little impact on results. Overall, these results suggest that concomitant exposure to perchlorate, thiocyanate, and low iodine markedly reduces thyroxine production.

Bruce *et al.,* 2013. Bruce *et al.* (2013) used data from the same NHANES study as Blount *et al.* (2006) and Steinmaus *et al.* (2007), but the original serum samples were re-analyzed for serum TT4, TSH, fT4, thyroglobulin, thyroglobulin antibody, thyroperoxidase antibody, and several other factors. Measurements of fT4, thyroid antibodies, and some other factors were not available for the earlier analyses. In addition, the authors evaluated the role of perchlorate, thiocyanate, and nitrate in iodide uptake inhibition using perchlorate equivalent concentrations (PECs). The premise of this method is that the relative potencies of each agent on inhibiting iodide uptake in the thyroid can be multiplied by the concentrations of these agents in urine to determine their total combined impact on iodide uptake and thyroid hormone production. The authors reported that the overall PEC was weakly and negatively associated with serum TT4 levels. Using the

logarithm of total PEC and logarithm of TT4, the regression coefficient (β) was -0.0251 (p=0.020). However, similar associations were not seen for the logarithm of TSH (β = -0.0182; p=0.665) or free thyroxine (β = -0.0066; p=0.554). Other variables in the regression models included age, fasting time, body mass index, calories, ethnicity, serum albumin, use of certain medications, and other factors.

Importantly, the relative potencies used in this paper were based on the results of the Tonacchera *et al.* (2004) laboratory study. In this study, human sodium-iodide symporter (NIS) was transfected into Chinese hamster ovary cells, which were then seeded in 24-well plates and cultured in Dulbecco modified Eagle's medium containing 10% fetal calf serum. When the cells reached 100% confluence, the medium was removed, and the cells were washed with physiologic solution and combined with 500 mL buffer A (Hanks' balanced salt solution containing 0.5% bovine serum albumin and 10 mmol N-2-hydroxy-ethylpiperazine-N-2-ethanesulfonic acid) containing 100,000 counts per minute of carrier-free $Na^{125}I$. After the cells were incubated for 45 minutes at 37°C, they were washed twice with 2 ml of ice-cold buffer A, and solubilized with 1 ml of 0.1 mol/L sodium hydroxide. ^{125}I uptake was then determined by measuring the radioactivity counts per minute from each well. The doses causing a 50% decrease in iodide uptake for perchlorate, thiocyanate, and nitrate were 1.22 µmol/L (121 µg/L), 18.7 µmol/L (1086 µg/L), and 293 µmol/L (18,166 µg/L), respectively, and the relative potency of perchlorate (on a molar basis) was reported to be 15 and 240 times that of thiocyanate and nitrate. [For comparison, median serum levels of perchlorate, thiocyanate, and nitrate found in a recent study in pregnant women in New Jersey were 0.223 µg/L, 936 µg/L, 2410 µg/L, respectively (Blount *et al.,* 2009)]. It is not known how well human NIS in Chinese hamster cells simulates human NIS in human cells, how well hamster ovarian cells simulate human thyroid cells, or how well conditions in these wells simulate the complex environment of the human body.

Other unknowns regarding the Tonacchera *et al.* (2004) findings include: how the effects seen for the very high concentrations of perchlorate and the other agents used in this study correlate to effects that may be caused by lower, more common serum concentrations of these agents; how the thyroid and NIS auto-regulatory mechanisms in humans may affect relative potencies; how the fairly short-term exposures in this study correlate with the longer-term, more chronic exposures commonly seen with drinking water chemical exposures; whether or not there may be age-related or other susceptibility effects; how well the urine concentrations of each of the agents assessed correlate with the actual concentration of these agents at the NIS; what role the endogenous production of nitrate might play in affecting these relative potencies, the use of urine nitrate concentrations as an exposure metric, or auto-regulatory mechanisms; or how the presence of other agents or conditions that may inhibit the NIS, affect auto-regulatory mechanisms, or affect the thyroid in other ways might change the relative potencies reported in Tonacchera *et al.* (2004).

Gold *et al.,* 2013. In this study, serum thyroid hormone levels (fT4 and TSH) and questionnaire information on past and current doctor-diagnosed thyroid disease were collected from women who lived in an area with past perchlorate contamination. Subjects included approximately 300 women who resided near a National Priority List site in Sacramento, California, where five public drinking water wells contained between 93-250 µg/L of perchlorate. These high concentrations were thought to have begun in 1988 and ended in 1997 when the wells were capped. The study also included approximately 500 women from two neighboring comparison communities, one of which had significant airborne pollutant exposure and one of which had no noted high air or water contaminant exposure. The study did not begin, and serum thyroid hormone levels and questionnaire data were not collected, until > 4 years after the highly contaminated wells were capped. Overall, no association was found between past perchlorate exposure and thyroid hormone levels or past or current thyroid disease. The fact that thyroid hormone levels were not collected until several years after the high exposures were stopped limits the usefulness of this study since most evidence suggests that iodide uptake inhibition of perchlorate ends fairly rapidly (e.g., within a day) after the exposure is stopped (Greer *et al.,* 2002). In addition, questionnaire information on the presence of doctor-diagnosed thyroid disease is probably not a sensitive marker of the adverse effects of perchlorate exposure and its use would miss more subtle effects including the small thyroid hormone changes linked to cognitive effects in children discussed above (see pages 39-56).

Carcinogenicity

Morgan and Cassady, 2002. Morgan and Cassady (2002) assessed observed and expected numbers of new invasive cancer cases for all sites combined and 16 cancer types among residents of the greater Redlands area between 1988 and 1998. The community is known to have drinking water contaminated with perchlorate and trichloroethylene (0.09-97 ppb measured in 1980). They reported no significant differences between observed and expected numbers for all cancers, thyroid cancer, or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus and the colon and rectum. More cases were observed for uterine cancer (standardized incidence ratio = 1.35; 99% CI, 1.06-1.70) and skin melanoma (standardized incidence ratio = 1.42; 99% CI, 1.13-1.77).

Li *et al*., 2001. These authors compared the prevalence of thyroid cancer in Clark County (Las Vegas) which had measurable perchlorate concentrations in public water supplies to Washoe County, which did not. The relative risk was 0.75 (95% CI, 0.35-1.59).

Other Toxicities

Acute Toxicity

The acute lethal oral dose of perchlorate for an adult human was estimated to be 15 g, or 214 mg/kg for a 70-kg person (Von Burg, 1995).

Subchronic Toxicity

Potassium perchlorate has been used to treat Graves' disease in humans, and most of the early data on perchlorate in humans are in patients with this disease. Graves' disease is an autoimmune disorder in which patients carry immunoglobulins in their blood that bind to the TSH receptors on thyroid cells and act like TSH to stimulate DNA synthesis and cell divisions leading to a hyperthyroid state. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the uptake of iodide into the thyroid and causes a discharge of accumulated iodide in the gland.

Godley and Stanbury (1954) report using potassium perchlorate to treat 24 patients with Graves' disease. Patients were treated with 600 to 1,200 mg/day for at least 11 weeks with a few patients treated for up to 52 weeks. Two patients developed gastrointestinal problems. In one patient, these effects occurred at 600 mg/day.

Crooks and Wayne (1960) administered potassium perchlorate at 600 to 1,000 mg/day to 200 patients with Graves' disease and observed one case of skin rash and three cases of nausea. In another group of 10 patients given 1,500 mg/day and 40 patients given 2,000 mg/day, five cases of skin rash, two cases of nausea, and one case of agranulocytosis occurred. Leukocyte counts returned to normal in the patient with the agranulocytosis when perchlorate treatment was stopped. The length of treatment was unclear but generally appears to have been less than 8 weeks.

Morgans and Trotter (1960) reported that three percent of 180 patients treated with 400 to 1,000 mg/day potassium perchlorate and 18 percent of 67 patients treated with 1,200 to 2,000 mg/day displayed a variety of adverse reactions that included skin rash, sore throat, gastrointestinal irritation, and lymphadenopathy. Based on the data reported by Crooks and Wayne (1960) and their own clinical observations, Morgans and Trotter (1960) recommended a daily dose of 800 mg/kg-day, a compromise between effectiveness and minimizing the toxic side effects of perchlorate.

Genetic Toxicity

No reports were found of studies that examined genetic endpoints (chromosomal aberrations, sister chromatid exchanges, etc.) in humans exposed to perchlorate.

Immunotoxicity

Weetman *et al*., (1984) investigated the effect of perchlorate on human T and B cell responses to mitogen *in vitro*. Perchlorate at concentrations of 0, 0.01, 0.1, 1.0 and 10 mmol/L (1.17 g/L) were tested in cultures "designed to assess B and T cell responses." Supernatant immunoglobulins G and M (IgG and IgM) were measured by enzyme-linked immunoassays after culture of cells for 10 days with pokeweed mitogen. The investigators found that perchlorate at 0.1 to 10 mmol/L inhibited IgG production and at 10 mmol/L inhibited IgM production. They concluded that perchlorate has significant immunosuppressive activity at pharmacologically relevant concentrations that is not due to simple cytotoxicity (assessed by ethidium bromide/acridine orange fluorescence). However, the perchlorate concentrations in this study are in fact very high. A later study in Chinese hamster ovary cells expressing the human NIS (Ajjan *et al.*, 1998) showed perchlorate inhibition of iodide uptake evident at 0.01 micromolar, progressing to complete inhibition at 20 micromolar (0.02 mmol/L), which is much lower than the doses used in Weetman *et al*. (1984). Thus the immune effects of the high concentrations used in the study of Weetman *et al*. (1984) appear of doubtful relevance.

Hematological Effects

Graves' disease patients treated with perchlorate doses in the range of 6 to 14 mg/kg-day for three to eight months occasionally developed fatal aplastic anemia (Fawcett and Clarke, 1961; Hobson, 1961; Johnson and Moore, 1961). The mechanism of this blood disorder is not known. The use of perchlorate to treat Graves' disease was discontinued because of these cases. Nonfatal agranulocytosis was reported in patients treated with 14 mg/kg-day perchlorate for 12 days (Southwell and Randall, 1960) or three months (Sunar, 1963). Barzilai and Sheinfeld (1966) reported that 8 of 76 patients treated with 14 mg/kg-day perchlorate for at least two months developed leukopenia or other side effects. There was also one case of fatal aplastic anemia and one of fatal agranulocytosis within this group of 76 patients (Barzilai and Sheinfeld, 1966). As similar adverse hematological effects were not observed in rodents exposed to 30 mg/kg-day or 100 mg/kg-day, humans may be more sensitive than rodents for this endpoint.

5. TOXICOLOGICAL EFFECTS IN ANIMALS

The primary effect of perchlorate exposure is the disruption of thyroid hormone regulation. This mode of action is supported by the results of a number of animal studies that show that perchlorate inhibits thyroidal iodide uptake, changes serum T3, T4, and TSH levels, and subsequently causes a range of toxic effects. The animal studies, described in the section below, illustrate a range of effects that can result from the modulation of thyroid hormone regulation by perchlorate. As previously (OEHHA, 2004), human data described in earlier sections provide a reliable basis for the establishment of the PHG.

Adverse Effects on the Thyroid

Subchronic Studies of Effects of Perchlorate on the Thyroid

In many animal studies, perchlorate has been shown to perturb thyroid hormone regulation, induce hypertrophy and hyperplasia in thyroid follicular cells, and cause an increase in thyroid weight.

Shigan (1963) administered ammonium perchlorate to “white rats” at 650 mg/kg-day for one month and did not observe noticeable cumulative properties. The author also exposed “white rats” to ammonium perchlorate for three months at 190 mg/kg-day and found the treatment affected the regulation of the involuntary nervous system, caused changes in the protein fractions of the blood serum, and disrupted the liver’s ability to produce glycogen for carbohydrate storage.

In a follow-up study, Shigan (1963) treated rabbits and “white rats” with 0, 0.25, 2.0, and 40 mg/kg-day of potassium perchlorate for 9 months. Many study details, such as the sex and number of animals in each dose group and the dosing medium, were not reported. In the two highest dose groups, the author found a significant increase in the amount of iodide excreted from the thyroid. It is not clear if the reported effect was seen in both species (U.S. EPA, 2002).

Caldwell *et al.* (1995) administered ammonium perchlorate to groups of Sprague-Dawley rats (six males and six females per group) in drinking water for 14 days at concentrations of 0, 1.25, 5.0, 12.5, 25, 50, 125 or 250 mg/L. The corresponding doses (male/female) in mg/kg-day are 0, 0.11/0.12, 0.44/0.47, 1.11/1.23, 2.26/3.06, 4.32/4.91, 11.44/11.47, and 22.16/24.86 mg/kg-day, respectively. At the end of the exposure period, thyroids were weighed, thyroid histopathology and morphometry examinations were performed, and thyroid hormone levels were measured with a radioimmune assay technique. The researchers reported that perchlorate exposure decreased circulating T3 and T4 and increased serum TSH. There is also evidence that rT3 (formed mostly in extrathyroidal tissues) and thyroglobulin levels were also increased. They also found that perchlorate exposure was associated with decreases in thyroid gland follicular lumen size and increases in relative thyroid weights. At the lowest

dose, 0.1 mg/kg-day, statistically significant changes in serum T4 were observed in both sexes; this level can be identified as a LOAEL.

Springborn Laboratories (1998) administered ammonium perchlorate via drinking water to male and female Sprague-Dawley rats (10 rats/sex/dose) at doses of 0, 0.01, 0.05, 0.2, 1.0, and 10 mg/kg-day for 14 and 90 days. An additional 10 rats/sex/dose were sacrificed after a 30-day recovery period following cessation of the 90-day exposure at doses of 0, 0.05, 1.0, and 10 mg/kg-day, to evaluate reversibility of any observed lesions. No statistically significant toxicological findings were observed among the groups with respect to clinical observations, body weights, food or water consumption, ophthalmology, hematology, or clinical chemistry. The researchers reported perchlorate exposure was associated with increased thyroid weights, thyroid follicular cell hypertrophy, and thyroid colloid depletion. Mean thyroid weights of male rats in the highest dose group were significantly increased after 14 and 90 days of exposure, while mean thyroid weights of female rats in the highest dose group were significantly increased after 90 days of exposure. No thyroid pathology was observed in other dose groups. After a 90-day exposure period followed by a 30-day recovery period, there was no increase in thyroid weight in either male or female rats in the 0.05, 1.0, and 10 mg/kg-day dose groups (Siglin *et al.*, 2000).

After 14 days of exposure, mean serum TSH levels were significantly increased in males at 0.2 mg/kg-day and higher, and in females at 0.05 mg/kg-day and higher, compared with the controls. Mean serum T4 levels were significantly decreased in both sexes at 10 mg/kg-day. Mean serum T3 levels were significantly decreased in males at levels of 0.01 mg/kg-day and higher. No statistically significant differences in T3 levels were observed in the female treatment groups.

After 90 days of treatment, mean TSH levels were significantly increased in males at 0.2 mg/kg-day and higher, and in females at 10 mg/kg-day only. Mean T3 and T4 levels were significantly decreased in both sexes at 0.01 mg/kg-day and higher. Based on this data set, a LOAEL of 0.01 mg/kg-day can be identified.

Following a 90-day exposure period and a 30-day recovery period, TSH levels were significantly increased in all three female recovery groups (0.05, 1.0, and 10 mg/kg-day), whereas no significant differences in TSH levels were observed in the male recovery groups. Mean T4 levels were significantly lowered in all three male recovery groups (0.05, 1.0, and 10 mg/kg-day), whereas no significant differences in T4 levels were observed in the female groups. Mean T3 level was significantly lower in females at 10 mg/kg-day. No statistically significant differences in T3 levels were observed in the male recovery groups (Siglin *et al.*, 2000). The authors did not speculate on the differences between the male and female data.

Siglin *et al.* (2000) noted there was a change in the mean TSH levels in the male and female control groups between 14 days and 120 days. They also found changes in mean T3 levels of control females over the course of the study. It was not clear if the observed variability in mean control hormone levels was reflective of normal age-related variations or due to other factors such as the relatively small sample sizes.

Studies of Perchlorate's Inhibitory Effects on Iodide Uptake by the Thyroid

The previous and subsequent section describes various studies that have documented the effects of perchlorate on thyroid and pituitary hormones as well as the thyroid of the treated animals. This section describes animal studies of perchlorate's effects on thyroidal iodide update.

Yu *et al.* (2000), working with the United States Air Force and U.S. EPA, investigated the inhibitory effects of perchlorate on thyroidal iodide uptake in rats. They injected perchlorate at 0, 0.01, 0.1, 1 or 3 mg/kg into groups of male Sprague-Dawley rats (six animals per dose and time point). At two hours post dosing, the rats were challenged with ^{125}I with carrier (33 μg/kg) by intravenous injection and euthanized at various time points post-dosing. Statistically significant thyroidal iodide uptake inhibition was found in the 1 and 3 mg/kg perchlorate dose groups at the 2, 6, and 9 hour time points. In addition, significant inhibition was also observed in the 0.1 mg/kg dose group at the 9 hour time point (Table 43).

Table 43. Percent Inhibition of Iodide Uptake in the Thyroid Gland of Male Rats (n=6) Dosed with Perchlorate (Yu *et al.*, 2000)

Time points	Perchlorate dose (mg/kg)	Mean iodide concentration in the thyroid (μg/g)	Thyroidal iodide uptake inhibition (%)[a]
2 hours	Control[b]	24.4	-
	0.01	21.3	13
	0.1	18.6	24
	1	7.4[c]	70
	3	3.0[c]	88
6 hours	Control[b]	46.5	-
	0.01	36.7	21
	0.1	32.0	31
	1	19.2[a]	59
	3	9.1[a]	80
9 hours	Control[b]	55.0	-
	0.01	49.2	11
	0.1[c]	39.2	29
	1	24.7[c]	55
	3	10.0[c]	82

[a]Percent inhibition = (control mean – dose mean) x 100 / (control mean)
[b]Dosed with ^{125}I with carrier only (33 μg/kg)
[c]$p<0.05$ compared to controls.

In a follow-up study, Yu *et al*. (2000) exposed groups of male Sprague-Dawley rats (6 animals per dose and exposure duration) to perchlorate in drinking water with target concentrations of 0, 1, 3, and 10 mg/kg-day continually for 1, 5, or 14 days. At the end of day 1, 5, or 14, rats were challenged once with 33 μg/kg ^{125}I with carrier and euthanized two hours later. Blood and thyroid gland were collected for analyses. A dose-related inhibition was noted in the one-day treatment group. The degree of inhibition was reduced over time and by exposure day 14, no inhibitory effect was observed in the 1 and 3 mg/kg-day groups. In a similar study, thyroid hormone profile of rats exposed to perchlorate was investigated. Male rats in groups of 8 were exposed to perchlorate in drinking water at 0, 0.1, 1, 3, and 10 mg/kg-day continually for 1, 5, or 14 days. In all treated groups, regardless of dose or exposure duration, TSH levels were increased compared to the control. The serum T4 levels were initially decreased in all dose groups except the lowest, 0.1 mg/kg-day. By 14 days, the 1 mg/kg-day dose group returned to control T4 values while T4 levels of the 3 and 10 mg/kg-day dose groups were still significantly depressed.

Yu *et al.* (2002) modeled the effects of perchlorate on the hypothalamus-pituitary-thyroid axis in the male rat. They found a high correlation between serum concentrations of perchlorate and percentage inhibition in thyroidal iodide uptake, irrespective of the route of administration. They found the hypothalamus-pituitary-thyroid axis responded quickly to perchlorate's blocking effects on thyroidal iodide uptake. Serum thyroid hormone levels decreased and serum TSH levels increased in response to perchlorate. Under the influence of TSH, the thyroid was up-regulated and was able to overcome the blocking effects of perchlorate by increasing its capacity to sequester iodide and produce hormones. Yu *et al*. (2002) noted that this is a dose-dependent phenomenon, which was overwhelmed by the blocking effects of high serum levels of perchlorate (corresponding to above approximately 1 mg/kg-day).

In Paulus *et al*. (2007), perchlorate-induced inhibition of thyroidal iodide uptake was measured in normally fed female Sprague-Dawley rats and in rats made iodine-deficient by long-term restriction of iodine in the diet (n = 10 rats per perchlorate-iodine group). In the iodine deficient animals, dietary iodine levels were 9-10 times lower than those in the normally fed animals. T4 levels in the iodine-deficient animals were 50 percent lower than in the normally fed animals, although TSH levels did not differ across the diet groups. Both groups were given a dose of ^{131}I via gavage, and ^{131}I uptake was measured in the thyroid. The proportion of administered ^{131}I taken up by the thyroid was greater in the iodine deficient rats than in the rats fed a normal diet.

Rats from each diet group were then given either no perchlorate or perchlorate at doses of 1.1, 5.6, or 28 mg/L, and percent of radioactive iodide uptake (%RAIU) was re-measured. In the normally fed rats, perchlorate produced significant inhibition of %RAIU at every dose group. In the iodine-deficient rats, %RAIU was decreased at all dose levels except the lowest dose of 1.1 mg/L (Figure 10), although the %RAIU reduction in the second highest dose group was not statistically significant. These results suggest that iodine-deficient animals are resistant to the iodide-uptake inhibiting effect of perchlorate compared to animals with adequate iodine intake. Based on this, the authors concluded that if the human NIS system reacts to iodine deficiency in a manner similar to the rodents in this study, iodine-deficient individuals may not represent a sensitive subpopulation for perchlorate toxicity. Importantly, this conclusion is based on a lack of findings in only one dose group. In addition, it is currently unknown whether these results apply to humans. Finally, the degree of iodine deficiency induced in the iodine deficient rats was fairly severe (the dietary iodine levels were one tenth of what they were in the normally fed animals). It is not known whether the effects seen in rats with this severe degree of iodine deficiency are relevant to the more moderate iodine deficiencies that are common in human populations.

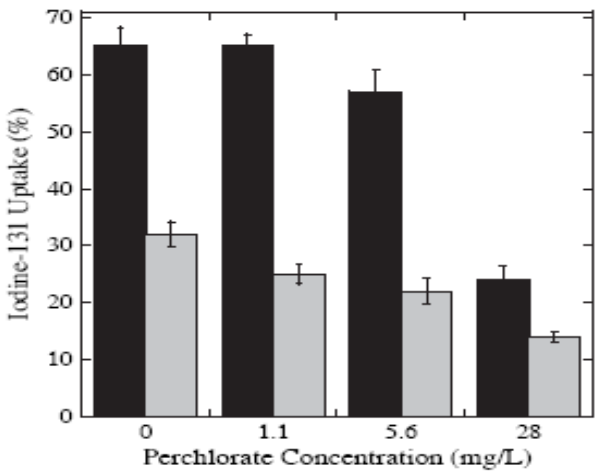


Figure 10. Dose-Response Relationship between Perchlorate and Radioactive Iodide Uptake in the Thyroid in Rats with Normal and Low Iodine Intake (Paulus *et al*., 2007). Gray bars are values from rats fed a normal diet, black bars are values from rats fed a low-iodine diet. Values shown are mean ± SEM.

Clewell *et al*. (2001; 2003; 2007) have developed and attempted to validate rat and human physiologically-based pharmacokinetic (PBPK) models for the estimation of the effect of life stage and species on perchlorate and iodide inhibition kinetics. The models have been used to estimate perchlorate distribution in the male, pregnant, fetal, lactating and neonatal rat, and predict resulting inhibitory effects on thyroidal iodide uptake. The authors conclude that the fetal rat thyroid is most vulnerable to inhibition. Clewell *et al.* (2007) reported that "the fetus is predicted to receive the greatest dose (per kilogram body weight) due to several factors, including placental sodium-iodide symporter (NIS) activity and reduced maternal clearance of ClO_4^-."

Developmental and Reproductive Toxicity

A number of toxicity studies have shown that perchlorate exposure causes a variety of adverse health effects in the offspring of the test animals.

Developmental Toxicity

Postel (1957) gave a one percent solution of potassium perchlorate to eleven guinea pigs in the second or third week of pregnancy. The control group consisted of three sows receiving a diet containing 0.48 µg/g of iodine, distilled water *ad libitum*, and 50 mg of ascorbic acid orally twice a week. The treated sows were divided into four groups which received 0, 8, 16, or 32 µg, respectively, of triiodothyronine (T3) by subcutaneous injection each day. Control sows and those receiving potassium perchlorate alone were given daily injections of saline solution. The overall mean dose of potassium perchlorate

was 1,520 mg/kg-day in the T3-injected sows and 740 mg/kg-day in the group receiving only potassium perchlorate. The mean exposure duration was 37 days (range 21-48 days). One hour before the removal of the fetuses, radioactive sodium iodide was injected subcutaneously into the sow. Radioactivity of the blood and thyroids of the sows and fetuses were analyzed.

Postel reported that massive enlargement of the fetal thyroid was observed in all the perchlorate treated groups, with or without T3 injection. The overall mean weight was 491 mg/100 g body weight, compared with a mean control fetal thyroid weight of 32 mg/100 g body weight. Perchlorate did not cause enlargement of the maternal thyroid in any of the treatment groups, with or without T3 injection.

Postel (1957) also reported that the thyroid/serum radioiodide concentration ratio was approximately 175 in fetuses and 50 in sows in the control group. It was suggested that this finding supports the concept that the normal fetal thyroid is in a relatively hyperplastic state. The thyroid/serum radioiodide concentration ratios of both the perchlorate-treated sows and their fetuses were significantly lower than those of the controls.

Lampe *et al.* (1967) gave perchlorate-treated food to 12 rabbits from the beginning of pregnancy through gestation day 21 or 28. The rabbits were dosed at 100 mg/kg-day. No concurrent controls were used. Ingestion of perchlorate was found to cause an increase in maternal and fetal thyroid weights. On the 21st day of treatment, the maternal thyroid weights in treated animals were nearly three times higher than control thyroids; fetal thyroids from the treated animals were nearly four times the control weights. Continued intake of perchlorate further enhanced the increase in thyroid weight, particularly in the fetus. On the 28th day of treatment, the maternal thyroid weights in treated animals were nearly four times higher than control thyroid weights, and fetal thyroid weights from treated animals were nearly nine times higher than control thyroid weights.

Sztanyik and Turai (1988) investigated the safety of using potassium perchlorate or potassium iodide as blocking agents to prevent the uptake of radioiodine by fetuses. They injected these compounds into pregnant albino rats (body weight 200 to 250 grams) in amounts sufficient to “significantly decrease” uptake of radioiodine by the fetuses (0.1 to 6.0 mg potassium perchlorate per adult rat). There was no evidence of embryo- or fetotoxicity at these doses. In summary, the results of this study, and those of Postel (1957) and Lampe *et al*. (1967), all provide evidence that perchlorate can pass through the placenta from the mother to the fetus and affect the fetal thyroid in test animals.

A developmental neurotoxicity study of ammonium perchlorate in rats was conducted by Argus Research Laboratories (1998a; 1998b; 1998c). Ammonium perchlorate was administered to groups of 25 female Sprague-Dawley rats via drinking water at target doses of 0, 0.1, 1.0, 3.0, and 10 mg/kg-day. The dosing period was from the beginning of gestation (GD 0) to postnatal day (PND) 22.

Five dams per group were selected for sacrifice and blood collection on PND 10. Pups (F1 generation) were counted and clinical signs recorded daily during pre- and post-weaning. Some of the pups were assigned to four different subsets for additional evaluations: Subset 1 for brain weight and neurohistological examination on PND 12; Subset 2 for passive avoidance testing, water maze testing, and blood collection for thyroid and pituitary hormone analysis; Subset 3 for motor activity evaluation and auditory startle habituation; Subset 4 for regional brain weight evaluation and neurohistological examination on PNDs 82 to 85. U.S. EPA (2002) analyzed the F1 data and concluded that perchlorate treatment was associated with: (a) brain morphometric changes in the 10 mg/kg-day dose group and possibly also the 3 mg/kg-day dose group; (b) thyroid colloid depletion, hypertrophy, and hyperplasia in the 0.1 and 3 mg/kg-day dose groups; (c) thyroid hormone (T3 and T4) changes in the 0.1 and 1 mg/kg-day dose groups; and (d) increases in motor activity in some dosed animals.

Argus Research Laboratories (1999; York *et al.*, 2001) reported a two-generation reproductive toxicity study in Sprague-Dawley rats. Male and female rats (30 rats/sex/group) of the first generation (P) were exposed to ammonium perchlorate in drinking water at 0, 0.3, 3, and 30 mg/kg-day. One male and one female were allowed a cohabitation period of a maximum of 14 days. Lactation day (LD)1, postpartum, was defined as the day of birth. Rats that did not deliver a litter were sacrificed on gestation day 25 and examined for pregnancy status. At the end of the 21-day postpartum period, all surviving P rats were sacrificed. Pups not selected for continued evaluation were also sacrificed on LD 21. The selected F1 pups were dosed during the post weaning, cohabitation, and lactation periods. All F1 generation dams and their litters (F2 generation) were sacrificed on LD 21. York *et al.* (2001) reported that perchlorate is not a reproductive toxicant in the doses tested. In both the P and F1 adult rats, there were no deaths, abortions, or premature deliveries. No changes were reported in any sperm parameters in either P or F1 adult male rats nor on mating or fertility parameters in either P or F1 adult female rats (estrous cyclicity, fertility index, number of days in cohabitation, and number of rats mated). Natural delivery and litter observations for both F1 and F2 generation pups were comparable among the treated and control groups. Treatment-related effects were not observed on the gestation index, the number of dams delivering litters, the duration of gestation, the average number of implantations, the average number of live pups, the viability and lactation indices, the sex ratios, or the pup body weights.

York *et al.* (2001) found that perchlorate exposure caused statistically significant, dose-dependent changes in thyroid weight, histopathology, and hormone levels in P, F1, and F2 generation rats. Relative thyroid weights were significantly increased in the 30 mg/kg-day dose group for both sexes in the P generation and for F2 generation pups. However, in the F1 generation adult rats, relative thyroid weights were significantly increased in all dose groups for females and in the 3 and 30 mg/kg-day dose groups for male rats. All three generations developed hypertrophy and hyperplasia of thyroid follicular epithelium that increased in incidence and severity in a dose-related manner. Dose-related changes in TSH,

T3, and T4 were also observed in the treated rats. However, these changes were inconsistent among the different generations, sexes, and ages of animals.

U.S. EPA (2002) noted that two male rats from the high dose group (30 mg/kg-day) in the F1 generation (second parental generation) in the study had adenomas of the thyroid. These males were dosed from conception to 19 weeks of age. Without incorporating historical data, the difference between 0/30 in the control and 2/30 in the 30 mg/kg-day is not statistically significant by standard tests (e.g., Fisher's exact). However, using two earlier reported background incidence rates of 3.6 percent and 3.9 percent for thyroid follicular cell adenomas in male Sprague-Dawley rats in 2-year studies and Bayesian analysis, U.S. EPA (2002) determined the increase in thyroid follicular cell adenoma at 19 weeks in male Sprague-Dawley rats exposed to 30 mg/kg-day to be statistically significant.

Effects of perchlorate on motor activity in Sprague-Dawley rats were studied by Bekkedal *et al*. (2000). The females were dosed with ammonium perchlorate in drinking water for two weeks at 0, 0.1, 1, 3, or 10 mg/kg-day prior to mating with the breeder males and through PND 10. As dosing was stopped on PND 10, it is likely that the pups were not directly exposed to perchlorate in drinking water. On PND 14, one male and one female were randomly selected from each litter to be used in the motor activity testing. These same animals were tested on PND 14, PND 18, and PND 22. Pups were individually tested in automated Opto-Varimex Activity boxes where 9 different measures of activity were recorded for 90 minutes on each test day. Data were analyzed in 9, 10-minute blocks using a repeated measures ANOVA.

Bekkedal *et al*. (2000) reported no statistically significant differences for any of the nine measures of motor activity, and there were no reliable interactions related to treatment. A general pattern in the results was noticed. The authors suggested that there was a divergence in activity between the control and treated groups which emerged late in the 90-minute testing sessions.

U.S. EPA and NIEHS used a Bayesian hierarchical model to analyze the motor activity data reported by Argus Research Laboratories (1998a) and Bekkedal *et al*. (2000). They built a linear mixed-effects regression model relating dose, sex, age, habituation time and a habituation time × dose interaction term to the expected number of ambulatory movements, with an animal-specific intercept included to account for within-animal dependency (U.S. EPA, 2002). U.S. EPA concluded that there was evidence of an increasing dose-response trend in motor activity in both data sets, and suggested that the lower limit on the estimated dose corresponding to a 10 percent increase in motor activity relative to control can be used as a surrogate for the NOAEL. Because of the variability in the Argus Research Laboratories (1998a) study, a NOAEL that relied on the Bekkedal *et al*. (2000) study was chosen at 1 mg/kg-day to represent effects on motor activity from these combined data.

Argus Research Laboratories (2001) studied the effects of perchlorate on thyroid and brain development both during gestation and postnatally. Perchlorate was administered in drinking water to female rats two weeks prior to cohabitation at 0, 0.01, 0.1, 1, or 30 mg/kg-day and continued through the day of sacrifice. F1 generation rats were not directly dosed but might have been exposed *in utero* during gestation and via maternal milk and maternal water during the postpartum period. The rats were selected only from female rats that had litters of at least 12 live offspring at the time of cesarean-sectioning (Part A) or at the time of the first tissue collection (Parts B and C). P generation rats assigned to Parts B and C that delivered a litter were sacrificed on either PND 9 (Part B) or PND 21 (Part C). The thyroid and brain from one male and one female pup per litter were selected for histological and morphometric evaluation, with one set evaluated on PND 4, PND 9, and PND 21. Details of the study and findings are described in the study report prepared by the Argus Research Laboratories (2001), and a summary of the findings and evaluations is provided below.

According to the report, there were no deaths, adverse clinical observations or necropsy findings during the premating, gestational and/or lactation periods that were treatment-related in Parts A, B, and C. There were 16 pregnant dams in Part A. No treatment-related changes were found in cesarean-sectioning or litter parameters. There were 15 or 16 pregnant dams for Parts B and C that delivered. Natural delivery was unaffected by the treatment and all clinical and necropsy observations in the F1 generation pups were considered unrelated to the treatment.

The absolute and relative thyroid weights of dams at the highest dose were increased. The absolute thyroid weights of some pups exposed at 1 and 30 mg/kg-day were increased. Furthermore, the absolute thyroid weights of the PND 9 male pups in the 0.01, 0.1, 1 and 30 mg/kg-day dose groups were significantly increased over the controls.

An exposure-related increase in the incidence and severity of decreased colloid was noted in dams in the 1 or 30 mg/kg-day groups. Similar observations were made on the fetuses at birth and pups at PND 4 and PND 9. An increased incidence of follicular cell hypertrophy and/or hyperplasia was found in dams in the 30 mg/kg-day dose group. An increased incidence of follicular cell hyperplasia was also found in the 1 mg/kg-day dams sacrificed on PND 21.

In Part A, maternal TSH levels were significantly increased and T4 levels were significantly decreased at all exposure levels. Fetal TSH levels were significantly increased at 1 and 30 mg/kg-day while T3 was significantly decreased at all exposure levels. Changes of both the maternal and fetal thyroid hormone levels occurred in an exposure-dependent manner.

Maternal and fetal thyroid and pituitary hormone levels were also affected by various doses of perchlorate in Parts B and C. Most changes occurred in an exposure-dependent manner. In the PND 21 male pups, TSH levels were

significantly increased and T4 levels were significantly decreased at all exposure levels. T3 levels were also significantly decreased in the 1 and 30 mg/kg-day groups. In the PND 21 female pups, TSH levels were increased at all exposure levels, reaching statistical significance in the 0.1, 1, and 30 mg/kg-day groups. T4 levels were decreased with increased exposure but did not reach statistical significance.

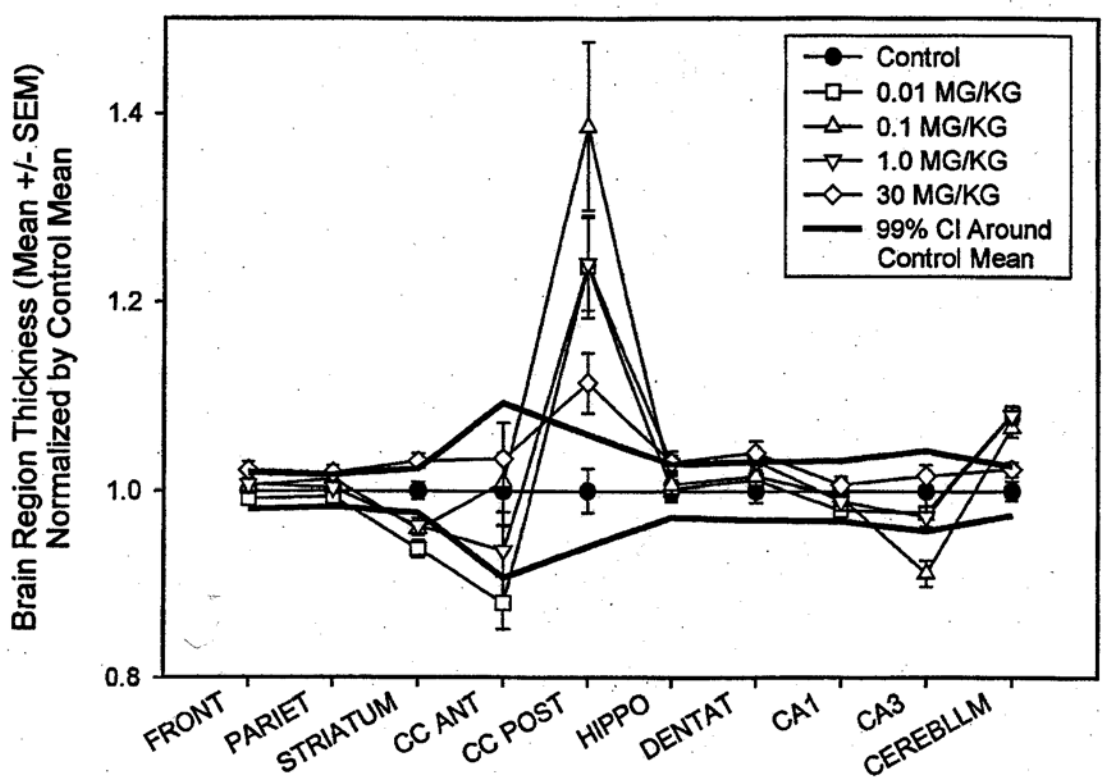


Figure 11. Profile Analysis of Brain Morphometry Measurements for PND 21 Rat Pup Brain Regions. The male and female linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis determines whether the vectors of measurements from each treatment group differ from each other and control in a dose-dependent fashion. The heavy line represents the ±99 percent confidence interval around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values (from U.S. EPA, 2002).

Size of various brain areas was also measured in brain sections from the PND 9 and PND 21 pups. Due to signs of disruption or damage found in the PND 9 sections that might have compromised the measurements, U.S. EPA (2002) relied upon the PND 21 measurements. In the PND 21 brains, the striatum, cerebellum, and corpus callosum in the exposed animals all showed significant differences from those of controls with the lowest administered dose of ammonium perchlorate, 0.01 mg/kg-day. As shown in Figure 11, different brain

regions show an inverted U or U-shape dose response. For instance, the corpus callosum showed a notable increase in linear extent of 24 percent or more at PND 21 in the 0.01, 0.1, and 1 mg/kg-day dose groups; however, this effect was not observed at the highest dose group, 30 mg/kg-day. Using these data, U.S. EPA (2002) identified a LOAEL of 0.01 mg/kg-day for the adverse effects of ammonium perchlorate on the developing brain in rats. This is equivalent to 0.0085 mg/kg-day for the perchlorate anion alone.

Thuett *et al.* (2002) studied the effects of *in utero* and lactational exposure to ammonium perchlorate on developing deer mice. Breeding pairs were dosed continuously with 0, 1 nM, 1 µM, or 1 mM of ammonium perchlorate in drinking water, from cohabitation until pups were sacrificed at PND 21. Pups from the second litter were used for evaluation. The researchers found the treated groups tended to have smaller litter sizes than did controls, but a greater survival percentage. They reported that perchlorate is a developmental toxicant and showed variable effects with increasing concentrations. Body weights of the pups in the 1 µM group were consistently lower than in the controls and in other treatments after PND1. They also reported that perchlorate treatment had an effect on the liver and heart weights. However, although liver weight alone was statistically different between treatments, liver weight when analyzed with body weight as a covariate showed no statistically significant difference. Heart weights for male pups were decreased in the 1 µM and 1 mM treatment groups. Heart weights decreased while body weight was increasing. Citing other study results, Thuett *et al*. (2002) suggested that an inadequate level of thyroid hormones during cardiac muscle development can alter cardiac function and/or heart size.

Reproductive Toxicity

Female rats were dosed with perchlorate in drinking water during gestation. The daily intake rates were estimated to range from 237 mg/rat to 615 mg/rat (Brown-Grant, 1966; Brown-Grant and Sherwood, 1971, as cited in U.S. EPA, 2002). These researchers observed no significant differences in litter size, number of pups, and pregnancy rate. Relative thyroid weights of the dams and litters were increased significantly compared with the controls.

A developmental toxicity study was performed on New Zealand White rabbits (Argus Research Laboratories, 1998d). It involved 25 naturally-mated does per group exposed to ammonium perchlorate in drinking water at 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day from gestation day 6 to gestation day 28. Observations were based on 22, 24, 23, 24, 24, and 23 pregnant does that survived to gestation day 29 in the 0, 0.1, 1, 10, 30, and 100 mg/kg-day dosage groups, respectively. Fetuses were delivered by cesarean section. The authors reported that doses as high as 100 mg/kg-day did not affect litter parameters. All values were within the historical ranges of the testing facility. The litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions, percent dead or resorbed conceptuses, percent male fetuses and fetal body weights were comparable and did not differ significantly in the six dosage groups.

All placentas appeared normal and no doe had a litter consisting of only resorbed conceptuses (Argus Research Laboratories, 1998d). U.S. EPA (2002) analyzed the maternal hormone data and noted statistically significant decreases in T4 for the 1, 10, 30, and 100 mg/kg-day dose groups. There were no statistically significant changes in T3 or TSH at any dose.

Argus Research Laboratories (1998d) also reported that no fetal alterations (defined as malformations and variations) were attributable to exposure to ammonium perchlorate at doses as high as 100 mg/kg-day: (a) the incidences were not dosage-dependent; (b) the observation occurred in only one or two high dosage group fetuses; or (c) the incidences were within the averages observed historically at the testing facility.

OEHHA notes that rabbit is probably not an appropriate animal model for the study of adverse developmental effects of perchlorate. Studies have shown that the placental iodide transport in rabbit is capable of generating a fetal serum-to-maternal serum iodide concentration of 5/1 to 9/1, thus facilitating the production of fetal thyroid hormone. A similar transport mechanism is not known to exist in human placenta (Hall and Myant, 1956 and Roti *et al.*, 1983, as cited in Fisher, 1996).

In a study by Argus Research Laboratories (2000), female rats were dosed at 0, 0.01, 0.1, 1.0 and 30.0 mg/kg-day ammonium perchlorate in drinking water beginning 15 days before cohabitation and continuing through the day of sacrifice. All rats were sacrificed on gestation day 21, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Preimplantation loss was noted at all dose levels: 12, 18, 20, 16, and 25 percent at the respective doses from 0 to 30 mg/kg-day. U.S. EPA (2002) noted that it was not clear whether these increases were statistically or biologically significant compared to control animals. OEHHA analyzed the data by the Mann-Whitney U test (since the data are not normally distributed) and found that the increase in preimplantation loss was statistically significant in the 30 mg/kg-day group compared to controls ($p<0.05$). A decrease in the number of live fetuses was also reported to be statistically significant ($p<0.05$) at 30 mg/kg-day, although no significant decrease was noted in the lower dose groups. Ossification sites per litter for sternal centers and forelimb phalanges were significantly reduced at 30 mg/kg-day.

Carcinogenicity

A number of animal studies have been reported that may be useful in determining the carcinogenic potential of perchlorate. However, the interpretation of the study results is hampered by the small number of animals per dose group, short exposure and observation durations, lack of multiple dose groups, and co-exposure to other cancer causing agents.

Gauss (1972) treated female NMRI mice with one percent potassium perchlorate in the diet or the control diet for 160 days. The one percent dose is equivalent to approximately 2,000 mg/kg-day based on standard assumptions. The investigator noted progressive changes in the thyroids of treated mice beginning with colloid loss, progressing to increases in size of nuclei and increased epithelial height, followed by appearance of hyperplasia and hypertrophy of the thyroid parenchyma. Later in the treatment period, hyperplastic follicles, areas of adenomatous tissue, adenoma complexes and secreting cystadenomas were observed. No progression to malignancy was observed during the study period.

Several Japanese investigators (Hiasa *et al*., 1987) tested potassium perchlorate for its ability to promote the carcinogenic activity of N-bis(2-hydroxypropyl)nitrosamine (DHPN). They divided the rats into four groups. Groups 1, 2, and 3 received 1,000 ppm potassium perchlorate, 1,000 ppm potassium iodide, or 1,000 ppm propylthiouracil in the diet, respectively. Group 4 was the control and received the basal diet throughout the study period of 18 weeks. At the beginning of the study, 50 percent of the rats in each group were injected with DHPN at 280 mg/100 g body weight as an initiator. Rats treated with both chemicals had a 100 percent incidence (20/20) of thyroid adenomas. Rats treated with potassium perchlorate alone had no thyroid adenomas. The incidence was five percent (1/20) in rats given DHPN alone. The investigators concluded that potassium perchlorate promoted the development of thyroid tumors in the rats treated with DHPN, but the chemical itself was not carcinogenic in this experiment.

Groups of male Wistar rats were exposed for two years to zero or one percent potassium perchlorate in their drinking water (Kessler and Kruskemper, 1966). Based on body weights and estimated water consumption, the one percent concentration was estimated to provide a dose of approximately 1,300 mg/kg-day. Animals were sacrificed and examined after 0, 40, 120, 220 and 730 days of exposure. Body weights of control and exposed animals were similar throughout the experiment, but thyroid weights of the exposed rats increased markedly compared to control rats at each examination interval. At 40 days, the exposed rats showed follicular cell hyperplasia, i.e., small follicles with high epithelia, large nuclei, numerous mitoses, colloid resorption and low-grade mesenchymal reaction. According to the authors, these changes are typical of thyroid glands stimulated by TSH for a relatively short time. Diffuse degenerative changes with fibrosis and increased colloid were observed after 200 days. Four of 11 rats treated with one percent potassium perchlorate for two years developed benign thyroid tumors. The twenty untreated controls had no thyroid tumors.

Pajer and Kalisnik (1991) divided 72 female BALB/c mice into 6 groups. Three groups were given 1.2 percent sodium perchlorate in drinking water, while three groups were controls. Eight or 32 weeks after the beginning of the study, one perchlorate and one control group of animals were irradiated with a total of four Grays of ionizing radiation (gamma rays) over a period of five days. Forty-six

weeks after the beginning of the experiment, 42 animals were sacrificed, while 30 had died during the experiment. The perchlorate dose to the treated mice was about 2,100 mg/kg-day based on standard assumptions for body weight and water consumption. Perchlorate treatment alone caused hypothyroidism with hypertrophic and hyperplastic thyroid epithelial cells as well as pituitary thyrotropic cells. Perchlorate and irradiation together caused effects similar to those caused by perchlorate treatment alone. Follicular cell carcinomas of the thyroid gland were found after perchlorate treatment (5/6 mice) and after perchlorate with irradiation (14/14), both statistically significant at $p<0.001$ versus controls (0/22).

The data indicate perchlorate caused thyroid follicular cell carcinomas in the treated mice. The study result is limited by the small number of animals in the perchlorate-only treated group, the design of the study, and the deaths of over 40 percent of the mice before the end of the experiment.

In a two-generation reproductive toxicity study in rats (Argus Research Laboratory, 1998b), two out of 30 male Sprague-Dawley rats (P2) in the highest dose group (30 mg/kg-day) were found to have adenomas of the thyroid. No such tumors were found in the control group or the other dosed groups (0.3 mg/kg-day and 3 mg/kg-day). In the study, the male rats were exposed to ammonium perchlorate in drinking water from conception to 19 weeks of age. As thyroid follicular cell adenomas are relatively rare in male Sprague-Dawley rats (the background incidence of this tumor reported in the literature was only 3.6-3.9 percent), U.S. EPA (2002) concluded that the increase in tumor incidence was treatment related.

In a number of subchronic perchlorate studies, increased thyroid follicular cell hypertrophy and hyperplasia were observed in some of the treated animals (Lampe *et al.*, 1967; Caldwell *et al.*, 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998a, 1999, 2001; Keil *et al.*, 1998). Summaries of these studies are provided in sections above. The data indicate that oral administration of perchlorate induces hyperplasia in the thyroid of rodents and if the exposures are lengthened, some of the lesions might progress to thyroid tumors.

Other Toxicities

Acute Toxicity

In acute toxicity testing, animals generally died within the first few days after oral administration of high doses of ammonium perchlorate (750 to 4,200 mg/kg). Autopsy findings included necrosis and hemorrhaging of the mucous membranes of the stomach. Intestinal damage, pulmonary edema, and vascular dilation and congestion of the spleen, brain and sinuses were also noted (Von Burg, 1995).

Table 44, showing acute LD_{50} values for perchlorate salts in several species, is modified from Von Burg (1995), compiled from Schilt (1979), U.S. EPA (1971), Shigan (1963), and Joesten and Hill (1966). The lethal dose for the various perchlorate salts when administered to mice by intraperitoneal injection varied over a 50-fold range.

Mannisto *et al.* (1979) administered potassium perchlorate to male Sprague-Dawley rats in drinking water for four days at concentrations of 0, 10, 50, 100 or 500 mg/L. At the end of the exposure period, they measured blood levels of TSH and thyroid hormones (T3 and T4). Significant changes (increased TSH and decreased T3 and T4) were observed in the 100 and 500 mg/L (15.3 and 76.3 mg/kg-day) exposure groups. In the 50 mg/L (7.6 mg/kg-day) exposure group, there was a significant decrease in concentration of T3 and T4; the TSH level was increased, but the increase was not statistically significant.

Table 44. Acute LD_{50} Values for Perchlorate Salts (Modified from Von Burg, 1995; Schilt, 1979; U.S. EPA, 1971; Shigan, 1963; Joesten and Hill, 1966)

Species	Route of Administration	Cation	Dose (mg/kg)
Rat	Oral	NH_4^+	3,500 to 4,200
Mouse	Oral	NH_4^+	1,900 to 2,000
Rabbit	Oral	NH_4^+	750 to 1,900
Guinea Pig	Oral	NH_4^+	3,310
Mouse	i.p.	Li^+	1,160
Mouse	i.p.	Mg^{++}	1,500
Mouse	i.p.	Na^+	1,150
Mouse	i.p.	Mn^{++}	410
Mouse	i.p.	Fe^{+++}	370
Mouse	i.p.	Co^{++}	160
Mouse	i.p.	Ni^{++}	100
Mouse	i.p.	Cu^{++}	29
Mouse	i.p.	Zn^{++}	76

Genetic Toxicity

Ammonium perchlorate was tested in a battery of genotoxicity tests, and found to be negative in all tests (U.S. EPA, 2002). Ammonium perchlorate was negative in the reverse mutation assay in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) with and without S9 activation (ManTech Environmental Technology, 1998). The Ames tests were later repeated by the National Institute of

Environmental Health Sciences. Strains TA102 and TA104 were added to cover the possibility that ammonium perchlorate causes mutation by producing reactive oxygen species or other DNA damaging radicals. The repeat tests also used the pre-incubation test methodology as it provides better contact between the test material and the target organism. Ammonium perchlorate was negative in the reverse mutation assay in *S. typhimurium* (TA98, TA100, TA1535, TA97, TA102, and TA104) with and without S9 activation, thus confirming the original Ames test results (Zeiger, 1998).

Ammonium perchlorate was negative in the L5178Y/TK$^{+/-}$ mouse lymphoma assay without S9 activation. Results of the mouse lymphoma assay with S9 activation were equivocal because of low frequency of mutations in the positive controls (ManTech Environmental Technology, 1998). The test was later repeated. In this assay, concentrations of ammonium perchlorate in the treatment medium of 50 to 5,000 µg/mL were negative in the L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay in the absence and presence of Arochlor-induced rat liver S9 (BioReliance, 1999). The results of the repeat study provided support for the negative results reported in the first study.

Ammonium perchlorate tested negative in *in vivo* micronuclei assays in mice and rats. In the mouse micronucleus assay, five male and five female Swiss CD-1 mice were dosed by gavage at 0, 62.5, 125, 250, 500, or 1,000 mg/kg for three consecutive days. No increases in the frequency of micronuclei were found for any dose group (ManTech Environmental Technology, 1998). There is some uncertainty whether the maximum tolerated dose was reached in the study. Typically, the assay is performed at 85 percent of the maximum tolerated dose, and the 1,000 mg/kg-day dose represents approximately 50 percent of the LD_{50}. Furthermore, there was no indication of toxicity to the bone marrow cells because the polychromatic erythrocyte/normochromatic erythrocyte ratio was not different from the controls. The test was later repeated by the National Institute of Environmental Health Sciences. Male B6C3F$_1$ mice were injected intraperitoneally with 0, 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in buffered saline. Five mice per group were treated daily for three consecutive days, and were sacrificed 24 hours after the last injection. All animals in the 1,500 and 2,000 mg/kg groups died after the first injection and 4/5 animals in the 1,000 mg/kg group died after the second injection. All animals in the 125, 250, and 500 mg/kg groups survived the treatment. No increases in micronuclei were seen at any of the test doses, and the trend test was not positive (Zeiger, 1999). The negative results of the repeat study support the results of the first study.

The 90-day subchronic bioassay using Sprague-Dawley rats also evaluated micronuclei induction (Siglin *et al.*, 2000). Ten rats per sex were treated with ammonium perchlorate in drinking water for 90 days at 10 mg/kg-day. The results indicate that ammonium perchlorate under the test condition was not mutagenic to the bone marrow cells of male and female Sprague-Dawley rats. The chemical was not toxic to the bone marrow cells at the dose tested, as it did

not reduce the ratio of polychromatic to normochromatic erythrocytes in male or female rats.

Based on the above *in vitro* and *in vivo* genotoxicity test results, ammonium perchlorate does not appear to be mutagenic or clastogenic. Therefore, genotoxicity is not considered a potential mode of carcinogenic action for perchlorate.

Immunotoxicity

Shigan (1963) administered ammonium perchlorate to rabbits and white rats in water at 190 mg/kg-day for three months. The mode of administration was not described. No effect was found on immune function as evaluated by leukocyte phagocytosis (Shigan, 1963).

A series of hematological and immunotoxicology experiments in female $B6C3F_1$ or CBA/J Hsd mice were conducted as part of the U.S. EPA's perchlorate testing strategy (U.S. EPA, 2002). In these experiments, mice were exposed for 14 or 90 days to ammonium perchlorate at doses between 0.02 and 50 mg/kg-day via drinking water. The mice were tested at intervals for immunotoxicological effects such as delayed type hypersensitivity and cytotoxic lymphocyte activity (Keil *et al*., 1998, 1999; Burleson Research Technologies, 2000).

In the hematological studies, no differences were observed between control and dosed mice in 14- or 90-day experiments for erythrocyte cell count, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentration, nor in leukocyte differential counts of neutrophils, monocytes, and lymphocytes. An increase in the percentage of reticulocytes was observed in the peripheral blood of mice exposed to 3 mg/kg-day of ammonium perchlorate in a 90-day study. No consistent alteration in the bone marrow stem cell assay was observed. An increase in the number of colony-forming units was observed in bone marrow cell cultures from mice dosed at 30 mg/kg-day in a 14-day study. However, in two other 90-day studies, this positive result was not confirmed. Upon reviewing the immunotoxicological studies, U.S. EPA (2002) found that three immune function parameters were altered by ammonium perchlorate exposure: (a) suppression of *in vitro* peritoneal macrophage phagocytosis of *L. monocytogenes*; (b) enhancement of the plaque-forming cell (PFC) assay response to sheep red blood cells (SRBCs); and (c) enhancement of the local lymph node assay (LLNA) response to 2,4-dinitrochlorobenzene (DNCB).

Decreased *in vitro* phagocytosis of *L. monocytogenes* by peritoneal macrophages obtained from mice dosed for 14 days at 1 or 30 mg/kg-day (ammonium perchlorate) was observed. In mice exposed for 90-days, phagocytosis was decreased in all dose groups (Keil *et al.*, 1998, 1999). However, similar effects were not observed in a 90-day perchlorate exposure followed by a 30-day recovery period study. These *in vitro* data suggest that perchlorate suppresses the phagocytic capacity of peritoneal macrophages, but

this suppression is reversed after a 30-day recovery period. It is difficult to interpret the biological significance of this data set because *in vivo* study results indicate ammonium perchlorate exposure did not alter the ability of mice to combat *L. monocytogenes* infection. It was suggested that while perchlorate may reduce the phagocytic capacity of peritoneal macrophages, the ability of macrophages from other sites (e.g., spleen, liver) to clear *L. monocytogenes* was not altered (U.S. EPA, 2002).

The PFC assay is routinely used for identifying immunosuppressive chemicals. The reason why the highest dose(s) of ammonium perchlorate, given over 90 days, enhanced this response is not known. The enzyme-linked immunosorbant assay (ELISA) data for mice exposed to up to 30 mg/kg-day for 14 or 90 days do not corroborate this enhanced response to SRBCs observed in the PFC assay. The data from Burleson Research Technologies (2000) indicate that exposure to perchlorate enhances the LLNA response to DNCB. While a dose of 50 mg/kg-day for 14 days enhanced the response, the same dose for 90 days suppressed the response. Lower doses of 0.06 and 0.2 mg/kg-day also increased the response; however, interpretation of these data is made difficult by the observation that 2 mg/kg-day did not affect the response in the 14-day study. OEHHA agrees with U.S. EPA (2002) that interpretation of the results is made difficult by (a) some technical problems encountered in the studies, (b) the apparent inconsistency of the high-dose study results, and (c) the unknown biological significance of the response enhancement.

Neurotoxicity

By interfering with the thyroid-pituitary axis, perchlorate can interfere with development of the central nervous system. Thyroid hormone plays an essential role in the development of the corpus callosum and other brain structures. As part of the U.S. EPA's program to evaluate the toxicity of perchlorate, neurodevelopmental tests on Sprague-Dawley rats were conducted by Argus Research Laboratories (1998a; 2001). The study design and findings have been summarized in the "Developmental and Reproductive Toxicity" section.

6. DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The primary effect of perchlorate exposure is the disruption of thyroid hormone regulation. This mode of action is supported by the results of a number of animal studies that show that perchlorate inhibits thyroidal iodide uptake, changes serum T3, T4, and TSH levels, causes thyroid enlargement, induces thyroid follicular cell hypertrophy and hyperplasia, and a number of other toxicological endpoints, as reviewed in the "Toxicological Effect in Animals" chapter. Similarly, the primary action of perchlorate in humans is inhibition of iodide uptake into the thyroid gland. The function of the thyroid gland is the production of thyroid hormone. Iodide is a key component in the structure of thyroid hormone, and by blocking its uptake into the thyroid, perchlorate can potentially cause decreased production of this hormone. Decreases in thyroid hormone have been associated with impaired neurodevelopment in children, increases in cardiovascular disease risk factors, and other adverse effects.

Selecting the Key Biochemical Effect

In developing PHGs, OEHHA is required by the California Safe Drinking Water Act of 1996 (Health and Safety Code Section 116365) to consider the existence of groups in the population that are more susceptible to adverse effects of drinking water contaminants than are typical healthy adults. The primary mechanism of perchlorate toxicity is inhibition of iodide uptake by the thyroid. Three groups that may be particularly susceptible to this effect of perchlorate due to alterations in iodine status are pregnant women, the developing fetus, infant or young child, or women with low iodine intake.

Thyroid stress of pregnancy: In his review paper, Glinoer (2001) suggested that pregnancy causes profound changes in thyroid function and represents a stress on the thyroid hormonal system. In the first trimester of gestation, there is an increased need for thyroid hormones and an increased need for iodine from the diet. When iodine nutrition levels are sufficient, physiological adaptation takes place. When iodine is restricted or deficient, adequate physiological adaptation is difficult to achieve and is progressively replaced by pathological alterations occurring in parallel with the degree of long-term iodine deprivation. Glinoer concluded, "Therefore, pregnancy typically reveals underlying iodine restriction and gestation results in an iodine-deficient status, even in conditions with only a marginally restricted iodine intake, such as is observed in many European regions."

Results of a prospective study reported by Kung *et al.* (2000) showed that in a borderline iodine-sufficient area (median urinary iodine level = 9.8 µg/dL), pregnancy can pose a stress on the thyroid, resulting in higher rates of maternal

goitrogenesis as well as neonatal hypothyroxinemia and hyperthyrotrophinemia. Thyroid enlargement in these women persisted and failed to revert completely even 3 months after delivery.

Susceptibility of the fetus, infant and young child: As reviewed above, several epidemiological studies provide evidence that iodine deficiency during pregnancy may adversely affect brain development and cause neurointellectual deficits in the offspring. These effects are not limited to areas with severe iodine deficiency and endemic cretinism; effects have been associated with levels of thyroid hormone that fall into what have traditionally been considered normal ranges. The severity of effects may depend on the timing and the severity of the iodine deficiency. In several studies conducted in areas with moderate or even mild iodine deficiency, mainly in southern Europe, it was shown that developmental abnormalities may occur in schoolchildren who are clinically euthyroid. Even borderline iodine deficiency might lead to impaired school performance in some children (Glinoer, 2001).

Since the thyroid is dependent on iodide for thyroid hormone production, inadequate iodine intake can lead to decreased thyroid hormone production. Studies in humans that have identified links between relatively small decreases in thyroid hormone levels during pregnancy and significant effects on cognition in the offspring include Pop *et al*. (1999, 2003), Haddow *et al*. (1999), Klein *et al*. (2001), 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). Importantly, some of these effects are seen at thyroid hormone levels that are within what has been traditionally defined as the normal range, and in pregnant mothers and offspring without any other evidence of clinical hypothyroidism.

One reason why the fetus, infant or the young child may be particularly susceptible to inadequate iodine intake and small changes in thyroid hormone levels is that the fetal and infant periods are critical times of brain and neurological development. Another reason may be that the fetus and infant do not have fully developed thyroids and have stores of thyroid hormone that are much lower than in adults (van den Hove *et al.,* 1999). These low stores may make them more susceptible to temporary decreases in iodine intake or other factors that may inhibit hormone production. Finally, some data suggest that many young children in the U.S. may not have an adequate iodine intake. Pearce *et al*. (2007) estimated that 47 percent of the breast milk samples in their study of 57 women from the Boston area did not contain enough iodine to meet the infant iodine intake recommended by the Institute of Medicine.

Women with low iodine intake in the U.S.: The rates of most thyroid diseases are much greater in women than in men. The reason for this is unknown, but it suggests that women might be more susceptible to environmentally-caused

thyroid problems than men. In Blount *et al.* (2006), statistically significant associations between increasing perchlorate and decreasing T4 and increasing TSH were seen in women but not in men. In addition, effects on T4 were only seen in women with evidence of somewhat reduced iodine intake (urinary iodine concentrations < 100 µg/L), but not in women with evidence of higher iodine intakes. These effects highlight the importance of gender and iodine status when assessing the potential impacts of perchlorate.

Urinary iodine concentration is an indicator of the adequacy of iodine intake for a population. According to the World Health Organization (WHO), the median urinary iodine concentrations in iodine-sufficient populations should be greater than 100 µg/L (WHO, 1994; as cited in Hollowell *et al.*, 1998). In the NHANES 2001-2, the geometric mean urinary iodine concentration in women was 126 µg/dL, which indicates an adequate iodine intake for the population as a whole. However, this level does not mean that every individual member of the population has an adequate iodine intake. In Blount *et al.* (2006), the perchlorate-T4 association was seen in women with spot urinary iodine levels below 100 µg/L, a group that included 37 percent of all women in the study. The fact that the women in this study were derived from an essentially nationally representative sample of all women in the U.S. suggests that a large number of U.S. women (e.g., 37 percent) have iodine intakes that may put them at risk for effects from perchlorate.

In summary, the primary mechanism of perchlorate toxicity is the inhibition of iodide uptake into the thyroid gland and a subsequent decrease in thyroid hormone production. Iodine deficiency and thyroid insufficiency have been linked to a number of significant adverse health effects including goiter, impaired cognitive development, and increases in cardiovascular risk factors. Some of these links have been seen at levels of iodine and thyroid hormones that have been considered to be within normal reference ranges. OEHHA has chosen to use reduced thyroidal iodide uptake as the key biochemical effect for the perchlorate PHG. This is the same key biochemical effect used in the OEHHA 2004 PHG perchlorate document and the same one used by the NRC in their report on the health implications of perchlorate exposure (OEHHA, 2004; NAS, 2005). The purpose of this PHG is to help prevent any inhibition of thyroidal iodide uptake that could potentially lead to the adverse effects described above.

Selecting the Critical Study

OEHHA used the 14-day perchlorate clinical dosing study of Greer *et al*. (2002) for its dose-response analysis in the 2004 perchlorate PHG document, and five percent inhibition of iodide uptake as the benchmark response (BMR). Greer *et al.* (2002) administered a daily oral dose of perchlorate to groups of male and female volunteers for 14 days at doses of 0.007, 0.02, 0.1, or 0.5 mg/kg-day. Reductions in iodide uptake by the thyroid gland were seen at all four dose levels, with statistically significant reductions at the highest three doses.

In this update, to determine a level of perchlorate exposure that would not inhibit thyroidal iodide uptake, OEHHA again has chosen the Greer *et al*. (2002) study as the critical study and applied the benchmark dose approach for identification of the point of departure. This was selected as the critical study 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 the key biochemical outcome, iodide uptake in the thyroid. Several other studies have linked perchlorate exposure to changes in thyroid hormones and these were evaluated as to whether they could be used for risk assessment. However, these were either based on ecologic measurements of perchlorate exposure or only a single or few urinary perchlorate measurements (e.g., Kelsh *et al.,* 2003; Blount *et al.,* 2006). Basing exposure on only a few measurements could bias true associations towards the null and lead to an underestimation of true risks. Nonetheless, we estimated a PHG using an analysis of the NHANES data, which is presented in the Appendix. Despite any possible exposure misclassification, this estimate comes quite close to that estimated using Greer *et al.* (2002) data.

Selecting the Point of Departure

The benchmark dose (BMD) is the dose associated with a predefined level of response, the benchmark response (BMR). For this analysis, a five percent decrease of mean radioactive iodide uptake by the thyroid is used as the BMR. A five percent decrease was selected because this is the lowest level of effect that is commonly detectable with statistical significance in many animal and human studies.

A statistical lower bound of the BMD, the 95 percent lower confidence limit (the BMDL), is used as the point of departure for defining an exposure level that is likely to be without an appreciable risk of deleterious effects in humans. Using the BMDL rather than the BMD 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).

U.S. EPA recommends BMD methods to estimate reference doses (RfDs), which are used along with other scientific information to set criteria and standards for noncancer human health effects. In the past, RfDs have mainly been determined from NOAELs, which represent the highest experimental dose for which no adverse health effects have been documented. As noted by U.S. EPA and many others, the BMD approach has several significant advantages over the NOAEL approach when appropriate dose-response data, like those in Greer *et al.* (2002), are available. Using the NOAEL to determine RfDs has long been recognized as having a number of limitations. One is that the NOAEL is limited to only one of the doses in the study and is highly dependent on study design; in particular, the dosages selected by the investigators. In most clinical studies, like Greer *et al.* (2002), only a relatively small number of dose levels are selected. Because the level selected as the NOAEL has to be one of the doses evaluated in the critical

study, the NOAEL is highly dependent on the dose levels chosen by the investigator.

Another weakness is that the NOAEL is highly dependent on the size of the study. The reason for this is that it is usually defined as the highest dose at which there is no *statistically significant* observable effect, and statistical significance is highly dependent on sample size. All else being equal, the larger the sample size, the more likely a finding of a given magnitude (e.g., a 5% decrease in iodide uptake) will be statistically significant. Thus, two studies (one large and one small) may both find the same 5% effect at a particular dose, but this 5% could be statistically significant in the larger study but not in the smaller study. In this example, the 5% effect could be real in both studies, but would be selected as the NOAEL in the smaller study for the sole reason that the investigators did not include more subjects. The same arguments apply to using a NOEL. In the Greer *et al*. (2002) study, a small decrease in iodide uptake by the thyroid was seen in those subjects receiving the lowest perchlorate dose. As seen in Figure 12, the magnitude of this effect is about what was expected based on the magnitudes of the effect sizes seen in the higher dose groups. In a larger study, this may have been statistically significant and considered a LOEL. However, the sample size was small (there were only seven subjects in the lowest dose group), and this small effect size was not statistically significant and thus considered a NOEL. It is possible that this effect was due to chance in Greer *et al*. (2002). However, it is also possible this effect is real or that the actual effect is even higher than observed but ended up being lower due to chance.

In the BMD approach, this is not an issue. This approach involves estimating effect sizes based on the entire dose-response curve, that is, all of the dose levels, all of the sample sizes at each dose level, and all of the effect sizes at each dose level. As such, it is not reliant on any single dose and therefore much less reliant on the doses chosen by the investigator, on the sample size at any single dose level, or the statistical significance of any single result. Sample size is still important in the BMD method in that the results of each dose are partially weighted by the number of subjects at each dose level and in calculating the BMDL.

A final advantage of the BMD approach is that because all dose levels are considered, the shape of the dose-response curve throughout the entire dose range, especially at the lower doses, is taken into account. This can potentially allow for a more accurate estimation of the true effects occurring in the lower dose ranges.

For all of these reasons, OEHHA has chosen to use the BMD approach rather than the NOEL approach. As discussed below, this had only a relatively small impact on the PHG.

Calculation of the BMD Using Greer *et al*. (2002)

OEHHA used the BenchMark Dose Software, version 2.0.0.33 (U.S. EPA, 2008a) to perform the analyses based on the human data reported by Greer *et al*. (2002) shown in Table 45. This is the same analysis, using the same data from Greer *et al.* (2002) that was used in the OEHHA 2004 perchlorate PHG document. A detailed discussion of the application of the software is provided in a U.S. EPA (2012) document, "Benchmark Dose Technical Guidance."

OEHHA tried several curve fitting models provided by the software and found the Hill model adequately describes the data (goodness of fit test, p=0.46), shown plotted in Figure 12. The fit is generally considered adequate when the p-value is greater than 0.10. Other models, including linear and polynomial models, fit the data poorly based on visual inspection and goodness of fit p-values.

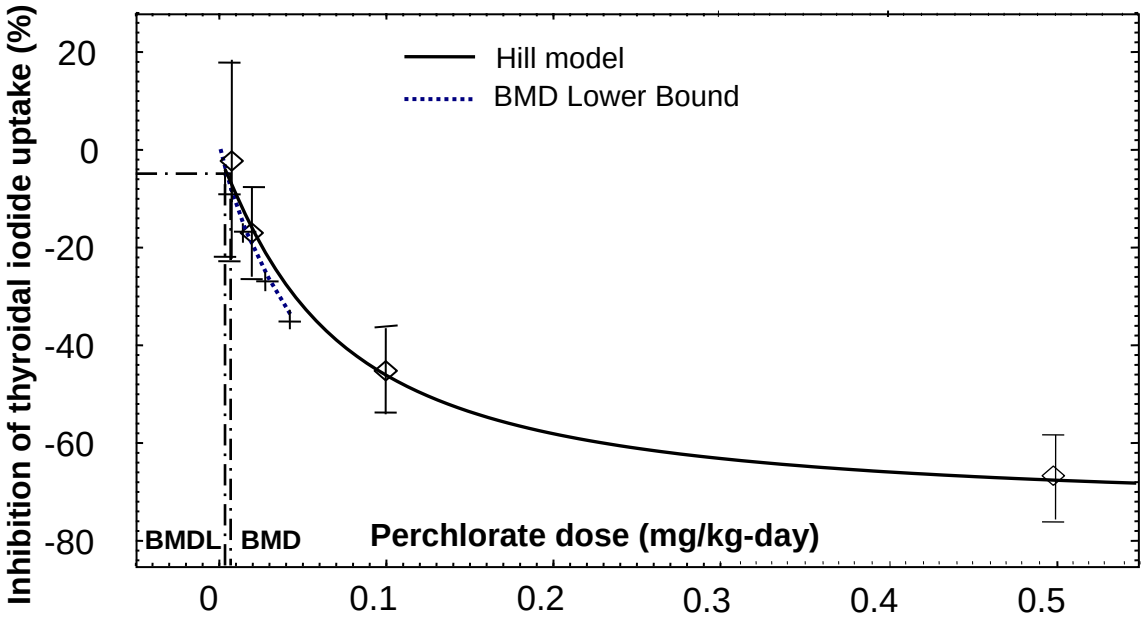


Figure 12. Analysis of the Greer *et al*. (2002) Data by the Benchmark Dose Approach. The Hill model was run with the following settings: intercept = zero, power parameter restricted to be greater than one, a constant variance model assumed. The BMR was a 5 percent decrease in iodide uptake.

The form of the response function estimated by the model is as follows:

Response = intercept + $(v \times \text{dose}^n) / (k^n + \text{dose}^n)$

where:

intercept = 0
v = -67.076

n = 1.43546
k = 0.0684664

The estimated BMD and BMDL corresponding to a five percent reduction of the mean thyroidal iodide uptake are 0.0068 mg/kg-day and 0.0037 mg/kg-day, respectively. These are the same BMD and BMDL calculated in the 2004 OEHHA perchlorate PHG document. It should be noted that the BMDL of 0.0037 mg/kg-day is lower than the lowest dose tested, 0.007 mg/kg-day, in the Greer *et al*. (2002) study.

Table 45. Human Data from Greer *et al*. (2002)

Average dose (mg/kg-day)	Change in 24 hr radioactive iodine uptake by the thyroid (%)		Number of subjects in each dose group
	Average	Standard deviation	
0.007	-1.844	22.019	7
0.02	-16.393	12.828	10
0.1	-44.693	12.32	10
0.5	-67.076	12.114	10

For comparison, significant changes in serum T3, T4, and TSH levels were observed even at the 0.01 to 0.1 mg/kg-day dose range for perchlorate in adult rodents. Rat fetuses and rat pups are reportedly more sensitive to the effects of perchlorate than adult rats. In several reproductive and developmental studies, colloid depletion of the thyroid, thyroid hypertrophy, and abnormal brain development were found in rat pups exposed to perchlorate *in utero* and after birth. Based on these study results (Springborn Laboratories, 1998; Argus Research Laboratories, 2001), a LOAEL of 0.01 mg/kg-day can be identified.

Carcinogenic Effects

There are two published epidemiological studies that investigated the association between perchlorate in drinking water and cancer (Li *et al*., 2001; Morgan and Cassady, 2002). Based on the reported data, it does not appear that perchlorate was associated with increased risks of cancer in the two study areas during the study periods, under the limitations of the studies.

Several subchronic oral studies in rodents showed that perchlorate induced hypertrophy and hyperplasia in the thyroid gland (Caldwell *et al*., 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998b, 1998d, 1999, 2001; Keil *et al*., 1998). In two chronic oral studies, perchlorate at relatively high concentrations (over 1,000 mg/kg-day) was shown to produce thyroid follicular cell tumors in rats (Kessler and Kruskemper, 1966) and mice (Pajer and Kalisnik, 1991). However, only benign tumors were observed in the

study reported by Kessler and Kruskemper (1966), and inadequate reporting and low survival of the control and exposed animals lowered confidence in the results reported by Pajer and Kalisnik (1991). Each study had only one perchlorate exposure group. In a developmental study reported by Argus Research Laboratories (1999), thyroid follicular cell adenomas were also observed in two male Sprague-Dawley rats (2/30) exposed to 30 mg/kg-day perchlorate *in utero* and after birth. No such tumors were found in the vehicle control (0/30). Though the incidence is not significant using standard tests (e.g., Fisher's exact test), the fact that the tumors were found in 19-week old rats and the historical incidence of this type of tumor in male Sprague-Dawley rats in 2-year studies reported in the literature is only 3-4 percent makes the finding noteworthy (U.S. EPA, 2002).

Complex anions structurally similar to perchlorate, such as pertechnetate (TcO_4^-), perrhenate (ReO_4^-) and tetrafluoroborate (BF_4^-), are also capable of inducing thyroid follicular cell neoplasia in test animals (Green, 1978, as cited in Paynter *et al.*, 1988). Based on the limited data available, there are reasons to believe that perchlorate is a potential carcinogen in rodents.

After reviewing thyroid carcinogenesis in rodents and in humans, U.S. EPA (1998b) in the "Assessment of Thyroid Follicular Cell Tumors" stated that "in spite of the potential qualitative similarities, there is evidence that humans may not be as sensitive quantitatively to thyroid cancer development from thyroid-pituitary disruption as rodents. Rodents readily respond to reduced iodide intake with the development of cancer, humans develop profound hyperplasia with "adenomatous" changes with only suggestive evidence of malignancy. Even with congenital goiters due to inherited blocks in thyroid hormone production, only a few malignancies have been found in humans."

One factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation is the influence of protein carriers of thyroid hormones in the blood. In humans, other primates, and dogs a high affinity thyroxine-binding globulin tightly binds T4 (and T3 to a lesser degree); this protein is missing in rodents, rabbits and lower vertebrates. As a result, T4 bound to proteins with lower affinity in the rodent is more susceptible to removal from the blood, metabolism, and excretion from the body. As shown in Table 46, the estimated serum half-life of T4 is much shorter in rats (<1 day) than in humans (5-9 days). This shorter T4 half-life in rats requires a higher level of serum TSH and T4 production rate than in the adult human (U.S. EPA, 1998b). Thus, it appears that the rodent thyroid gland is chronically stimulated by TSH levels above basal levels to compensate for the increased turnover of thyroid hormones, and this in turn could move the gland towards increased growth and potential neoplastic change more readily than in humans. It is interesting to note that adult male rats have higher serum TSH levels than females, and they are often more sensitive to goitrogenic stimulation and thyroid carcinogenesis. In humans, there is no sex difference in hormone levels, but females much more frequently develop thyroid cancer (U.S. EPA, 1998b).

Some of the studies summarized in this document provide some evidence of a quantitative difference in the thyroid responses of humans and rodents to perchlorate, based on applied dose. Several 14-day drinking water studies showed significant depression in serum T3, T4, and elevation in serum TSH levels in rodents exposed to doses as low as 0.01 or 0.1 mg/kg-day (Caldwell *et al.*, 1995; Springborn Laboratories, 1998; Keil *et al.*, 1998; Yu *et al.*, 2000). By contrast, serum T3, T4, and TSH levels in healthy adult humans that are not iodine deficient are much less sensitive to perchlorate exposure. For instance, after exposure to perchlorate in drinking water as high as 12 mg/kg-day for 1, 2, or 4 weeks, no significant changes in serum T3 and T4 levels were found in male volunteers. Serum free T4 and TSH levels were significantly depressed following perchlorate exposure when compared to those before exposure (Brabant *et al.*, 1992; Mattie, 2000). A significant reduction in intrathyroidal iodine concentration was also noticed in the study reported by Brabant *et al.* (1992). Lawrence *et al.* (2000) found no change in serum T3, T4, and TSH in male volunteers exposed to perchlorate in drinking water at 0.14 mg/kg-day for 1 and 2 weeks. Greer *et al.* (2002) exposed male and female volunteers to perchlorate in drinking water at 0.02, 0.1, or 0.5 mg/kg-day for 2 weeks and collected blood samples on days 1, 2, 3, 4, 8, and 14. Statistically significant depression of iodide uptake by the thyroid was observed in the upper two dose groups. However, no significant depression in serum T3 and T4 nor elevation in serum TSH was observed. No dose-response relationships were noticed for these thyroid and pituitary hormones. These data show that though a similar mode of action of perchlorate is operative in rodents and humans, the sensitivities of serum T3, T4, and TSH levels of the two species to perchlorate may not be the same. However, this remains to be fully elucidated. Greer *et al*. (2002) and the other clinical studies cited above only evaluated healthy adults, not sensitive members of the human population such as pregnant women and their fetuses, women with low iodine status, people with thyroid disease, or infants. There is some epidemiological evidence that lower levels of chronic perchlorate exposure seen in the NHANES data (Steinmaus *et al.,* 2010) affect newborn thyroid function.

Table 46. Inter- and Intraspecies Differences of T3, T4, and TSH Levels and Sensitivity to Thyroid Cancer (Modified from U.S. EPA, 1998b)

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T4 half-life	5-9 days	0.5-1 day
T3 half-life	1 day	0.25 day
$\frac{\text{T4 production rate}}{\text{kg body weight}}$	1 ×	10 × that in humans
TSH	1 ×	6-60 × that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	Sexes equal	Male $\leq$ 2 × Female
Sensitivity to thyroid cancer	Female = 2.5 × Male	Male > Female

In evaluating a thyroid carcinogen, it is important to determine the mode of action as it impacts the choice of models in high-to-low dose extrapolation. In the "Assessment of Thyroid Follicular Cell Tumors," U.S. EPA (1998b) stated that in order to show the antithyroid activity of a chemical is the cause of thyroid tumors observed in rodents, it is necessary to demonstrate the following:

1. Increases in thyroid growth;
2. Changes in thyroid and pituitary hormones (considered to be the most important);
3. Location of the sites of antithyroid action (documents where in the body the chemical under assessment leads to perturbations in thyroid-pituitary function);
4. Dose correlations among various effects (to determine where the growth curve for the thyroid gland deviates from the normal pattern of cell replacement and how this relates to doses producing tumors); and
5. Reversibility of effects following treatment cessation during the early stages of disruption of the thyroid-pituitary axis (shows that permanent, self-perpetuating processes have not been set into motion).

The available toxicity data of perchlorate appear to have fulfilled the five requirements described above. Several *in vitro* and *in vivo* genotoxicity studies have been performed on perchlorate. Under the testing conditions, none of the tests indicates perchlorate is a genotoxic agent. Perchlorate is known to inhibit the uptake of iodide in the thyroid, thereby causing a reduction in the hormones T3 and T4. Subchronic and chronic drinking water studies showed that perchlorate exposure depressed serum T3 and T4 but elevated serum TSH

levels in rodents and rabbits. At higher exposure levels, thyroid follicular cell hypertrophy, thyroid follicular cell hyperplasia, and increased thyroid weights were also observed in adults as well as postnatal rats, as described in the chapter “Toxicological Effects in Animals.”

There is also evidence that the thyroid follicular cell hypertrophy and hyperplasia observed in rats exposed to ammonium perchlorate might be reversible. In the study reported by the Springborn Laboratories (1998), absolute and relative thyroid/parathyroid weights were significantly increased in male rats exposed to 10 mg/kg-day for 14 days as well as 90 days. However, no significant increases in both absolute and relative thyroid/parathyroid weights were observed in male rats exposed to 10 mg/kg-day for 90 days, followed by a 30-day recovery period. Similarly, absolute and relative thyroid/parathyroid weights were significantly increased in female rats exposed to 10 mg/kg-day for 90 days, but no significant increases in terms of both absolute and relative thyroid/parathyroid weights were observed in female rats exposed to 10 mg/kg-day for 90 days, followed by a 30-day recovery period.

The available data suggest that thyroid tumors observed in rodents exposed to perchlorate via the oral route are likely to be caused by the disruption of thyroid-pituitary homeostasis. It follows that if there were no thyroid and pituitary hormone changes, and no thyroid follicular cell hypertrophy and hyperplasia, there would be no thyroid tumors. For this reason, the perchlorate dose determined for prevention of a detectable decrease in T4 in humans (noncarcinogenic effect) is reasoned to be protective against thyroid tumors as well.

There are no adequate data from human studies to evaluate the cancer potency of perchlorate. Several studies in laboratory animals have reported increases in thyroid tumors following perchlorate exposures, although these have involved doses that are orders of magnitude higher than the exposure levels seen in the dose-response data OEHHA used for BMD modeling. Furthermore, there are difficulties in estimating the cancer potency of perchlorate based on animal cancer data because of differences in iodine deficiency and thyroid disease status (background rates) in laboratory animals compared to humans. For these reasons, a quantitative dose-response evaluation was not performed for the possible carcinogenic effects of perchlorate. It is reasoned that by setting the perchlorate PHG low enough to avoid impacts on thyroid hormone status, all other potential adverse thyroid effects, including benign and malignant thyroid tumors, will be prevented.

7. CALCULATION OF THE PHG

The PHG is derived in this section by first calculating an Acceptable Daily Dose, or maximum amount that can be consumed without toxic effects, using the BMD value derived from the human study by Greer *et al*. (2002) in the last chapter. This is then converted into an acceptable drinking water perchlorate concentration. In making these calculations, population subgroups that may be much more susceptible to the effects of perchlorate than healthy adults and perchlorate exposures from sources besides drinking water are taken into account. The resulting updated PHG will help prevent perchlorate-related reductions in thyroidal iodide uptake and subsequent decreases in thyroid hormone production that may be associated with adverse health effects.

A quantitative dose-response evaluation was not performed for the possible carcinogenic effects of perchlorate. By setting the perchlorate PHG low enough to avoid impacts on thyroid hormone status, all other potential adverse thyroid effects, including benign and malignant thyroid tumors, are assumed to be prevented.

Acceptable Daily Dose (ADD)

For estimation of a health-protective concentration of perchlorate in drinking water, an acceptable daily dose (ADD) of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the dose leading to the key biochemical effect from human or animal studies to the estimation of a lifetime ADD that is unlikely to result in or lead to any toxic effects. For this purpose, the following equation can be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL/BMDL in mg/kg-day}}{\text{UF}}$$

where,

ADD = estimated maximum daily dose that can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL/BMDL= no-observed-adverse-effect level, lowest-observed-adverse-effect level, or lower limit on the benchmark dose estimated from the critical study;

UF = uncertainty factor(s).

For this case, OEHHA chose 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).

An uncertainty factor of 10 is applied to help account for interindividual variability in the population that was not captured by the Greer *et al*. (2002) study. This factor is used to account for uncertainty in interindividual differences in toxicodynamic and toxicokinetic factors that may impact susceptibility. It is not intended to account for drinking water intake rates and actual estimates of perchlorate intake and exposure, which are incorporated separately. The Greer *et al.* (2002) 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 with the other potential susceptibility factors. Potentially susceptible groups include:

- Neonates and infants, who may be susceptible to perchlorate for several reasons:

    a. Infancy is a critical period for development of the brain and nervous system, processes that are critically reliant on adequate supplies of thyroid hormone. The role that thyroid hormone plays in these critical developmental processes highlights the potential importance of any agent, like perchlorate, whose mechanism involves alterations in thyroid hormone production.

    b. Some data suggest that neonates have stores of thyroid hormone and iodide that are much less than those in adults (Van den Hove *et al*., 1999). This would make neonates less able than adults to respond to any factor that temporarily decreases iodine intake, iodide uptake, or thyroid hormone production.

    c. Data from several studies (Kelsh *et al.,* 2003; Brechner *et al,.* 2000; Steinmaus *et al.,* 2010; and others) provide evidence of a possible link between perchlorate in drinking water during pregnancy and thyroid hormone levels in newborns.

    d. Preterm infants may be the subgroup most at risk for impairment in thyroid hormone production caused by a reduction in iodine intake or iodide uptake by the thyroid. Intrathyroidal stores of thyroid hormone and iodide are markedly less in preterm infants than in full term infants (van den Hove 1999). In addition, preterm infants have immature pituitary-thyroid function, and lose the contribution of maternal thyroid hormone at birth. Preterm infants also can have immature gastrointestinal function which could reduce their iodine intake.

    e. Since the primary mechanism of perchlorate toxicity is reduced iodide uptake by the thyroid, individuals who are already iodine deficient may be particularly susceptible to perchlorate toxicity. Importantly, the breast milk of many women may not provide an adequate iodine intake for the breast-fed child.

- Women with low iodine intakes. In Blount *et al*. (2006), statistically significant associations between increasing perchlorate and decreasing T4 were seen in women with urinary iodine levels below 100 µg/L, but not in women with higher iodine levels. Estimates from NHANES 2001-2 suggest that 37 percent of all women in the U.S. have urinary iodine levels below 100 µg/L (Blount *et al*., 2006).

- The developing fetus. Studies have shown that decreases in maternal T4 during pregnancy, even relatively small ones, can lead to significant cognitive deficits in the offspring.

- Lactating women. Lactating mothers are considered a potentially sensitive subpopulation for effects of perchlorate because their need for iodine is greater than other adults. They are therefore at greater risk of getting an insufficient amount of iodine from the diet. The NAS (2001) suggests an Estimated Average Requirement and a Recommended Dietary Allowance of iodine almost two-fold higher for lactating mothers than for other adults.

- Pregnant women, who also have increased iodine requirements.

- People with thyroid diseases.

- People with high levels of thiocyanate, which typically comes from food or tobacco smoking. Data from Steinmaus *et al*. (2007) suggest that the magnitude by which perchlorate reduces T4 levels is about two times greater in people with high thiocyanate levels than in people with average or low thiocyanate levels (Table 40).

In summary, an uncertainty factor of 10 is applied to account for all sensitive groups, including infants, who may be more susceptible to perchlorate than the 37 healthy research volunteers in Greer *et al*. (2002). OEHHA notes that the uncertainty factor of 10 that used here is the same as the uncertainty factor of 10 recommended and used by the NRC to calculate its most recent perchlorate reference dose for U.S. EPA (NAS, 2005).

OEHHA considered an additional uncertainty factor of three to account for the short duration of the Greer *et al*. (2002) study. However, there is evidence in this study that iodide uptake is inhibited fairly quickly after exposure begins and the inhibition does not increase or increases only slightly as exposure continues. That is, in the three highest dose groups (those in which statistically significant reductions in iodide uptake were seen), the greatest proportion of the inhibition occurred by the second day of dosing and either did not worsen or worsened only slightly by day 14 of dosing. Furthermore, it can be argued that if there is no reduction in thyroidal iodide uptake, there will be no concomitant reduction in stored iodide, and extending the exposure duration is not likely to have an impact on the thyroid function. For this reason, OEHHA decided against an additional factor to account for the short duration of the critical study.

Given an uncertainty factor of 10 to account for increased susceptibility in infants,

$$\text{ADD} = \frac{3.7\ \mu g/kg\text{-}day}{10} = 0.37\ \mu g/kg\text{-}day$$

Public Health Protective Concentration (C)

Calculation of the updated public health protective concentration (C) for perchlorate in drinking water uses the following equation for noncarcinogenic endpoints:

C = ADD µg/kg-day × (BW/WC) × RSC

where:

(BW/WC) = the ratio of body weight (kg) and tap water consumption rate (L/day); the ratio for the 95th percentile of infants age 0-6 months is estimated to be 4.2 kg-day/L (OEHHA, 2012; Table 47); and

RSC = relative source contribution. A value of 0.73 (73 percent from water) is used to account for exposure of infants to perchlorate in their diet.

The 95th percentile water consumption rate and body weight ratios are based on water intake estimates derived by OEHHA (2012). These rates were based on data from the Continuing Survey of Food Intake of Individuals (CSFII) for the years 1994-1996, 1998. The CSFII was a large multistage probability sample collected by the U.S. Department of Agriculture involving over 20,000 individuals from throughout the U.S. The CSFII survey collected data on food and beverage intakes for two 24-hour non-consecutive periods, 3-10 days apart. These data were based on recall of dietary and water intake information, and it is possible that intake is overestimated in some people, and underestimated in others. These errors could balance each other out. Overall, their true impact is unknown although there is no reason to believe they were large. Because these data are based on the average of two separate assessments of drinking water intake in each individual collected 3-10 days apart, they are more likely to represent true longer-term intake patterns than data collected using a single day survey.

Using the CSFII data, the U.S. EPA Office of Water estimated the amount of water consumed by each individual (U.S. EPA 2004). For all demographic groups except infants, this included both water consumed directly from the tap for drinking (direct water) and water from the tap used to prepare beverages and foods, either at home or at a food service establishment (indirect water). OEHHA used these U.S. EPA estimates for those subjects reported to be drinking water consumers only since these are the people most likely to have the greatest exposures from local public drinking water sources (OEHHA, 2012).

For infants, OEHHA also used data from the CSFII but derived water intake rates based on the water used by infants consuming reconstituted formula. In order to identify infants who received reconstituted formula, OEHHA reviewed the food descriptions provided for the formula consumed by each infant in the CSFII. The amount of reconstituted formula consumed was then multiplied by the percent of indirect water in each type of reconstituted formula in order to calculate the amount of water consumed by each infant. Sample weights provided in the CSFII dataset were used to weight each individual's intake according to the number of infants in the population that he/she represented and these data were used to calculate distributional characteristics including 95th percentiles. The 95th percentiles rather than the means or medians were used to calculate the health protective concentration since this accounts for the fact that some people have much higher drinking water intake rates (and thus much higher intake of chemicals in their drinking water) than others. Further details and justification of these estimates are provided by OEHHA (2012).

Water intake rates for the various population groups used to calculate health-protective concentrations are shown in Table 47. OEHHA is focusing on infants in these calculations based on the variety of data discussed above showing that this group may be particularly susceptible to perchlorate. This includes the studies from California and elsewhere that provided evidence that thyroid hormone levels in infants were adversely affected by relatively low perchlorate exposure levels (Kelsh *et al.,* 2003; Brechner *et al*., 2000; Buffler *et al.,* 2006; Steinmaus *et al*., 2010; Li *et al.,* 2000a; Crump *et al.,* 2000). It also includes the data discussed above 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 day's worth compared to several weeks' worth in adults) (van den Hove *et al*., 1999; Pearce *et al.,* 2007). In addition, formula-fed infants have much higher water intake rates than older children and adults. Thus, their exposure to perchlorate from drinking water on a body weight basis at a given perchlorate concentration in drinking water will be considerably higher than in children or adults.

Food and water are the primary sources of exposure to perchlorate for most people. Perchlorate has been detected in a wide variety of foods, including fruits, vegetables, grains, dairy milk, and human breast milk (Kirk *et al*., 2005; Pearce *et al*., 2007; Murray *et al*., 2008). Perchlorate levels in urine from NHANES 2001-2, as reflected in the analysis of Blount *et al*. (2006), are generally supportive of the analysis of estimated intakes of perchlorate from foods provided by the U.S. FDA (U.S. FDA, 2009). Together, these data demonstrate that food is the primary source of perchlorate for the general population.

Estimates of perchlorate intake from food were derived from the U.S. FDA's Total Dietary Survey 2005–2006 as reported in U.S. EPA, 2008b. The RSC was defined as the proportion of the ADD not derived from food, and was calculated using the following equation:

RSC = (ADD – estimated perchlorate intake derived from food) / ADD

For most adults, including pregnant and lactating women, the mean perchlorate intake from food was estimated to be 0.10 µg/kg-day. At an ADD of 0.37 µg/kg-day, the RSC then equals 0.73, or 73 percent of the acceptable dose available for consumption in tap water.

The U.S. FDA Total Diet Survey did not provide information on perchlorate intake in infants less than 6 months old. In addition, this survey did not report estimates of perchlorate intake in analyses that only included infants who are formula-fed. Since the intake of perchlorate from food in formula-fed infants could be substantially higher than that in breast-fed infants, failure to specifically evaluate this group could have resulted in a PHG that was not adequately protective in formula-fed infants. Schier *et al.* (2010) 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. Perchlorate concentrations in formula varied by formula type and ranged from 0.03-5.05 µg/L, and the estimated geometric mean intake of perchlorate from the different formula types ranged from 0.03 to 0.29 µg/kg-day, with an average of 0.10 µg/kg-day. Using this average and the equation above, an RSC of 0.73 is also estimated for infants.

Using this RSC, the health-protective concentration (C) for infants is estimated to be:

C = 0.37 µg/kg-day x 4.2 kg-day/L x 0.73
C = 1.13 µg/L = 1 µg/L (ppb) (rounded)

Table 47 shows the values of C calculated for infants compared to those for other subpopulations. A relative source contribution of 73 percent and an uncertainty factor of 10 were used in all calculations.

Table 47. Estimated Health Protective Water Concentrations (C) of Perchlorate for Various Subpopulations, Assuming Intakes of Perchlorate in Food of 0.10 µg/kg-day[a] and an RSC of 0.73[b]

Group	Drinking Water Intake (L/kg-day)[c]	BW/WC (kg-day/L)	C (µg/L)
Infants (< 6 months old)	0.237	4.2	1.13
Pregnant women	0.043	23.3	6.29
Lactating women	0.055	18.2	4.92
Females (age 15-44)	0.038	26.3	7.10
Adults (16+)	0.045	22.2	6.00

[a]Based on estimated adult intakes using data from the 2005-6 U.S. FDA Total Dietary Study (U.S. EPA, 2008b) and infant intake from the data of Schier *et al.* (2010).
[b]Calculated as: (ADD – estimated food perchlorate intake) / ADD).
[c]95th percentile intake for drinking water consumers including both direct and indirect water for groups other than infants. For infants, drinking water intake is based on water used to reconstitute infant formula (OEHHA. 2012).

Based on these estimates, OEHHA calculated a PHG of 1 ppb (µg/L). OEHHA concludes that this level is health protective for lifetime exposure to perchlorate in drinking water, and is protective of sensitive populations including infants, pregnant women and their fetuses, those with low intake of iodine or high intake of other thyroid iodide uptake inhibitors, and other potentially susceptible groups.

A benchmark dose analysis was also done using the NHANES 2001-2 data discussed above (Blount *et al.,* 2006), and this provided results similar to those obtained using the Greer *et al*. (2002) data (see Appendix). The NHANES 2001-2 analysis required several data transformations, which greatly increased its complexity. The analysis using the Greer *et al.* (2002) data was used to calculate the final updated PHG since it is an experimental study where exposure levels are known for more than just a single point in time.

8. RISK CHARACTERIZATION AND CONCLUSION

Exposures to Perchlorate

In NHANES 2001-2, an essentially nationally representative sample of people ages ≥12 years in the U.S., perchlorate was detected in the urine of every one of the 2,820 people tested (Blount *et al*., 2007). This suggests that probably everyone in California, as well as everyone in the U.S., is exposed to perchlorate through some source.

The primary routes of exposure are through food and water. In a recent study by the U.S. FDA, perchlorate was found in many commonly consumed foods including dairy products, fruits, and vegetables (U.S. FDA, 2009). In fact, perchlorate was detected in at least one sample of over 70 percent of the individual food items tested. 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). In California, from 2010 to 2013, detectable levels of perchlorate were reported in 248 public drinking water sources (Table 2) (SWRCB, 2014). The Colorado River is also known to be contaminated with perchlorate, and this water source is used by several large public drinking water agencies in California. For both the U.S. EPA and the California SWRCB surveys discussed above, analyses were based on detection limits of 4 ppb, and it is possible that millions more people are exposed at levels below this. This highlights the potential widespread perchlorate exposure in California that may be occurring from public drinking water sources.

Currently, the actual number of people that are likely to be affected by perchlorate from public drinking water in California is unknown. However, given the data above suggesting that hundreds of public water sources in California contain perchlorate, and given that potential susceptible groups including infants and women with low iodine intake represent a large fraction of the entire population, the number of people that may be affected by perchlorate exposure through their public water supplies is likely to be quite large.

Perchlorate in water can be naturally occurring as well as a result of industrial contamination. The fact that it can occur naturally does not decrease the potential health consequences of exposure. Many naturally occurring chemicals such as arsenic, lead, and asbestos are also toxic and measures are taken to reduce human exposures. Thus, the PHG is based on perchlorate exposure in drinking water whether its presence is due to natural or man-made events.

Health Impacts of Perchlorate

There is a continuum in the possible health effects from perchlorate exposure resulting from its effects on the thyroid, and some susceptible groups or individuals may have perchlorate-related effects at exposures that are lower than those causing similar effects in healthy individuals. For fetuses, infants and children, there is the potential for abnormal growth and development. Of particular concern are effects on brain development. Decreases in thyroid hormone production in infants are related to decreases in IQ. Much of the knowledge on the potential effects of perchlorate comes from extensive data on iodine deficiency in humans.

OEHHA considers effects on thyroid hormone production and subsequent changes to be adverse. Iodide uptake inhibition is the key event that leads to other possible effects from perchlorate exposure. Prevention of iodide uptake inhibition prevents progression to the adverse health effects of perchlorate. The inhibition of iodide uptake by perchlorate has been clearly documented in humans. The National Academy of Sciences (NAS, 2005) deemed "inhibition of iodide uptake by the thyroid as the basis of the perchlorate risk assessment to be the most health-protective and scientifically valid approach." OEHHA agrees with this approach and used it in developing its original 2004 PHG for perchlorate.

The Greer *et al.* (2002) study was used to characterize perchlorate's inhibition of iodide uptake for both the current PHG and the 2004 PHG. It might be argued that the magnitudes of the effects seen in the Greer *et al*. (2002) study were relatively small, and might not be noticeable in otherwise healthy individuals. However, this ignores the impact of these effects on a population basis. Any downward shift in the mean level of the thyroid hormone T4 in a population could increase the number of people who fall into the range of T4 values that are associated with high risks of either subtle or overt thyroid-related disease and toxicity (Miller *et al.,* 2009).

The purpose of the perchlorate PHG is to identify a level of perchlorate in drinking water that prevents any perchlorate-related reduction in thyroid iodide uptake that might lead to decreases in thyroid hormone production. Evidence suggests that even small decreases in thyroid hormone levels may be associated with significant adverse effects, including altered cognitive development in children and increased cardiovascular risk factors in adults. Importantly, these changes have been seen at thyroid hormone levels that are within what have been traditionally defined as normal reference ranges, and have occurred in people without any other evidence of overt thyroid disease. These findings suggest that small changes in thyroid hormone levels may be associated with at least some increased risk of thyroid-related adverse outcomes.

Health Value for Perchlorate from the Greer et al. (2002) Study

OEHHA used the BMD approach rather than the no-observed-effect level (NOEL) approach for the following reasons. OEHHA, like U.S. EPA, has chosen to use the BMD approach as the standard method in dose calculations, provided there are sufficient data to do so. As the U.S. EPA (2012, p. viii) notes, the BMD approach "involves dose-response modeling to obtain BMDs, i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data, incorporates and conveys more information than the [NOAEL; no-observed-adverse-effect level] or [LOAEL; lowest-observed-adverse-effect level] process traditionally used for noncancer health effects." The NAS (2009, p. 129) in its review of U.S. EPA risk assessment practices similarly has recognized this as a refinement that makes better use of the dose-response evidence available than do calculations based on NOAELs.

The NOEL approach depends heavily on a single chosen dose level used in a study, and how closely it is placed relative to the next highest dose. The NOEL approach treats small and large studies the same, even though small studies, with their limited number of subjects, are less likely than large studies to find health effects at any given dose. In this case, the small group sizes in the Greer *et al*. (2002) study make it more difficult to detect an effect. For these reasons reliability of the NOEL approach can vary depending on the size of the study. In contrast, the BMD approach provides a systematic method for calculating a dose at a low specific effect level. The BMD approach uses all the dose-response evidence in the study, incorporates the shape of the dose-response curve, and takes into account the number of subjects in the study, thus providing a more reliable calculation. The advantages of using the BMD approach for this PHG are particularly important given the limited number of subjects that participated in the Greer *et al*. (2002) study.

Identifying Infants as a Sensitive Population

OEHHA, as part of its periodic review of perchlorate, assessed exposure patterns in bottle-fed infants as well as the special susceptibility of infants and children. OEHHA determined that infants, along with pregnant women and fetuses, are likely to be more susceptible to the effects of perchlorate.

The major change in the current PHG calculation relates to the updated information on infants as a sensitive population. OEHHA's review and updating of the perchlorate PHG considered the data from several human studies published after the 2004 PHG and the 2005 NAS review. These studies provided qualitative support for recognizing that infants are likely to be significantly more susceptible to perchlorate than healthy adults. Evidence for this includes the following:

1. New data suggest 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). Perchlorate-related toxicity is likely to be greater in infants who are already deficient in iodine.
2. OEHHA's analysis of data from studies conducted in California and elsewhere 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).
3. 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). Because of these low stores, infants may be less able to tolerate transient periods of decreased iodide uptake and decreased thyroid hormone production compared to adults.
4. Human data suggest that perchlorate can interact with other contaminants to produce a greater effect than that caused by perchlorate alone (Blount *et al.*, 2006; Steinmaus *et al*., 2007), and infants are exposed to these same contaminants.

In the 2004 PHG, an uncertainty factor of 10 was applied to address all sensitive groups (pregnant women, lactating women, thyroid compromised adults) except infants, where an uncertainty factor of 3 was used. However, given the evidence discussed above, the extent and ways infants are likely to be more susceptible to perchlorate than healthy adults, and the fact that the Greer *et al.* (2002) study included only healthy adults, OEHHA has increased the uncertainty factor applied to infants from the factor of 3 used in the 2004 PHG to a factor of 10 in this updated PHG. This is one factor that resulted in the lowering of the updated PHG. The other factor driving the change in the PHG value is the use of updated drinking water intake rates for infants, described below.

Water Consumption by Infants

The water consumption rate by infants is one of the factors that influence the final PHG. The recent analysis by OEHHA (2012) of data available from a large survey of food and beverage intake conducted by the U.S. Department of Agriculture shows that drinking water intakes per kilogram of body weight are higher in infants than previously thought. This means that infants are likely to have greater perchlorate exposure per kilogram of body weight for a given concentration of perchlorate in drinking water than was estimated in the 2004 PHG.

OEHHA used its new analysis and chose the 95th percentile drinking water intake rate instead of an average rate in order to protect the infant population,

and not just those with average drinking water consumption. Infants who drink more water will have a greater exposure to perchlorate and thus may be at greater risk. In this case the bottle-fed infant will have the greatest intake of water and exposure to water contaminants.

The 95th percentile value of 0.237 L/kg-day for drinking water intake per body weight for infants aged 0-6 months (OEHHA, 2012) was used in the PHG calculations. For comparison, U.S. EPA (2009) typically assumes that infants and children drink 1 liter (L) per day and that they weigh 10 kilograms, that is 0.1 L/kg-day. Using the U.S. EPA value would result in a PHG of 2.7 ppb instead of 1 ppb.

Comparison with Other Agencies

Currently, there is no federal drinking water standard (MCL) for perchlorate. The current California MCL is 6 ppb. Several other states have action levels or MCLs in the range of 1-26 ppb as shown in the table below.

Table 48. State MCLs or Advisory Levels (ppb) for Perchlorate (ASTSWMO, 2011)

Alabama	24.5	Missouri	10.9
Alaska	26	New Jersey	5
Arizona	14	New Mexico	1
California	1 (PHG) 6 (MCL)	New York	5
Florida	4	Nevada	18
Hawaii	25	North Carolina	2
Kansas	10.9	Texas	17
Maine	1	Vermont	2 (prevent) 4 (enforce)
Maryland	1	Virginia	15
Massachusetts	2	Wisconsin	1 (prevent) 0.1 (enforce)

PHGs published by OEHHA are for use by the California State Water Resources Control Board (SWRCB) in establishing primary drinking water standards, that is, the California MCLs. In developing the PHG for perchlorate, OEHHA's approach follows that used by the NAS (2005) to develop its reference dose in several key areas:

1. Both OEHHA and NAS identified the Greer *et al.* (2002) human study as the critical study for evaluating the effects of perchlorate.
2. Both OEHHA and NAS chose iodide uptake inhibition in the Greer *et al*. (2002) study as the key effect on which to base their calculations.
3. Both OEHHA and NAS noted that the subjects in the Greer *et al*. (2002) study were healthy adults and concluded that some people may be more

susceptible to perchlorate than these healthy adult subjects. For this reason, both OEHHA and NAS applied an uncertainty factor of 10 to calculate a dose that would address inter-individual variability among humans and be protective of those who are likely to be sensitive to the effects of perchlorate.

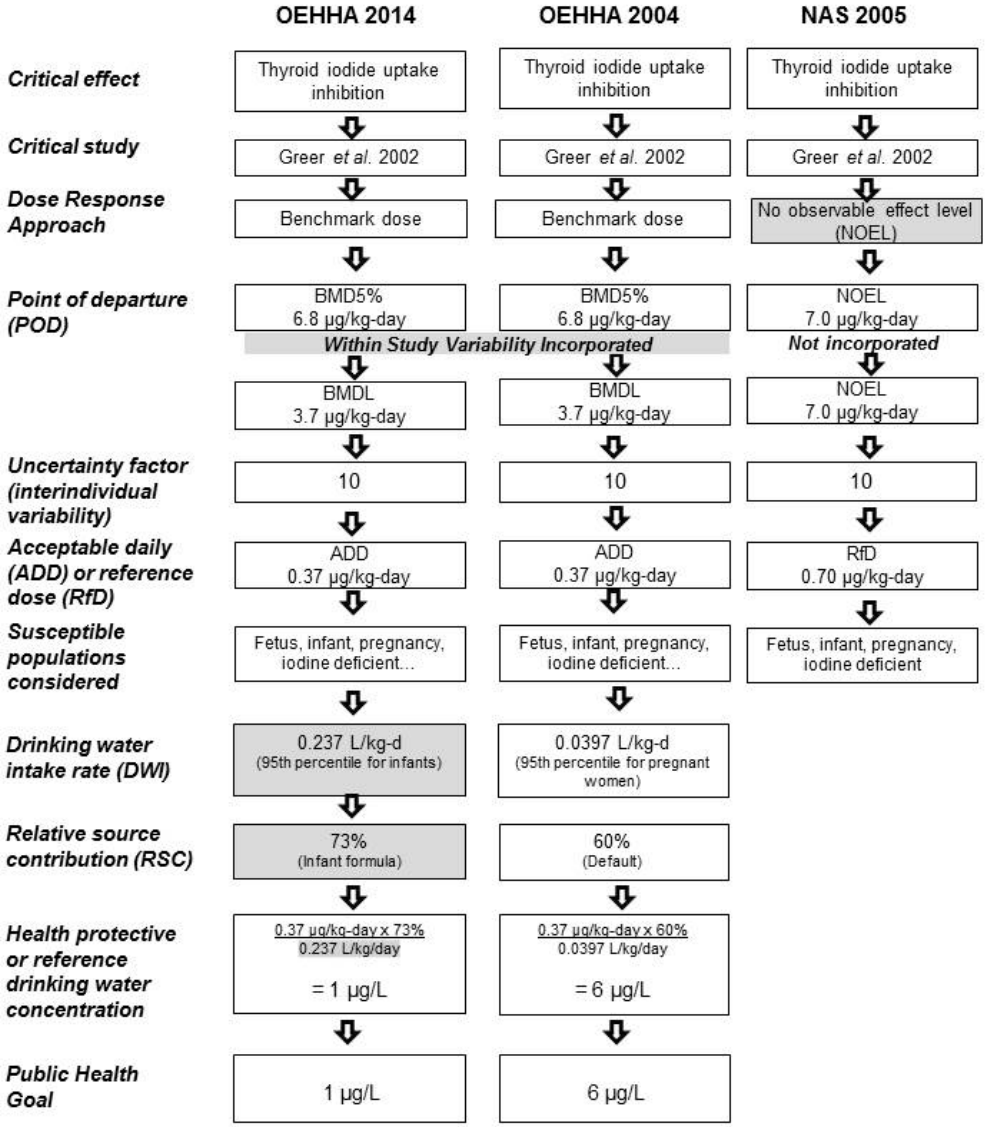
4. Both OEHHA and NAS identified the same populations likely to be more sensitive to perchlorate exposure: fetuses, preterm newborns, infants, developing children, pregnant women, people who have compromised thyroid function resulting from conditions that reduce thyroid hormone production, and people who are iodine-deficient.

There is only one substantive difference between the OEHHA and NAS analyses. The NAS used the no-observed-effect level (NOEL) approach. They determined that the NOEL was 0.007 mg/kg-day, the highest dose in the Greer *et al*. (2002) study that was not associated with a statistically significant response. OEHHA used the Benchmark Dose (BMD) approach and calculated a point of departure of 0.0037 mg/kg-day. As discussed above, the BMD method is a statistical method that is now widely recognized as a better approach because it incorporates more dose-response information from the study than the NOEL method (NAS, 2009; U.S. EPA, 2012).

From the NOEL, the NAS calculated a reference dose of 0.0007 mg/kg-day (the NOEL divided by the uncertainty factor of 10). OEHHA similarly calculated an ADD of 0.00037 mg/kg-day (the BMD-derived point of departure divided by the same10-fold uncertainty factor). Thus the two approaches differ by less than a factor of 2. The NAS did not take the next step to calculate an acceptable drinking water level for perchlorate.

A comparison of the NAS methods to those of this PHG and OEHHA's 2004 perchlorate PHG are shown in Figure 13.

Figure 13. Comparison of the Methods Used to Calculate OEHHA's Current and 2004 Perchlorate PHGs with the Methods Used by NAS (2005)



Conclusion

The primary toxic mechanism of perchlorate is a reduction in iodide uptake into the thyroid gland. If severe enough, this can lead to reduced thyroid hormone production. Adequate supplies of thyroid hormone are vital for a variety of physiologic processes, and even small reductions in thyroid hormone have been associated with increased cardiovascular disease risk factors, abnormal fetal brain development, and altered childhood cognition. The purpose of this updated PHG of 1 ppb is to identify a level of perchlorate in drinking water that prevents perchlorate-related reductions in thyroidal iodide uptake and subsequent decreases in thyroid hormone production that may be associated with any of these adverse health effects.

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APPENDIX 1: ALTERNATIVE BENCHMARK DOSE CALCULATIONS USING BLOUNT *ET AL*., 2006 DATA

Study Description

Blount *et al*. (2006) was a cross-sectional study of urinary perchlorate levels and serum levels of thyroid hormones in 2,299 men and women ≥ age 12 who took part in the 2001-2002 National Health and Nutrition Examination Survey (NHANES). NHANES was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) and was designed to assess the health and nutrition status of the non-institutionalized population of the U.S. This survey involved a complex multistage sampling design with some over-sampling in certain areas and among certain subgroups, but was designed to provide results that are nationally representative. Information that was collected as part of this survey included questionnaire data on demographic information, smoking, health history, and medication use. A single serum measurement of T4 and TSH and a single measurement of urinary perchlorate and iodine concentration were also collected. Other information collected included urinary creatinine, thiocyanate, and nitrate; and serum levels of albumin, cotinine, and c-reactive protein.

The authors assessed the relationship between serum thyroid hormone levels and urine perchlorate concentrations using a linear regression analysis adjusted for potential confounding variables and co-variates including age, urinary creatinine, estrogen use, c-reactive protein, cotinine, ethnicity, menopause, premenarche, pregnancy, fasting time, body mass index, and kilocalorie intake. Several factors including urinary perchlorate and creatinine and serum TSH were logarithm transformed to normalize their distributions. Exclusions included subjects with missing data on co-variates, a history of thyroid disease, current use of thyroid medications, extreme values of T4 or TSH (n = 3), and subjects missing perchlorate measurements.

No association was found between T4 or TSH and perchlorate in men. In women, separate analyses were done for urinary iodine levels above and below 100 µg/L. This level was chosen since it is used by the World Health Organization to define iodine deficiency in a population. Thirty-seven percent of the women in this study had urinary iodine levels below 100 µg/L. The results of the analyses in women are shown in Table A1. A statistically significant association was seen between increasing TSH and increasing perchlorate in women with iodine levels above and below 100 µg/L. A statistically significant association was also seen between decreasing T4 and increasing perchlorate in women with urinary iodine levels below 100 µg/L but not in women with iodine levels above 100 µg/L.

Table A1. Associations between Thyroid Hormone Levels and the Logarithm of Urinary Perchlorate in Women with High and Low Levels of Urinary Iodine (Blount *et al.,* 2006)

	N	β	SE	p-value
Urine iodine < 100 µg/L				
T4	348	-0.8917	0.1811	<0.0001
Logarithm of TSH	356	0.1230	0.0373	0.0010
Urine iodine ≥ 100 µg/L				
T4	724	0.2203	0.3687	0.5503
Logarithm of TSH	697	0.1137	0.0506	0.0249

Abbreviations: β, regression coefficient; N, number of subjects, SE, standard error of the regression coefficient.
Differences in the numbers of subjects with an iodine category are due to differences in the number of subjects missing data on co-variates in each analysis. Except for perchlorate and creatinine, only co-variates with p-values < 0.10 were retained in each model.

Selection of the Key Biochemical Effect, Critical Study, Benchmark Response (BMR), and Control Group

Defining the Key Biochemical Effect

Thyroid hormone. As discussed in the previous sections, perchlorate has been associated with a variety of health and physiological effects. Focus is placed on perchlorate's impacts on the thyroid gland for the following reasons:

- These effects have been reported at lower exposure levels than the other significant health effects associated with perchlorate. By preventing low-dose thyroid effects, these other effects should also be prevented.
- Thyroid hormone is a very important hormone for homeostatic control in humans and alterations in thyroid hormone production have been associated with serious adverse outcomes, including diminished cognitive development and IQ in children and increased cardiovascular disease risk factors in adults.
- As discussed below, detailed dose-response data on perchlorate and thyroid hormone levels meeting the major tenets of causal inference are available from Blount *et al*., 2006.

Is this adverse? While some people may not consider decreased T4 or increased TSH to be an "adverse" health outcome in isolation, as detailed in the PHG document, decreases in T4 are very closely associated with outcomes that are clearly adverse such as childhood cognitive development and alterations in lipid metabolism (which are closely linked to cardiovascular diseases).

Statistically significant dose-response relationships have been seen between T4 and each of these outcomes in studies which involve at least some subjects with T4 or TSH values at levels traditionally considered to be within normal reference ranges (discussed in previous sections).

Selecting the Critical Study

The data on women with urinary iodine levels below 100 µg/L evaluated in Blount *et al.* (2006) and collected as part of NHANES 2001-2 were used for the point of departure calculations. These data showed a clear, statistically significant dose-response relationship in a group of subjects that are an essentially representative sample of all subjects ages 12 years and older in the U.S. Because this is basically a representative sample, the levels of perchlorate exposure seen in this group are likely to be those commonly found in the general U.S. population. Other reasons for selecting this study include:

- Its relatively large sample size, which helps ensure adequate statistical power.
- Its inclusion of a relatively large number of people who are potentially susceptible to perchlorate (i.e., women with low iodine intake). Other clinical dosing or occupational studies did not specifically examine susceptible groups. Using these other studies could therefore underestimate effects in susceptible populations.
- It is a human rather than an animal study.
- The individual data are publicly available.
- The findings linking perchlorate to thyroid effects are consistent with the major tenets of causal inference (reviewed in earlier sections).

In the NHANES 2001-2 dataset, the association between T4 and perchlorate was particularly strong in women with both low iodine levels and high thiocyanate levels (Steinmaus *et al*., 2007). OEHHA did not use the high thiocyanate group for our dose-response analysis since it involved only a relatively small number of subjects (n = 78).

Using raw data from NHANES: The main outcome variables in the Blount *et al*. (2006) study are serum levels of T4 and TSH. Levels of these hormones, as well as urinary levels of perchlorate and data on numerous potential co-variates are available for each individual in the study on the NHANES website. Since these data are publicly available OEHHA performed its own calculations using the individual data rather than relying on the grouped data and final results presented by Blount *et al*. (2006). As we have shown, the results of our analyses (Steinmaus *et al*., 2007) are very similar to those of Blount *et al*. (2006). The minor differences are probably due to differences in the statistical packages used and differences in the way certain potential co-variates were defined. Performing

the calculations with the raw data allowed OEHHA to confirm the statistical analyses of Blount *et al*. (2006) and allowed a more thorough evaluation of specific aspects of causal inference (presented previously).

The Benchmark Dose Approach

The benchmark dose (BMD) approach was used to calculate a point of departure (POD). The advantages of this approach over the NOAEL approach are discussed elsewhere (U.S. EPA, 2000). The BMD is defined as the dose (or exposure) that causes a prescribed response rate (Crump, 2002). A statistical lower bound of the BMD, usually the 95% lower confidence limit (the BMDL), is used as the point of departure for defining an exposure level that is likely to be without an appreciable risk of deleterious effects in humans. Using the BMDL rather than the BMD helps to account for the uncertainty inherent in a given study and according to the U.S. EPA, "assures (with 95% confidence) that the desired response is not exceeded (U.S. EPA, 2000)." The BMD and BMDL are calculated by fitting a mathematical model to dose-response data and determining the dose, and its 95% CI, that is associated with a predefined benchmark response (BMR).

BMD for continuous data. In the past, the BMR has been typically defined as a 10 percent increase in the proportion of subjects exhibiting a predefined adverse response over the baseline response (the proportion exhibiting the response in a control or unexposed group). The 10 percent level is based primarily on the level of response that can be detected with sufficient statistical power in typical sized animal and human studies. Benchmark dose calculations can also be done for response data that are continuous. The 2004 OEHHA perchlorate PHG, which used a 5 percent decrease in radioactive iodide uptake, is an example of using a response variable that is continuous.

Selecting the BMR

Several approaches can be used to define a BMR:

An absolute level of T4 that is generally considered to be adverse: There is no single value of T4 or TSH that most clinicians would consider to define "adverse." Sometimes, the upper and lower 2.5th percentiles of T4 values collected in large population samples like NHANES have been used to define "normal" reference ranges. However, these levels are not absolute: some people with T4 levels outside these reference ranges will not exhibit thyroid disease, while some with T4 values within the normal reference range will. As discussed in the PHG document, several studies have shown linear associations between T4 and disease-related markers at T4 values well within normal reference ranges. For these reasons, there is no single absolute value of T4 which can be used to separate those likely to have significant risks of thyroid-related disease from those likely to have no risk of disease. Regardless, T4 percentile cut-off points are related to the risks of thyroid disease. That is, at least at the lower spectrum of T4 levels, the lower the percentile a subject's T4 value falls into, the greater

their risk of thyroid-related disease. Because of this, OEHHA evaluated the use of various percentiles of T4 in NHANES (the lower 2.5th, 5th, and 10th percentiles) as cutoff points for defining people likely to have a substantial risk of thyroid-related disease. This is discussed in further detail below.

Dichotomizing the data: One way of defining a BMR would be to dichotomize the outcome data; that is, define a serum level below which T4 would be considered adverse and above which it would not be considered adverse. As mentioned above, this could be defined as the lower 2.5th or 5th percentiles of T4 levels in a large population sample. Once dichotomized, the BMR can then be defined as an increase of 10 percent in the proportion of subjects that exhibit T4 levels below this cut-off point compared to the proportion seen in an unexposed (or relatively unexposed) control group. The problem with this approach is that much of the detailed information that is part of the continuous data set is lost, with a potential reduction in statistical power. The hybrid approach discussed below is one method for overcoming this problem.

Absolute or percentage change: Another approach for defining a BMR is to use a level of change in T4 that is generally considered to be biologically significant. For example, a 10 percent decrease in T4 from the mean T4 of an unexposed control group might be considered to be an important change. The BMD would then be the perchlorate dose associated with this 10 percent decline in T4. One problem with this method is that there is no single level of change in T4 that is universally accepted as being adverse or important.

Any change or any detectable change: Given the linear relationship between T4 and lipid metabolism and the linear relationship between T4 and childhood cognition that may occur within normal ranges of T4, it might be suggested that *any* decrease in T4 should be considered important. This is especially relevant on a population basis where any measurable decrease in population mean T4 would likely cause an increase in the number of people with T4 levels in the adverse range. For this reason, OEHHA evaluated the possibility of defining a BMR as a relatively small decrease in T4 (e.g., 1 percent and 5 percent) from the mean T4 in an unexposed control group. The problem with this method is that the selection of 1 percent or 5 percent could be considered arbitrary. In other words, one might ask why a 0.5 percent decrease or a 0.0001 percent decrease was not selected.

Because of this, OEHHA considered another approach: defining a BMR as any decrease in T4 that is detectable statistically. This is similar (albeit not the same) to the BMR approach used for outcome data that are dichotomous, where the BMR is typically defined as an increase of 10 percent in the proportion of subjects eliciting a predefined adverse effect. The value of 10 percent is used because it is the level of response that can be seen with sufficient statistical power in study designs and sample sizes commonly used in animal and human studies (U.S. EPA, 2000). Standard sample size calculations (Hulley and Cummings, 1988) and the variance in T4 seen in the 385 women with low iodine

in NHANES 2001-2 were used to estimate the change in T4 that is likely to be detectable with sufficient statistical power in this group of women. OEHHA's calculations suggest that a study this size would have 86 percent power to detect about a 10 percent decrease in mean T4 comparing a group the size of the lower 10th percentile (i.e., 38 subjects) to the remaining subjects (i.e., 385 – 38 = 347 subjects). This power is close to the 80 percent level that most researchers would consider minimally adequate. The lower 10th percentile was used in these calculations because subjects in the lower 10th percentile of urine perchlorate were used as the "unexposed" control group in the BMD calculations (discussed below). Based on these statistical power estimates, a 10 percent decrease in mean T4 was selected as the BMR, although other levels of BMR were assessed for comparison purposes.

Selecting an Unexposed or Lesser Exposed Control Group

Since all of the subjects in NHANES 2001-2 had detectable levels of perchlorate, there is no truly unexposed group. Because of this, the following methods for defining a control group were considered:

a. *Lowest detected dose:* One method is to use the lowest dose detected as the baseline or control dose (0.19 µg/L). The problem with this method is that the control T4 level would be based on only one subject (only one of the 385 low iodine women had a urine perchlorate level of 0.19 µg/L) and therefore it would not be appropriately robust.

b. *Predicted T4 for the lowest detected dose:* Another method is to use the regression model to predict a mean T4 expected for a perchlorate dose of 0.19 µg/L. Since this T4 estimate is essentially based on the regression model (and all of the data that went into it), it would be more robust. The problem with this method is that the control group mean T4 would be based on a model prediction rather than on an actual set of real values.

c. *Predicted mean T4 for a perchlorate level of zero.* Another method would be to use the regression model and enter perchlorate as zero. The problem with this method is that the model includes the logarithm of perchlorate (log-perchlorate) and there is no logarithm of zero.

d. *A log-perchlorate of zero.* Another method could be to use the regression equation to estimate a mean T4 at a log-perchlorate of zero. The problem with this method is that a log-perchlorate of zero corresponds to a perchlorate level of 1 µg/L, and there is no obvious rationale for using a urinary concentration of 1 µg/L as the control level.

e. *Actual data.* The other option evaluated was using actual data from the low iodine women in NHANES 2001-2 and defining the control group as those subjects below a particular percentile cut-off point for perchlorate dose. Subjects with perchlorate values in the lower 5th, 10th, and 20th percentiles of

creatinine-adjusted urinary perchlorate concentrations were used for this. It was decided to use a control group defined as those subjects in the lowest 10th percentile of creatinine-adjusted perchlorate residuals. This was chosen because: 1) the lower 10th percentile was the basis of the statistical power calculations used to define the BMR of 10 percent (described above); 2) it was necessary to categorize perchlorate into ten equal size dose groups in order to use these data in the BMDS (described below).

The Standard Deviation and Hybrid Approach

One standard deviation (SD): The U.S. EPA suggests in the absence of any other idea about what level of response to consider adverse, a change in the mean of the outcome (T4) equal to one standard deviation from the control mean can be used (U.S. EPA, 2000). They also recommend that regardless of what approach is used, the standard deviation approach be presented for comparison. Crump and others have shown that for a response variable that is normally distributed, a decrease of one SD corresponds to an increase of about 10 percent in the proportion of subjects falling below the 2nd percentile value of an unexposed comparison group (Crump, 1995).

Hybrid approach: The hybrid approach uses the distribution of a continuous variable (i.e., a mean T4 and its standard deviation) to estimate the proportion of subjects falling below a predefined percentile cutoff point (Gaylor and Slikker, 1990; Crump, 1995). This is done by converting the mean T4 in the unexposed group and the BMR into units on the standard normal deviation scale.

Crump and others present a method for determining the fraction of the standard deviation of the mean outcome value (i.e., T4) that corresponds to a particular proportion of subjects that fall below (or above) a particular percentile cutoff point. For example, if a T4 value below the 2.5^{th} percentile in an unexposed control population is considered adverse, then a decrease in T4 corresponding to 0.82 times the T4 standard deviation corresponds to a BMR of 10 percent (i.e., a 10 percent increase in the proportion of people that will fall below the 2.5^{th} percentile cut-off point). The equation is:

$$BMR = Q \times SD, \text{ where}$$

$$Q = N^{-1}[1-P(0)] - N^{-1}[1-P(0) - BMR]$$

N^{-1} is the inverse of the standard normal distribution (i.e., the z-score for the probability of 1-P(0) or 1-P(0)-BMR), and P(0) = the percentile below which is considered adverse (e.g., P(0) = 0.025 if being below the 2.5^{th} percentile is considered adverse).

The Hybrid Approach Calculations

Standard deviations: The standard deviation in T4 for the 385 low iodine women in NHANES 2001-2 in the lower 5th, 10th, and 20th percentiles of creatinine-

adjusted perchlorate residuals were 1.87, 1.86, and 1.72, respectively. The similarity of these numbers shows that the variance in T4 is essentially independent of perchlorate dose.

Calculating Q. Values of Q were calculated for several levels of P(0) and BMR. Values for P(0) corresponding to the lower 2.5th and 5th percentiles of T4 were used since these have traditionally been used to define the lower bounds of "normal" T4 reference ranges. A P(0) value corresponding to the lower 10th percentile was also evaluated since significant cognitive effects were seen in children in the lowest 10th percentile of T4 or fT4 (Pop *et al*., 1999, 2003: Kooistra *et al*., 2006). BMRs of 5 and 10 percent were chosen since 5 and 10 percent are thought to represent levels that can be detected statistically in typical sized animal and human studies (U.S. EPA, 2000). Values of Q for these levels of BMR and P(0) are shown in Table A2.

Table A2. Hybrid Table: Calculating Values of Q

T4 considered adverse	P(0)	1-P(0)	N_A^{-1}	BMR	1-P(0)-BMR	N_B^{-1}	Q
< 2.5th percentile	0.025	0.98	1.960	5%	0.93	1.440	0.52
	0.025	0.98	1.960	10%	0.88	1.150	0.81
< 5th percentile	0.05	0.95	1.645	5%	0.90	1.282	0.36
	0.05	0.95	1.645	10%	0.85	1.036	0.61
< 10th percentile	0.1	0.90	1.282	5%	0.85	1.036	0.25
	0.1	0.90	1.282	10%	0.80	0.842	0.44

Abbreviations: BMR, the benchmark response or the increase in the proportion of people who fall below the T4 level considered adverse; N_A^{-1}, standard normal deviate for 1-P(0); N_B^{-1}, standard normal deviate for 1-P(0)-BMR; P(O), the percentile of T4 in the control group below which would be considered adverse.

Benchmark Dose Calculations

Transforming NHANES 2001-2 Data for the BMDS

The U.S. EPA BMD software (BenchMark Dose Software (BMDS) version 2.0) was used for the POD calculations. The following data transformations were required:

Creatinine adjustment: The BMDS does not allow the use of co-variates such as urine creatinine concentration. As shown above, urine creatinine was the only individual co-variate that caused a greater than 10 percent change in the perchlorate-T4 regression coefficient (Table 39). Because the addition of urinary creatinine appears to improve the model, it was decided that this variable should be incorporated into the POD calculations. This was done by calculating creatinine-adjusted perchlorate residuals ("perchlorate residuals") using the Proc

Reg statement in SAS with the logarithm of urinary perchlorate concentration (log-perchlorate) as the dependent variable and the logarithm of urinary creatinine concentration as the independent variable (logarithm of creatinine or log-creatinine). The association between serum T4 and the creatinine-adjusted perchlorate residuals was only slightly different than the association seen in the fully adjusted model.

OEHHA considered using the urine perchlorate:creatinine ratio. However, the association between T4 and this ratio in the low iodine women was not as strong as when perchlorate residuals were used and was only borderline statistically significant (unadjusted regression coefficient between T4 and the perchlorate:creatinine ratio = -1.18; SE = 0.66; p = 0.07). This is likely due to the factors discussed previously for the iodine/creatinine ratio. That is, it creates a variable that is not only dependent on perchlorate, but also on all the factors that determine an individual's urinary creatinine level (e.g., muscle mass, diet, physical activity, and many other factors). Including all these other influences into the perchlorate exposure variable can lead to misclassification of true perchlorate exposure.

Other co-variates and potential modifying factors: None of the other co-variates used in Blount *et al*. (2006) caused important changes in the perchlorate-T4 regression coefficient so these were not used in the BMD calculations. In addition, the NHANES complex sampling weights were not used because they had only a small impact on regression coefficients and their standard errors (Table A3), and the incorporation of these weights would have significantly complicated the model.

Table A3. T4-Log-Perchlorate Regression Coefficients Using Different Methods of Analysis

Method	Adjusted	Weights[a]	β	SE	p
Blount *et al*., 2006	Full	Yes	-0.89	0.18	<0.0001
Steinmaus *et al*., 2007	Full[b]	Yes	-0.73	0.22	0.004
	Full[b]	No	-0.87	0.27	0.0016
	Creatinine Only	No	-0.81	0.27	0.0026
	Unadjusted	No	-0.67	0.23	0.0041
BMDS	Creatinine Only	No	-0.79	0.28	na[c]

Abbreviations: BMDS, benchmark dose software; β, regression coefficient; SE, standard error; na, not available.
[a]NHANES sampling weights applied
[b]Only independent variables with p-values < 0.20 were entered and retained in the model except for log-creatinine which was retained in the model regardless.
[c]p-value not given. The 95% CI of the regression coefficient is -1.34 to -0.24

Figure A1 shows the linear relationship between serum T4 and the creatinine-adjusted perchlorate residuals. The effects of possible outlying values were assessed by removing certain data points. Removing the leftmost point in this figure (T4 = 10.1 µg/dL, urine perchlorate = 0.24 µg/L, urinary creatinine = 146 mg/dL) changed the regression coefficient from 0.8122 to 0.7924. Removing the rightmost data point in this figure (T4 = 7 µg/dL, perchlorate = 100 µg/L, creatinine = 40 mg/dL) changed the regression coefficient from 0.8122 to 0.8135.

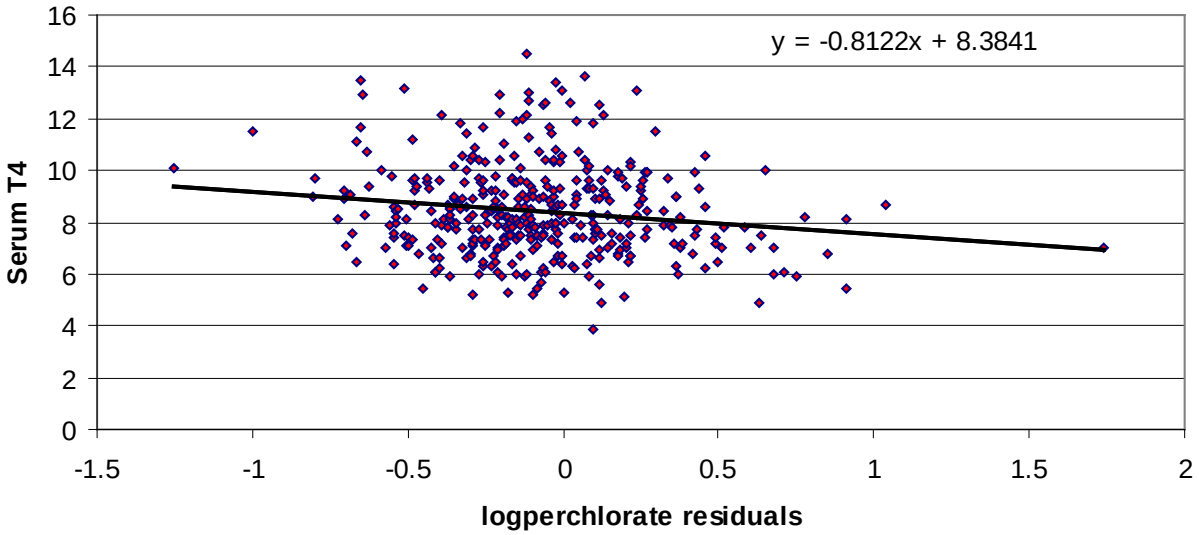

Figure A1. Serum T4 and Creatinine-adjusted Perchlorate Residuals in 385 Women with Urinary Iodine Levels < 100 µg/L, NHANES 2001-2

Categorizing dose: The BMDS allows for the use of individual data on dose and response and both can be entered as continuous variables. In controlled human trials, such as the Greer *et al*. (2002) study, doses are usually categorized into a relatively small number of dose groups. In NHANES 2001-2, each individual subject had their own urinary perchlorate concentration. Since there were 385 subjects in this study, this resulted in hundreds of "dose groups." Entering this many dose groups into the BMDS led to implausibly wide confidence intervals around the BMD and implausibly low BMDL values. To make the calculations computationally easier, perchlorate residuals were ranked into 10 equal sized dose groups and each individual in each group was assigned the mean perchlorate residual value for that group. Categorizing data into groups such as these can potentially cause a loss of information and decrease study power but this did not seem to be the case here. Table A4 compares the perchlorate-T4 model parameters calculated in SAS Proc Reg using the individual data versus those calculated by the BMDS using the 10 dose groups. As seen, the regression coefficient and its standard error are essentially the same in both models. This shows that using the 10 dose groups instead of the 385 individual dose points resulted in little loss in statistical power and essentially no loss in our ability to accurately measure the dose-response relationship.

Since the choice of using 10 dose groups was somewhat arbitrary, the effect of using more or fewer dose groups was evaluated. Table A4 shows the results of the BMDS calculations using 5 or 20 equal size dose groups. As seen, when five dose groups were used it appears that too much information was lost and the dose-response relationship becomes less strong. Using 20 dose groups didn't substantially change the model compared to when 10 groups were used.

Table A4. Association between T4 and Perchlorate Residuals: Comparing Individual Data to Dose Groups

Method	Intercept (SE)	β (SE)
SAS Proc Reg: individual data	8.04 (0.56)	-0.81 (0.27)
BMDS linear model:		
10 dose groups	8.88 (0.17)	-0.79 (0.28)
5 dose groups	8.68 (0.16)	-0.54 (0.30)
20 dose groups	8.99 (0.20)	-0.83 (0.27)

Abbreviations: β, regression coefficient; SE, standard error

Positive numbers for dose: The BMDS does not accept negative values for dose. The dose metric was perchlorate residuals, which had a mean of zero and some positive and some negative values. To prevent having any negative values for dose, the mean of the lowest dose group was subtracted from the means of each dose group to obtain a new dose variable for each group. This resulted in a value of zero for the lowest dose group and positive values of dose for all other groups. Since this maintained the absolute difference between doses it did not affect the perchlorate-T4 residual slope or its standard error.

Following these transformations, BMDS was used to calculate a BMD and BMDL. The BMD and BMDL are the creatinine-adjusted logarithm of urine perchlorate residuals minus the perchlorate residuals in the lowest dose group. These numbers were converted back into a urinary perchlorate level in µg/L using the following steps:

1. Adding the mean perchlorate residual in the lowest dose group (note that this is actually a negative number so adding it is the same as subtracting its absolute value). This gives the creatinine-adjusted residuals of the logarithm of urine perchlorate concentration.

2. Because the residuals have a mean of zero, and some positive and some negative values, they have no practical meaning in terms of an actual perchlorate concentration. To convert the residuals back to an actual value of log perchlorate, the log-perchlorate concentration that was predicted for the mean log-creatinine level of the study population as a whole was added using the method recommended in Willett and Stampfer, (1998). The predicted log-perchlorate concentration was calculated using SAS Proc Reg with log-perchlorate as the dependent variable and log-creatinine as the independent variable (the same equation used to calculate the residuals).

3. This number was then converted to a urine perchlorate level in µg/L by taking the inverse log.

Using the BMDS

The linear model using the continuous data option was selected in the BMDS. The individual data for serum T4 was entered as the response variable and the mean values for each dose group (as described above) were entered as the dose variable. As discussed above, subjects in the lowest 10th percentile of perchlorate residuals were used as the "unexposed" control group and had a transformed dose value of zero. The BMR was set at a relative decrease in T4 of 10 percent. Other options selected were: the constant variance and BMD calculation boxes were checked. Parameter assignments were all set at "default," the degree of polynomial was set at 1, the confidence level was set at 95%, and the adverse direction was set at "down."

BMDS Results

The following table shows the BMD and BMDL calculations where the BMR is defined as a 10 percent decrease in T4 from the mean T4 in the "unexposed" control group. For comparison purposes, BMD calculations are also shown for other definitions of BMR:

- The hybrid approach was used, with Q values corresponding to a 5 percent (Q = 0.25) or 10 percent (Q = 0.44) increase in the proportion of subjects below the lower 10th percentile of T4 in the "unexposed" control group. The lower 10th percentile was chosen because this was the cut-off used to define the "low" fT4 group in Kooistra *et al*. (2006), Pop *et al*. (1999), and Pop *et al*. (2003), three studies that found statistically significant associations between low maternal gestational fT4 and childhood cognitive deficits.

- Calculations are shown for BMRs defined as 20-30 percent decreases in T4 because this was the level of difference in T4 and fT4 associated with statistically significant declines in child cognitive development in other studies.

- Calculations for a BMR equal to a one standard deviation change in the mean T4 of the control group are shown because U.S. EPA recommends that these always be shown for comparison purposes.

Table A5. BMD and BMDL Results for Various Levels of BMR

BMR	1%	5%	10%	20%	30%	0.25SD[a]	0.44SD[a]	1SD
BMD (from the BMDS)	0.11	0.56	**1.13**	2.26	3.38	0.56	0.98	2.23
BMDL (from the BMDS)	0.07	0.37	**0.73**	1.46	2.19	0.35	0.62	1.40
Step 1: Add the lowest dose								
Lowest dose	-0.544	-0.544	**-0.544**	-0.544	-0.544	-0.544	-0.544	-0.544
BMDL log-perchlorate residual	-0.471	-0.179	**0.186**	0.916	1.647	-0.194	0.072	0.857
Step 2: Convert residual to log-perchlorate								
log-perc at mean log-creat	0.251	0.251	**0.251**	0.251	0.251	0.251	0.251	0.251
Add mean log-perc to residual log-perchlorate	-0.220	0.072	**0.437**	1.167	1.898	0.057	0.323	1.108
Step 3: Take inverse log								
BMDL in µg/L (urine)	0.60	1.18	**2.73**	14.69	78.99	1.14	2.11	12.83
Convert urine µg/L to intake								
Age[b]	35	35	**35**	35	35	35	35	35
Weight[b]	66.7	66.7	**66.7**	66.7	66.7	66.7	66.7	66.7
Height[b]	160.9	160.9	**160.9**	160.9	160.9	160.9	160.9	160.9
k (constant, for females)	1.64	1.64	**1.64**	1.64	1.64	1.64	1.64	1.64
Gram creatinine/day estimate	1.190	1.190	**1.190**	1.190	1.190	1.190	1.190	1.190
Creatinine g/L[b]	0.53	0.53	**0.53**	0.53	0.53	0.53	0.53	0.53
Intake at BMDL (in µg/day)	1.4	2.6	**6.1**	33.0	177.3	2.6	4.7	28.8
Intake at BMDL (in µg/kg-day)	0.020	0.040	**0.092**	0.495	2.659	0.038	0.071	0.432

[a]The T4 standard deviation (SD) in the control group was 1.87 so 0.25SD = 0.4675 and 0.44SD = 0.8228.
[b]The median levels in all 385 low iodine women in NHANES 2001-2 were used for these values.

Converting urinary perchlorate concentrations to levels of perchlorate intake

In all of these POD calculations perchlorate dose is expressed in terms of a urinary concentration of perchlorate. These values were used to calculate the BMD and BMDL. These were converted into an estimated daily intake of perchlorate using the method presented in Blount *et al*., 2007. This method is based on equations from Crockcroft and Gault (1976) and modified by Mage *et al*. (2004) and estimates perchlorate intake using urinary perchlorate and creatinine concentrations combined with estimates of daily creatinine output which are based on age, height, gender, and weight. This equation is:

$$\text{Daily perchlorate intake} = \frac{\text{µg urine perchlorate}}{\text{gram urine creatinine}} \times \frac{\text{gm creatinine}}{\text{Day}} \times \frac{1}{\text{kg}}$$

Where gm creatinine / day is estimated by:

10^{-6} x k x (140 – age(year)) x weight(kg)$^{1.5}$ x height(cm)$^{0.5}$

Where k = 1.64 for females. The median values in the 385 low iodine women were used for age (35), height (160.9 cm), weight (66.7 kg), and urine creatinine concentration (53 mg/dL). The results of these calculations are shown at the bottom of Table A5.

Summary

OEHHA calculated an intake BMDL of 0.092 µg/kg-day for a 10 percent decrease in T4. This BMDL is reasonably close to that calculated using the hybrid approach corresponding to an increase of 10 percent in the proportion of subjects with T4 values below the lower 10th percentile. The BMDS graphical displays of both of these analyses are shown in Figures A2 and A3.

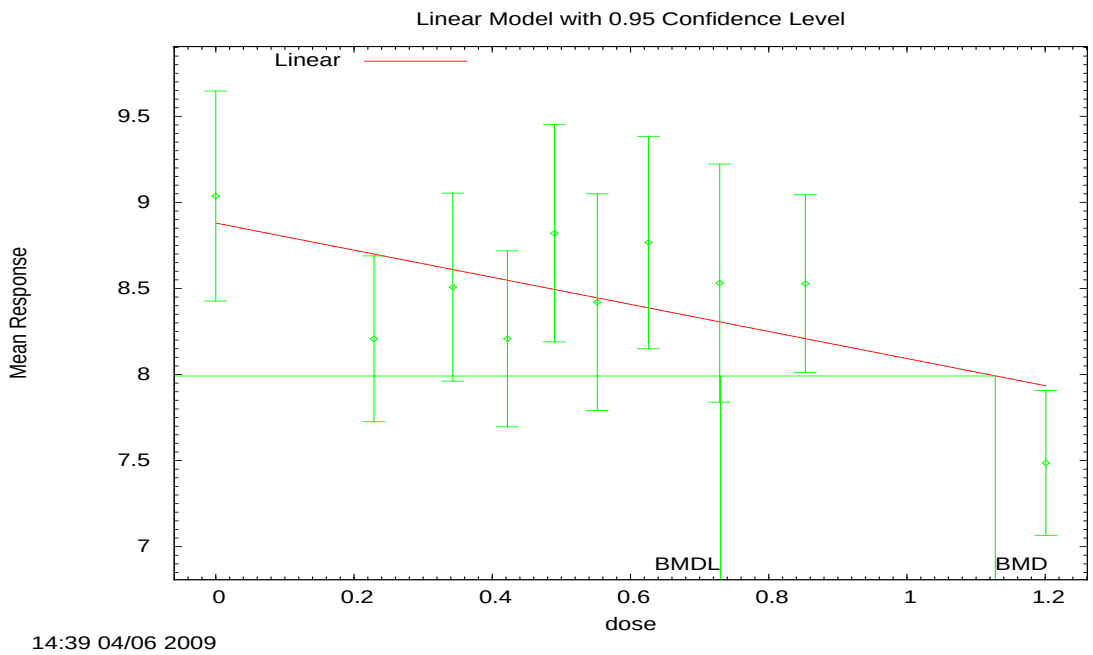

Figure A2. BMDS Output for Serum T4 (Mean Response) and Transformed Log Perchlorate Residuals (Dose) for a BMR of 10 Percent Decrease in T4

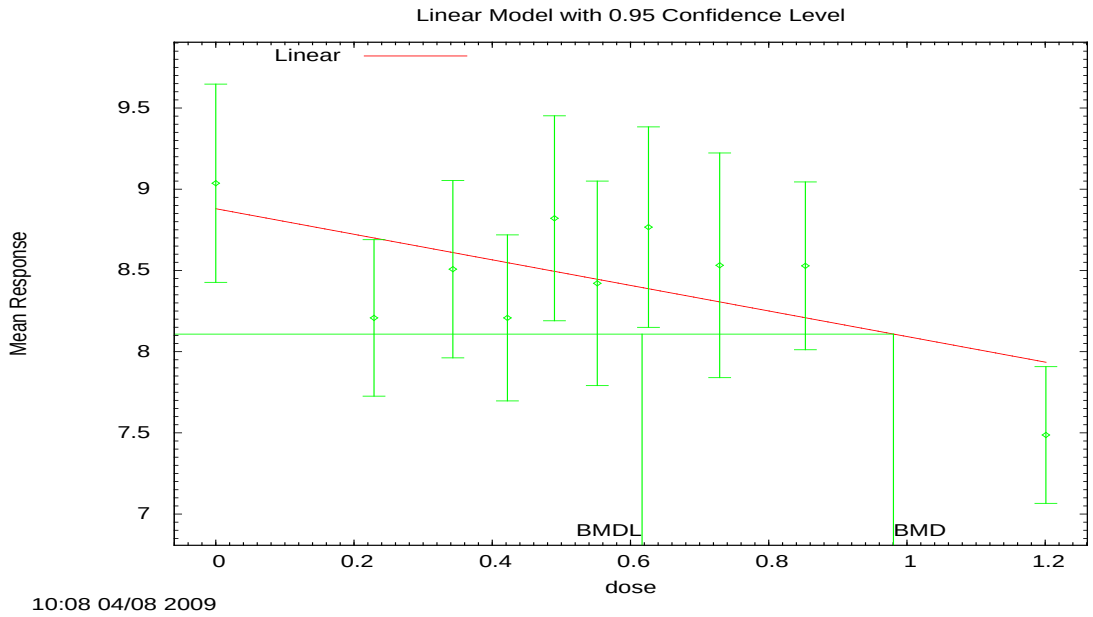

Figure A3. BMDS Output for Serum T4 (Mean Response) and Transformed Log Perchlorate Residuals (Dose) for a BMR of 0.44 x the T4 Standard Deviation of the Control Group

Calculation of the PHG Using NHANES 2001-2002 Data

Acceptable Daily Dose

For estimation of a health-protective concentration of perchlorate in drinking water, an acceptable daily dose (ADD) of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the dose leading to the key biochemical effect from human or animal studies to the estimation of a lifetime ADD that is unlikely to result in or lead to any toxic effects. For this purpose, the following equation can be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL/BMDL in mg/kg-day}}{\text{UF}}$$

where,

ADD = estimated maximum daily dose that can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL/BMDL = no-observed-adverse-effect level, lowest-observed-adverse-effect level, or lower limit on the benchmark dose estimated from the critical study;

UF = uncertainty factor(s).

For this case, the ADD is estimated from the lower limit of the two-sided 95 percent confidence interval of the perchlorate dose estimated to cause a 10 percent reduction in serum T4 levels, as observed in Blount *et al.* (2006). In this study, statistically significant associations between increasing perchlorate and decreasing T4 were seen in women with urinary iodine levels below 100 µg/L but not in women with higher iodine levels.

An uncertainty factor of four is used, including a factor of 2 to account for uncertainty due to the use of cross-sectional data to derive the dose-response estimates, and a factor of 2 to account for uncertainty due to possible increased susceptibility related to factors that were not already taken into account (e.g., exposure to other thyroid-inhibiting agents like thiocyanate, thyroid diseases, additional susceptibility in the fetus and young children). Thus,

$$\text{ADD} = \frac{0.092\ \mu\text{g/kg-day}}{2 \times 2} = 0.023\ \mu\text{g/kg-day}$$

Public Health Protective Concentration

Calculation of a public health protective concentration (C, in mg/L) for perchlorate in drinking water uses the following equation for noncarcinogenic endpoints:

C = ADD µg/kg-day × (BW/WC) × RSC

C = 0.023 µg/kg-day x 23.3 kg-day/L x 1

C = 0.54 µg/L = 0.5 µg/L (ppb) (rounded)

where:

(BW/WC) = the ratio of body weight (kg) and tap water consumption rate (L/day); the ratio for the 95th percentile of the pregnant woman population is estimated to be 0.0233 kg-day/mL or 23.3 kg-day/L (OEHHA, 2012 and Table 47 above); and

RSC = relative source contribution; a value of 1 is used for pregnant women. See the explanation for this value below.

Uncertainty factors

Susceptibility: Potential susceptible subpopulations were listed previously. It is likely that much of the susceptibility due to inadequate iodine intakes is already incorporated by using data on women with low iodine levels for these BMD calculations. In addition, some of the susceptibility due to several of the other factors listed above is likely already included in these calculations since NHANES is a population based study and involves at least some subjects who fall into the susceptible subgroups listed above. Because of this, OEHHA did not use the uncertainty factor of 10 that would typically be applied to account for inter-individual variability when dose-response data are derived from healthy individuals. However, some potentially susceptible groups were not included or were not specifically evaluated in the NHANES data set used. These include fetuses, infants and young children, people with thyroid diseases, and subjects exposed to high levels of other NIS inhibitors. An uncertainty factor was added to account for the potential susceptibility in these groups. Data from Steinmaus *et al*. (2007) suggest that the magnitude by which perchlorate reduces T4 levels is about 2 times greater in people with high thiocyanate levels than in people with average or low thiocyanate levels. Based on these findings an uncertainty factor of 2 was added, although it should be noted that the kinetics in rodents may be different than in humans, particularly with respect to the maternal-fetal transfer of chemicals.

Database weaknesses: An uncertainty factor was also added to account for the fact that this dose-response assessment is based on cross-sectional data. As discussed above, the single measurements of serum T4 and urinary perchlorate that were collected in NHANES 2001-2 may not be completely accurate measures of true long-term T4 levels or perchlorate exposure. Because this bias is most likely to be non-differential, the resulting impact on the perchlorate-T4 regression coefficient is likely to be towards a regression coefficient of zero, and true associations might actually be stronger than the one observed. Beaton *et al*. (1979) published an equation for predicting the magnitude of this bias:

$\beta_t = \beta_0 (1 + R^2_x/n_x)$,

where β_t is the estimated true regression coefficient after correcting for misclassification, β_0 is the observed regression coefficient, R^2_X is the ratio of intra- to inter-individual variances for the misclassified variable x, and n_x is the number of replicate measures of x per subject that were collected in the study. Since only a single urine and serum sample were collected from each subject in NHANES, $n_x = 1$, and the degree of bias is primarily related to R^2_x. Information on the value of R^2_x is not available in the NHANES 2001-2 data set, and OEHHA could not find a similar published study that specifically provided values of R^2_x for urinary perchlorate concentrations in humans.

In one study however, Ohira *et al*. (2008) compared 24-hour urine perchlorate measurements to creatinine-adjusted spot urine perchlorate concentrations in 14 breastfeeding mothers and reported that the average deviation between these two measures was 105 percent. In this NHANES study group, the mean spot urine perchlorate concentration was 2.86 µg/L. If one assumes the average deviation in the NHANES population was similar to that of Ohira *et al*. (2008), then the average deviation between a single spot urine perchlorate concentration and 24-hour sample concentrations in the NHANES study group would be about 3.0 µg/L (i.e., 105 percent x 2.86 µg/L).

Assume that:

1. The *mean* perchlorate concentration in spot samples from a large group of subjects (x_m) will be about equal to the *mean* perchlorate concentration in 24-hour samples from this same large group (this is probably a valid assumption because while a spot concentration may vary from a 24-hour concentration in any given individual, the spot concentration and 24-hr concentration means in the group should be about the same), and

2. The mean perchlorate concentration in 24-hour samples is a much better reflection of true perchlorate intake than perchlorate concentration from a spot urine sample (more on this later).

Then an estimate of the variance due to intra-individual variability associated with the use of spot samples might be estimated as:

Variance = $\sum (x_m - x_i)^2 / n - 1$,

where n is the number of subjects in the study, and $x_m - x_i$ is the average deviation between spot urine and 24-hour urine concentrations (3.0 µg/L). Given

this, the estimate of intra-individual variance is:

$$= 385 * (3.0\ \mu g/L)^2 / (385-1)$$

$$\approx 9\ \mu g/L$$

The total variance (intra- and inter-individual variance) in the spot urine perchlorate concentrations in the NHANES 2001-2 data set (all 385 low iodine women) was 32.9 µg/L. These data suggest that R^2_x might be somewhere near:

$$R^2_x = \text{variance}_{\text{intra-individual}}/\text{variance}_{\text{inter-individual}}$$

$$= 9\ \mu g/L / (32.9\ \mu g/L – 9\ \mu g/L) = 0.38$$

The corrected regression coefficient could be somewhere near:

$$\beta_t = \beta_0 (1 + R^2_x/n_x)$$

$$= 0.79 (1 + 0.38/1) = 1.09$$

That is, the corrected regression coefficient between perchlorate and T4 is about 1.09 times larger than the observed coefficient.

It should be noted that these calculations are estimates. They are based on the assumptions that 24-hour perchlorate levels are an accurate indicator of true long-term exposure and that the variance in the NHANES population is similar to that of Ohira *et al*. (2008). In addition, they do not take into account other sources of variance like laboratory imprecision. Despite this, these estimates do provide some general idea of the likely magnitude of the bias due to the use of spot urine samples and provide at least some assurance that a factor of two is likely to cover the uncertainty associated with this database insufficiency.

With regards to intra-individual variability in serum T4 measurements, Andersen *et al*. (2001) examined this issue in 16 healthy men by collecting monthly measurements of serum T4 over a period of one year. They reported an individuality index (defined as $SD_{analytical+intraindividual} / SD_{interindividual}$) of 0.54. A ratio of the respective variances (i.e., R^2_x) would be about $(0.54)^2 = 0.29$. This possible 29 percent increase in the perchlorate-T4 regression coefficient is also within the two-fold uncertainty factor that was incorporated.

Relative source contribution

Recent food analyses have greatly expanded the data available on exposures to perchlorate in food, as described in the Exposure section of this document. Perchlorate has been detected in a wide variety of foods, including fruits, vegetables, grains, dairy milk, and human breast milk (Kirk *et al*. 2005, Pearce *et al*., 2007; Murray *et al*., 2008). Perchlorate levels in urine from NHANES, as reflected in the analysis of Blount *et al*. (2006), are generally supportive of the

FDA analysis. Together, these data demonstrate that food is the primary source of perchlorate for the general population.

At an ADD of 0.023 µg/kg-day and a range of average perchlorate exposure levels in food of about 0.09 to 0.39 µg/kg-day, the health-protective level is already exceeded by perchlorate from food. Mean exposures from food for women of childbearing age are about 0.1 µg/kg-day, according to the estimates of Murray *et al.* (2008). Thus it does not seem appropriate to allocate any specific fraction of total exposure to drinking water; all sources should be limited because the threshold of effect is already exceeded by the exposures derived from food. The concept of zero as a relative source contribution from water is unattainable. We have used 1 as a placeholder, indicating that no specific "acceptable" fraction can be calculated.

Drinking water at a PHG level of 0.6 ppb would provide about one/tenth the average exposure to perchlorate that would be obtained from food, for women of child-bearing age. One could say that this is equivalent to a relative source contribution of 0.1, which is below the guidelines of 0.2 to 0.8 recommended by U.S. EPA. However, these guidelines are intended for exposures that yield total daily doses below the effect threshold. In this case, since effects on thyroid hormone homeostasis are observed at common environmental exposures, the operating principle should merely be to decrease exposure to perchlorate as much as practical, and ensure adequate dietary iodine.