# DRAFT

## For Peer Review Only

# Public Health Goal for Perchlorate In Drinking Water

Prepared by

**Office of Environmental Health Hazard Assessment**
**California Environmental Protection Agency**

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NOT A REGULATORY STANDARD

# PREFACE

**Drinking Water Public Health Goals**

**Pesticide and Environmental Toxicology Section**

**Office of Environmental Health Hazard Assessment**

**California Environmental Protection Agency**

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These 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 California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, 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 which can cause chronic disease shall be based upon currently available data and shall be set at levels which 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 California Department of Health Services (DHS) 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 DHS are to consider economic factors and technical 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 DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

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

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# DRAFT

# PUBLIC HEALTH GOAL FOR PERCHLORATE IN DRINKING WATER

## SUMMARY

A Public Health Goal (PHG) of 6 ppb (6 µg/L) is proposed based on the inhibitory effect of perchlorate on the uptake of iodide by the thyroid gland. The inhibition occurs at a transport molecule called the "sodium-iodide symporter" that is responsible for the active transport of iodide into the thyroid. Iodine is an essential trace element and can be rate limiting in thyroid hormone synthesis. Because of the inhibitory effect, many adverse health effects associated with low-dose perchlorate exposure are expected to be similar to those caused by iodine deficiency. This evaluation included a review of the toxicity data on perchlorate and adverse health effects associated with iodine deficiency. The proposed PHG is developed to protect the general population, including sensitive subgroups, from the consumption of perchlorate in drinking water.

Four sensitive sub-populations are identified in this evaluation: (i) pregnant women who are marginally iodine deficient or iodine deficient; (ii) fetuses of these pregnant women; (iii) infants and small children with low dietary iodide intake; and (iv) individuals suffering from hypothyroidism. Since there are defined members of the population suffering from iodide deficiency and/or hypothyroidism, it is essential that their illness not be exacerbated. Furthermore, since fetuses are at risk from permanent neurological effects, it is important to select an endpoint that will not increase the risk or potential for these effects to occur in children.

A number of human studies (Lawrence *et al*., 2000, 2001; Greer *et al*., 2000, 2002) documented the inhibitory effect of perchlorate on iodide uptake by the thyroid. Greer *et al*. (2000; 2002) administered a daily oral dose of perchlorate to groups of volunteers at 0.007, 0.02, 0.1, or 0.5 mg/kg-day. At the end of a 14-day exposure period, reduction in thyroidal iodide uptake was statistically significant in the three higher-dosed groups. Based on this study, a No Observed Adverse Effect Level (NOAEL) of 0.007 mg/kg-day can be identified. In two similarly designed studies, Lawrence *et al*. (2000, 2001) administered a daily dose of 10 mg or 3 mg of perchlorate in water to groups of male volunteers. Following a 14-day exposure period, reduction of thyroidal iodide uptake in the high-dose study was statistically significant while the reduction in the low-dose study, associated with a 10 percent inhibition of uptake, was not significant. The low dose corresponds to approximately 0.043 mg/kg-day, which is slightly higher than the LOAEL identified in the Greer *et al*. study (2002) of 0.02 mg/kg-day. Given the inherent variability in the measurements, we assume an actual NOAEL for human uptake in the lower range of these doses. Rounding up the lowest estimate from the Greer *et al*. studies (2000, 2002), OEHHA estimated a NOAEL of 0.01 mg/kg-day for the inhibition of thyroidal iodide uptake by perchlorate through the oral route.

The proposed PHG of 6 ppb is based on the NOAEL of 0.01 mg/kg-day (10 µg/kg-day), with an overall uncertainty factor of 30. The calculation assumes a body weight of 65 kg for a pregnant woman, a daily water consumption rate of 2 L/day and a relative source contribution of 60 percent.

An uncertainty factor of 10 is used to account for individual variability in the target populations due to (a) the small number of subjects in each dose group of the studies selected; (b) relatively high iodine intake levels of the subjects studied, compared to the sub-populations at greatest risk; (c) variation in exposure, such as seasonal variation in dietary iodide intake or exposure to goitrogens in certain foods and (d) variation in genetic makeup. Individual variability is evident in the Greer *et al*. study (2002); apparent decreases in thyroidal iodide uptake were noticed in some volunteers exposed to the lowest dose tested, 0.007 mg/kg-day. There is also an ecological study indicating a low level of perchlorate in drinking water was correlated with a reduction of thyroid hormone in newborns in California (Schwartz, 2001).

An uncertainty factor of 3 is used to compensate for the quality of the database and concerns about extrapolating the 14-day study results to lifetime exposure. The standard default uncertainty factor for extrapolating from short-term data to chronic exposure is 10. While confidence in the study results reported by Lawrence *et al*. (2000, 2001) and Greer *et al*. (2002) is limited because of the short exposure duration, the inhibitory effect of perchlorate most likely precedes other adverse effects of perchlorate. Fourteen days may not be long enough to evaluate the influence of perchlorate on thyroid hormones since there is a large reserve of iodide in humans. As perchlorate competes with iodide for absorption sites on thyroid cells, high dietary iodide intake might have elevated the LOAEL and NOAEL for perchlorate inhibition of thyroidal iodide uptake. Furthermore, there are some concerns for the lack of information on the degree and consequences of the potentially reduced secretion of iodide into the breast milk resulted from mother's exposure to perchlorate.

A significant fraction of the United States' population has been shown to have low iodide intake. Low urinary iodine concentrations (<5 µg/dL) were found in 11.7 percent of the population. Urinary iodine concentrations are an indicator of the adequacy of iodide intake for a population. The median urinary iodine concentrations in iodine-sufficient populations should be greater than 10 µg/dL, and no more than 20 percent of the population should have urinary iodine concentration less than 5 µg/dL. Urinary iodine data from the National Health and Nutrition Examination Survey III [NHANES III (1988-1994)] showed that the overall median (±standard error) urinary iodine concentration of the U.S. population was 14.5±0.3 µg/dL (Hollowell *et al*., 1998). Among women of childbearing age, 14.9 percent had urinary iodine concentration below 5 µg/dL. This is roughly equivalent to a dietary iodide intake of less than 68 µg/day (daily iodine intake [µg] = urinary iodine [µg/L] x 0.0235 x body weight [58 kg]) (NAS, 2001).

A number of human studies have shown that pregnancy itself puts stress on the thyroid (Crooks *et al*., 1967; Glinoer *et al*., 1990, 1992; Smyth *et al*., 1997; Caron *et al*., 1997; Brent, 1999; Kung *et al*., 2000). In areas of marginal iodine deficiency (intake level

<100 µg/day), there is an increased risk of abnormally low serum triiodothyronine (T3) and T4 levels, thyroid enlargement as well as goiter in pregnant women. In two prospective studies (Romano *et al*., 1991; Pedersen *et al*., 1993), it has been shown that the pregnancy-related thyroid enlargement could be prevented by administering iodide salts to the pregnant women. These results confirm that iodine deficiency is the main causative factor of thyroid enlargement during pregnancy.

More significantly, developing fetuses and neonates are considered particularly sensitive to iodine deficiency. It is because of the irreversible changes that can occur during this period of rapid structural and behavioral development. Severe iodine deficiency (<25 µg/day) during pregnancy can cause perinatal death and cretinism (WHO, 1994; as cited in Hollowell *et al*., 1998). Cretinism is characterized by mental deficiency, deaf mutism and spastic diplegia[1]. Even moderate to mild iodine deficiency during pregnancy has been linked to adverse neuropsychological development and a reduction of IQ of the child (Glorieux *et al*., 1988; Man and Jones, 1969; Rovet *et al*., 1987; Tillotson *et al*., 1994; Vermiglio *et al*., 1990; Bleichrodt and Born, 1994). Studies indicate that mild hypothyroidism or small decrements in maternal free T4 within what is generally considered the "normal" range (lowest 10 percent) during the first trimester are associated with impaired neuropsychological development in the child (Pop *et al*., 1999; Haddow *et al*., 1999). Adverse effects of perchlorate exposure on the development of fetal brain have also been observed in rats. In a study reported by Argus Research Laboratories (2001), ammonium perchlorate in water was administered to female rats throughout the gestation period at target doses of 0, 0.01, 0.1, 1 or 30 mg/kg-day. Several brain areas (e.g., corpus callosum) in the treated male and female pups (combined) varied significantly from controls in size. Different brain regions appeared to show an inverted U or U-shaped dose-response relationship. Based on this data set, U.S. EPA (2002) identified a LOAEL of 0.0085 mg/kg-day for effects of perchlorate (or 0.01 mg/kg-day for ammonium perchlorate) on fetal development.

As pregnant women who have low dietary iodine intake are already at risk of developing goiter and having children with reduced intelligence quotient (IQ), any additional stress on thyroid function is deemed undesirable. Similarly, individuals with existing thyroid problems, such as low serum T4 or/and high serum TSH are also identified as a sensitive sub-population. Thyroid glands of these individuals are not functioning properly; any additional stress on the thyroid or thyroid/pituitary system may increase the severity of the illness. Infants and small children are considered at higher risks regarding exposure to perchlorate. The iodide reserve in the thyroid of infants and children is less than that in adults; for this reason, infants and children are likely to be more sensitive to inhibition of thyroid iodide uptake by perchlorate (Delange and Ermans, 1991). There are indications that not only is perchlorate transferred from nursing mothers to their infants through breast milk, but also perchlorate can decrease the secretion of iodide into the breast milk, which is the sole source of iodide for breast-feeding infants.

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[1] Bilateral paralysis of both sides of any part of the body.

The *in vitro* and *in vivo* genotoxicity studies on perchlorate are negative. There are data to indicate that perchlorate is not metabolized in the body and does not bioaccumulate in laboratory animals and humans. The biological half-life of perchlorate in rodents is short, ranging from 2 to 20 hours. The biological half-life in humans is similar, approximately 5-8 hours. Short-term and subchronic drinking water studies showed that perchlorate exposure decreased serum T3 and T4 levels and increased serum thyroid stimulating hormone (TSH) levels in rodents and rabbits. At high perchlorate exposure levels, thyroid follicular cell hypertrophy, thyroid follicular cell hyperplasia, increased thyroid weights, and thyroid tumors were also observed in the treated animals. In one study, it was shown that the increases of absolute and relative thyroid/parathyroid weights observed in rats exposed to ammonium perchlorate were reversible (Springborn Laboratories, 1998; Siglin *et al*., 2000).

These data indicate that thyroid tumors observed in rats and mice orally exposed to perchlorate are likely to be caused by the disruption of thyroid-pituitary homeostasis. It is therefore reasoned that by preventing the early event of perchlorate inhibition of thyroidal iodide uptake one would also prevent the subsequent events, such as changes in serum T3, T4, and TSH levels, thyroid enlargement, thyroid follicular cell hypertrophy and hyperplasia, as well as thyroid tumors.

In their recent draft perchlorate risk assessment, U.S. EPA (2002) proposed a human health-protective drinking water equivalent level (DWEL) of 1 ppb for perchlorate. This value is derived from the estimated LOAEL of 0.0085 mg/kg-day, based on the apparent brain morphometric changes observed in a rat developmental neurotoxicity study (Argus Laboratories, Inc., 2001). U.S. EPA (2002) applied an overall uncertainty factor of 300 to calculate a draft oral reference dose (RfD) of 0.03 µg/kg-day, and assumed a default human body weight of 70 kg and a drinking water consumption rate of 2 L/day to calculate the DWEL of 1 ppb or 1 µg/L U.S. EPA defines RfD "as an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of adverse effects over a lifetime." The proposed OEHHA PHG value of 6 ppb, while higher than the draft U.S. EPA DWEL, is within the projected uncertainty range of the U.S. EPA estimate. The major difference between the two calculations is the use of rat data by U.S. EPA versus human data by OEHHA.

Currently, there is no Federal or State maximum contaminant level (MCL) for perchlorate. There is a State action level of 4 ppb (DHS, 2002).

## INTRODUCTION

Many adverse effects observed in test animals and humans exposed to perchlorate are related to the disruption of thyroid hormone regulation. The principal hormones secreted by the thyroid are thyroxine (T4) and triiodothyronine (T3); both hormones are iodine-containing amino acids. While T4 is produced only by the thyroid gland, about 80 percent of T3 is formed in the peripheral tissues 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 substrates for thyroid hormone synthesis are iodide and tyrosine. Tyrosine is generally not the rate-limiting substrate. Iodine is a trace element and can be rate limiting in thyroid hormone synthesis. Ingestion is the main route of iodine intake. Once ingested, iodine is reduced to iodide ($I^-$) in the gastrointestinal tract and readily absorbed into the bloodstream either through passive diffusion or active transport.

Thyroid tissue has a special ability to concentrate iodide selectively from the surroundings where the concentration is very low ($10^{-8}$ to $10^{-7}$ M). The thyroid can actively transport iodide into the organ such that intrathyroidal iodide concentrations can be several hundred-fold higher than those of the external medium. Such concentrations are presumably required to promote efficient hormone synthesis because patients lacking the ability to concentrate iodide have goiters and are hypothyroid (Wolff, 1998). A protein model has been proposed of the manner in which sodium and iodide ions are actively transported into the thyroid gland. The molecule that is responsible for this transport has been named the sodium-iodide symporter (NIS). Recently mouse symporter molecule has been cloned and expressed into normally noniodide-transporting cells; after the modification, these cells showed perchlorate-sensitive iodide accumulation capability (Perron *et al*., 2001). These researchers found evidence to believe that sodium-iodide symporter is also present in extra-thyroidal tissues, such as stomach, lactating mammary gland, and to a lower extent in small intestine, skin, and brain.

In humans, a majority of T4 and T3 in plasma is protein-bound. In normal plasma, the T4 binding distribution is 80 percent of T4 binding 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. Only a small percentage of T4 and T3 in plasma is free, 0.03 percent and 0.3 percent, respectively. Only the free hormone enters cells, exerts its biologic action, and determines thyroid physiologic status (Dillmann, 2000).

Control of the concentrations of T4 and T3 in the blood is mainly regulated by a negative feedback 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). The hypothalamus stimulates the pituitary through thyrotropin-releasing hormone (TRH) to produce thyroid-stimulating hormone (TSH), which then prompts the thyroid gland to produce the thyroid hormones T4 and T3. The stimulated thyroid actively transports inorganic iodide into the follicular cell and converts it to an organic form and then into thyroid hormone molecules, which can influence target organs throughout the body. The secreted T4 and T3 are metabolized in the liver and other tissues. Some thyroid hormone derivatives are excreted in the bile, and some of the iodine in them is reabsorbed. Cells in the hypothalamus and pituitary gland respond to levels of circulating thyroid hormones, such that when thyroid hormone levels are high, there is a signal to reduce the output of TRH and TSH. Similarly, when thyroid hormone levels are reduced, the pituitary is prompted to deliver more TSH to the thyroid gland to increase the output of thyroid hormone. 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 monitored in the serum of experimental animals and humans by radioimmunoassay and serve as biomarkers of exposure and effect of agents that disrupt thyroid-pituitary status (U.S. EPA, 1998a,b; 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).

Too much or too little thyroid hormone can lead to illnesses. 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 biosynthesis (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.

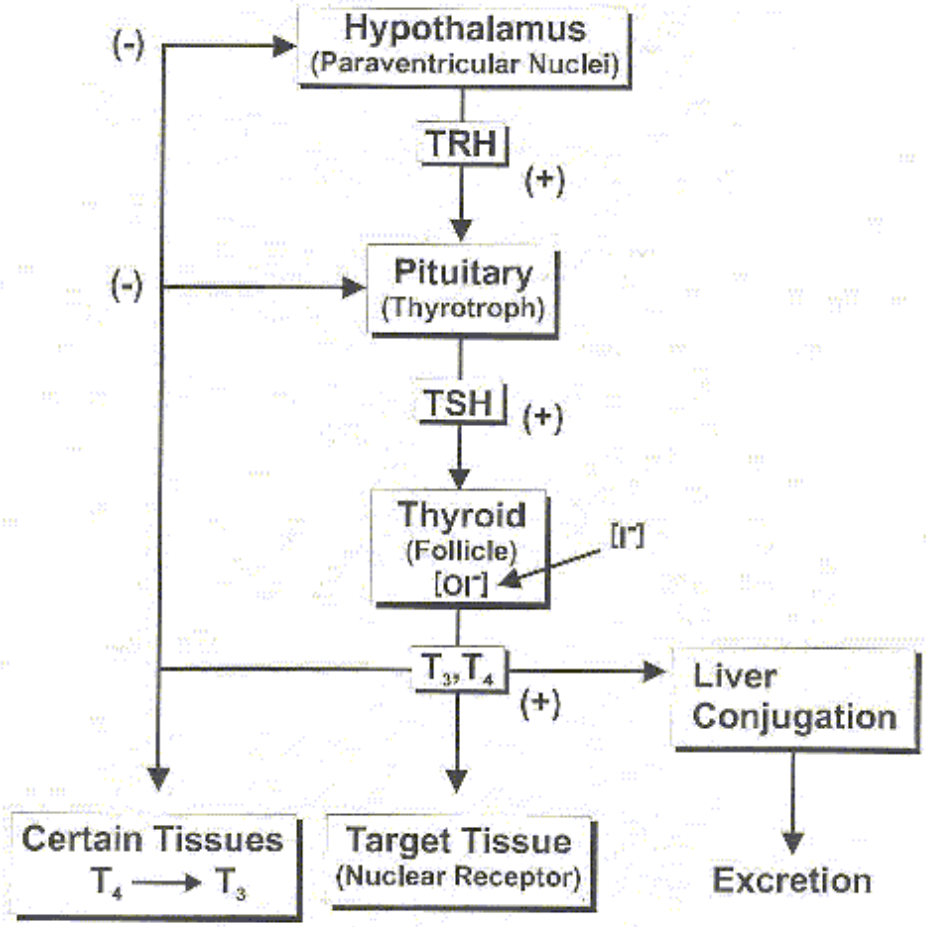


Figure 1. Hypothalamic-pituitary-thyroid axis (from U.S. EPA, 1998b)

The most severe neurologic impairment resulting from iodine deficiency is cretinism caused by iodine deficiency. 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. Recently, a number of studies indicated that even mild to moderate iodine deficiency can reduce maternal serum thyroid hormone levels and impair the brain development of the offspring (Glorieux *et al*., 1988; Man and Jones, 1969; Rovet *et al*., 1987; Tillotson *et al*., 1994; Vermiglio *et al*., 1990; Pop *et al*., 1999; Haddow *et al*., 1999; Bleichrodt and Born, 1994).

It has been suggested that fetal damage during development is inversely related to maternal serum T4 levels in the first and second trimesters. Maternal serum free T4 is able to pass through the placenta and is converted to T3 in the fetal brain. The T3 generated in situ is believed to be necessary for the development of brain, specifically the cerebral cortex, the extrapyramidal system, and the cochlea (Porterfield, 2000). The availability of a minimum level of maternal free T4 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 (low level of serum T4), superimposed on major neurodevelopmental events.

It is important to note that a normal level of maternal T3 does not seem to prevent the potential damage of a low supply of T4. This may be explained by the fact that placenta is rich in Type III deiodinase which catalyzes the conversion of T4 to reverse T3 and T3 to 3,3'-diiodothyronine (T2). Both reverse T3 and T2 are not biological active. A majority of the T3 in fetal brain is derived from the local generation of T3 from T4 by Type II deiodinase.

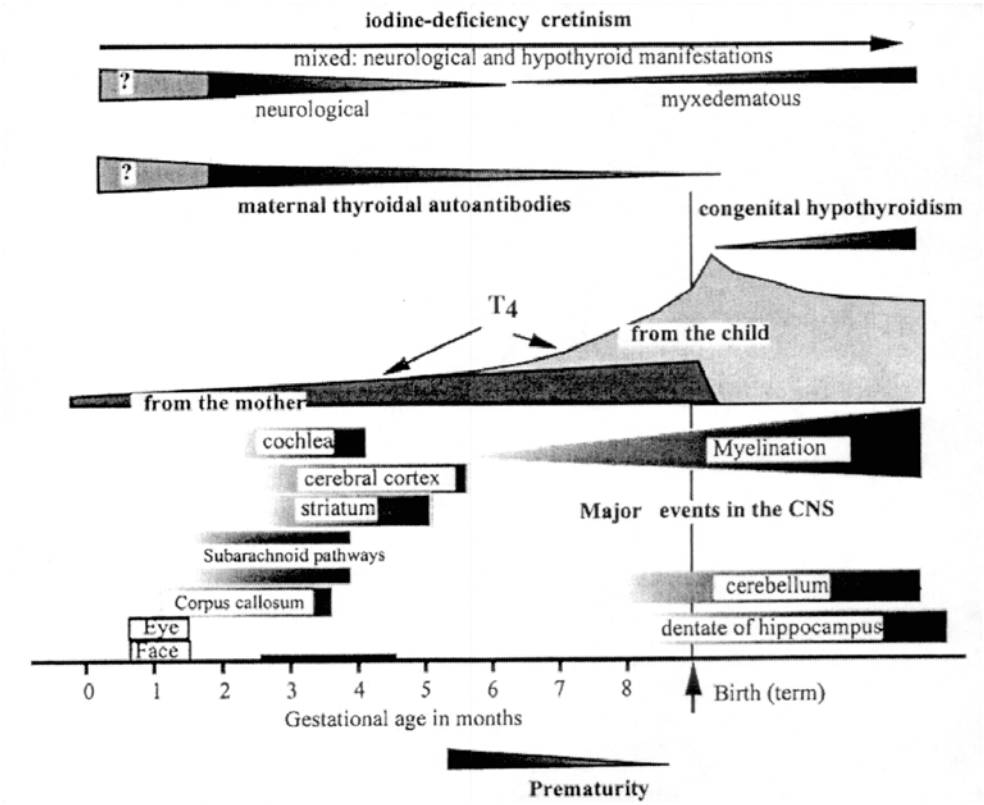


Figure 2. Approximate timing of major insults to the brain resulting from hypothyroxinemia, superimposed on major neurodevelopmental events. Conditions resulting in early maternal hypothyroxinemia, combined with later impairment of the fetal thyroid, are the most damaging, with central nervous system (CNS) damage that is irreversible at birth. The most frequent cause is maternal iodine deficiency and the presence of maternal autoimmune thyroid disorders. Unless iodine deficiency is also present, the CNS damage in congenital hypothyroidism is preventable by early postnatal treatment because the normal maternal thyroxine level has avoided damage to the brain until birth. However, normal maternal concentrations of T3 with low T4 do not protect the fetal brain because of its dependence on intracerebral regulation of local T3 availability by deiodinating pathways using T4 as a substrate. Interruption of the contribution of maternal T4 in premature infants with an immature thyroid may also underlie their increased risk of neurodevelopmental problems. The question mark indicates that we do not know whether very early CNS development, corresponding to a period when the general morphogenesis of the prosencephalon (neurulation and segmentation) is being determined, is thyroid hormone sensitive or not (from Morreale de Escobar *et al*., 2000).

## 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 ion. Perchlorate salts are ionic, and dissociate completely when dissolved in water. This risk assessment is for the perchlorate anion in water, regardless of the identity 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, 2000).**

| Property | Value or Information |
|---|---|
| Molecular Weight | 117.49 |
| Color/Physical State | white orthorhombic crystals |
| Melting Point | 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 has been and continues to be 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 ($NaClO_4$). The sodium perchlorate 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, 1998a).

## ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

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 use, large volumes of perchlorate have been disposed of since the 1950s. Some of this has leached into soil, and into aquifers used as drinking water sources. 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 to air also occur as a consequence of burning old rocket fuel that has been taken out of service. No data were found on levels of perchlorate in ambient air.

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

### *Soil*

Perchlorate used in rocket fuel has a finite shelf life. Because of this, large volumes of rocket fuel containing perchlorate have 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 result of past disposal practices, soil and groundwater near the facilities that had been engaged in rocket fuel manufacturing and disposal are contaminated. Another way in which soil can become contaminated is by irrigation with perchlorate-contaminated water.

A report of TRC Environmental Corporation (1998) raised the concern that some chemical fertilizer may be contaminated with perchlorate. U.S. EPA (2001) recently tested a variety of fertilizers collected from representative sites around the nation and did not find perchlorate contamination to be a problem. Based on these test data, there is insufficient evidence for fertilizers to be viewed as contributors to environmental perchlorate contamination. The only exceptions are those products derived from Chilean caliche (a natural perchlorate source).

### *Water*

Drinking water sources have become contaminated with perchlorate as a consequence of soil pollution in areas where solid rocket fuel has been 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 of 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) 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. EPA Method 314.0 (Federal Register, 2000) now exists for analysis of perchlorate in water to achieve this low detection level.

Since March 1997, DHS has sampled several hundred drinking water systems and drinking water wells throughout California. Perchlorate has been found in a large number of drinking water sources in California (Table 2). Contaminated drinking water wells have been found in eastern Sacramento County (up to 260 ppb) near Aerojet General Corporation's facility, and in Los Angeles County (up to 159 ppb) at an Aerojet facility (Azusa), the Whittaker-Bermite site (Santa Clarita), and the Jet Propulsion Laboratory (Pasadena), among others (DHS, 2000). Additionally, perchlorate has been detected in monitoring wells in Lincoln, Tracy, San Jose, Hollister, and the Los Angeles area, as well as at Edwards Air Force Base and El Toro Marine Corps Air Station in southern California (U.S. EPA, 1998a). Colorado River water (on the southwestern boundary of the State) is also contaminated with low levels of perchlorate (5-9 ppb) (DHS, 2000). In general, almost all of the areas where perchlorate contamination has been detected have had some activity involving rocket engines or fuel.

**Table 2. Reported perchlorate detections in California [1,2] (from DHS, 2000).**

| Systems and Sources [3] | Number Sampled | Perchlorate Detections | Perchlorate > 18 µg/L [4] |
|---|---|---|---|
| Public water systems | 273 [5] | 44 (16%) | 23 (8.4%) |
| Drinking water sources | 1,589 | 164 (10%) | 46 (2.9%) |

1. As of July 3, 2000.
2. Perchlorate is "detected" if found at least twice.
3. From monitoring by community and non-transient non-community public water systems (~4,900 systems with ~11,000 ground water sources and ~800 surface water sources).
4. The action level for perchlorate in California during this sampling period; the current action level is 4 µg/L (DHS, 2002).
5. These systems collectively serve 14.5 million people, about 43 percent of the state's population. Of all those systems' drinking water sources, 40 percent have been sampled.

As there is no existing drinking water regulation for perchlorate, DHS (2000) established an action level of 18 µg/L (ppb) for perchlorate. In early 2002, based on the draft risk assessment on perchlorate released by U.S. EPA (2002) and the detection limit that can be generally achieved, DHS (2002) reduced the action level to 4 µg/L (ppb). Action levels are health-based advisory levels and not enforceable standards. When they are exceeded, water systems are required to notify local governing agencies and are recommended to issue a consumer notice. In addition, DHS recommends that a source of drinking water be taken out of service if perchlorate concentration exceeds 40 µg/L (ppb) (DHS, 2002).

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.

## *Food*

No survey data were found on perchlorate levels in raw or processed foods. However, it has been suggested that perchlorate can be used as a growth promoter in leguminous plants (Verteletskaya *et al*., 1974; as cited in Burg, 1995), livestock (sheep and cattle) and poultry (Yakimenko *et al*., 1981; as cited in Burg, 1995). The cited USSR research indicated that weight gains in test livestock of 3-31 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, decreasing their metabolic rates.

There are preliminary results showing that some vegetables (e.g., lettuce) may bioaccumulate perchlorate. In a recent 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 rise steadily over the first 50-60 days, and then generally level off. The amount of perchlorate detected in the leaves correlates with dose. For example, at about 50 days into the study, the lettuce irrigated with 10.0 µg/ml (ppm) perchlorate exhibits a perchlorate content of about 300 µg/g on a wet mass basis (U.S. EPA, 2001). However, it was cautioned that the perchlorate concentrations used in the study are much higher than the concentrations in perchlorate-tainted irrigation water and that results obtained under laboratory growing conditions cannot be directly extrapolated to edible agricultural produce.

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.

There are concerns that breast milk may represent an exposure pathway for infants. Human mammary gland during lactation has been shown to express the sodium-iodide symporter (NIS) and may have the capability to actively secrete perchlorate into the breast milk (Vayre *et al*., 1999; Tazebay *et al*., 2000). There are no quantitative data available regarding the secretion of perchlorate into breast milk by nursing mothers, but this type of information is available for pertechnetate ($TcO_4^-$). Iodide and pertechnetate have similar ionic characteristics and are actively taken up by the NIS (Zuckier *et al*., 2001). Actually, it has been suggested that pertechnetate has a greater ability than perchlorate in inhibiting iodide transport into thyroid tissues *in vitro* (Wolff, 1964; as cited in Wolff, 1998). In three clinical case studies (Wayne *et al*., 1986; Mountford and Coakley, 1987; Ahlgren *et al*., 1985), $^{99m}TcO_4^-$ was injected into nursing mothers (one patient in each study) to obtain scintigraphs of the thyroid. Following the injection, breast feeding was stopped for at least 24 hr and the breast milk was collected for analysis. As the biological half-life of $^{99m}TcO_4^-$ is estimated to be approximately 3-5 hr, most of the injected dose would be excreted within 24 hr. They found the total amount of radioactivity in the milk ranged from 3 to 11 percent of the injected $^{99m}TcO_4^-$ dose. Considering the high food consumption rate of infants (on a body weight basis), this may represent an opportunity for a slightly higher relative dose to nursing infants than to their mothers, although data are too limited to estimate perchlorate dose by this pathway.

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). As there are no data to quantify the extent of the reduction of iodide in breast milk at low levels of perchlorate exposure, this source of uncertainty is considered covered in the limited data uncertainty factor.

## 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) administered orally 1-2 g perchlorate to patients and recovered 70 percent of the dose in the urine in 12 hr and 85-90 percent in 24 hr. 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 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 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). 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 $4.3 \pm 0.3$ in both rats and rabbits, at about 6 hours after the injection. These data demonstrate the fact that thyroid concentrates perchlorate ions in rat and rabbit. There were also indications of retention of perchlorate in the salivary gland and in the testes, when the perchlorate level in blood fell to a very low level.

Chow *et al*. (1969) compared the uptake of radiolabeled perchlorate and iodide ions with stable ions in male Sprague-Dawley rats. The male rats were injected with 0.1, 0.2, or 5.0 meq/kg potassium perchlorate (14, 28, or 690 mg/kg, respectively) 2 hr prior to sacrifice. It was found that 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. The apparent accumulation of perchlorate in the thyroid, as reflected in the thyroid/blood ratio, was found to be inversely related to the perchlorate dose (U.S. EPA, 2002).

Chow and Woodbury (1970) also sstudied perchlorate accumulation by the thyroid at low doses. They administered perchlorate by intra-peritoneal 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 hr after dosing. The levels of perchlorate measured in the thyroid and plasma indicate that perchlorate was best accumulated in the thyroid at the low dose. At higher doses (at or above 14 mg/kg), the level of perchlorate in the thyroid was lower than in the plasma (U.S. EPA, 2002).

It has been shown that perchlorate inhibits iodide transport into the thyroid. This is because in addition to iodide, thyroid tissues can also select several related mono-valent anions. Measurement of the ability to be concentrated by thyroid tissues, or to inhibit iodide transport, has resulted in the following potency series for mono-valent 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). It is not clear whether this anion sequence, measured at very high concentrations, has any necessary mechanistic relation to what occurs in the thyroid, at low concentrations. Anbar *et al*. (1959) showed that the inhibition of iodide transport by perchlorate is a truly competitive process. They injected intraperitoneally $^{36}Cl$-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 3).

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 by-product 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 3. The ratio between concentrations of iodide and perchlorate ions in the thyroid (from Anbar *et al*., 1959).**

| Iodide dose (mmol) | Perchlorate dose (mmol) | Ratio* $I^-/ClO_4^-$ | | |
|---|---|---|---|---|
| | | 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 |

*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 iodide diet for 4-5 weeks prior to the time of perchlorate administration. The level of perchlorate in the thyroid peaked at four hours after administration, then declined to approximately five percent of its peak value at 96 hours. The decay followed an exponential function with a half-life of 20 hr. When the levels of radioactivity in the serum and the urine are plotted against time, they also follow an exponential function with a half-life of approximately 20 hr. 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 hr after the administration of perchlorate is shown in Table 4.

**Table 4. Percent dose of $^{36}$Cl per g tissue at 96 hr after intraperitoneal injection of $^{36}$Cl-perchlorate (from Goldman and Stanbury, 1973).**

| Organ | Percent dose/g tissue |
|---|---|
| Thyroid | 0.142±0.1 * |
| Kidney | 0.125±0.09 |
| Spleen | 0.098±0.03 |
| Liver | 0.048±0.04 |
| Brain | Background |

* mean±standard deviation; each value represents five animals.

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 hr and accounted for a calculated 96 percent of the dose. The second-phase half-life, which accounted for only 4 percent of the administered dose, ranged from 72 to 80 hr. Yu *et al*. (2000) injected perchlorate intravenously at 0.01, 0.1, 1, or 3 mg/kg to male Sprague-Dawley rats and monitored the serum concentration of perchlorate over time. In

this study, the estimated early- and terminal-phase half-lives of perchlorate in rat were 2-3 hr and 12-26 hr, respectively.

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. Another important difference may be explained by the fact that the rats in the study reported by Goldman and Stanbury (1973) had been maintained on a low iodide 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 indicated by urinary perchlorate concentration, increased over the three days of work exposure, with a decrease 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 hours and 8.2 hours.

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-hr period.

### *Physiological/Nutritional Role*

Perchlorate has no known nutritional role. In 1952 investigators observed that perchlorate displaces iodide from the rat thyroid (Wyngaarden *et al*., 1952). Since then

perchlorate has been widely used in 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).

It was reported that when ammonium perchlorate was added to the feed of farm animals, weight gain was increased by 3-31 percent. The optimum dose ranged from 2 to 5 mg/kg (Grayson, 1978; Yakimenko *et al*., 1981; as cited in Burg, 1995). This is most likely a non-nutritive effect associated with inhibition of thyroid hormone production (hypothyroidism).

## TOXICOLOGY

As perchlorate competitively blocks iodide from entering the thyroid gland, many of the adverse effects of perchlorate exposure in the low dose range are similar to those of iodine deficiency. For this reason, an overview of some of the adverse health effects of iodine deficiency is provided in this section.

In areas of inadequate iodine intake, thyroid hormone synthesis and secretion both decline. The pituitary gland responses by secreting more TSH, which in turn causes thyroid hypertrophy and iodine deficiency goiter. Children who are born in areas of severe iodine deficiency may suffer from cretinism. The main cause of the disease is iodine deficiency, but it is aggravated by dietary goitrogens, selenium deficiency, and autoimmune hypothyroidism. The manifestations of endemic cretinism range from goiter or mild mental retardation in euthyroid subjects to severe mental deficiency and neurologic defects in those with variable degrees of hypothyroidism. Two subtypes of endemic cretinism have been described, neurologic cretinism and myxedematous cretinism[2]. Neurologic cretinism is more common. It is characterized by delayed growth of long bones, neurological complications such as deaf mutism, mental retardation, and spasticity. Goiter is sometimes associated with the illness, but not myxedema. Myxedematous cretinism is less common. It is characterized by delayed growth of long bones, myxedema, and sometimes goiter but with fewer neurologic problems than are seen in neurologic cretinism. Neurologic damage in the absence of neonatal hypothyroidism has been postulated to be due to maternal hypothyroxinemia early in gestation (Burrow *et al*., 1994) (also see Figure 2).

Even moderate to mild iodine deficiency or hypothyroidism during pregnancy has been linked to adverse neuropsychological development and a reduction of IQ of the child (Glorieux *et al*., 1988; Man and Jones, 1969; Rovet *et al*., 1987; Tillotson *et al*., 1994; Vermiglio *et al*., 1990; Haddow *et al*., 1999; Bleichrodt and Born, 1994).

The presence of maternal thyroid peroxidase antibodies (TPO-ab) during pregnancy with no abnormalities in free T4 (fT4) or TSH has been found to be associated with a significant risk for reduction in scores on the McCarthy Scales of Children's Abilities.

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[2] Hypothyroidism leads to a slowing of metabolic processes and in its most severe form to the accumulation of mucopolysaccharides in the skin, causing a non-pitting edema termed myxedema.

The General Cognitive Scale, which reflects IQ scores, showed a decrement of over 10 points in those with antibodies present (Pop, 1995). At least 10 percent of women of childbearing age have these antibodies present (Westman, 1994). In a further study, Pop *et al*. demonstrated that TPO-abs were an effective marker for fT4 in the lowest 10 percent (Pop, 1999). In that study it was found that 25 percent of women with elevated TPO-ab at 12 weeks gestation had fT4 in the lowest $10^{th}$ percentile (compared with 8 percent of those negative) and of those that were antibody positive at 32 weeks, 46 percent were in the lowest 10th percentile at 12 weeks. A positive correlation was again found between being in the lowest $10^{th}$ percentile of fT4 at 12 weeks gestation and risk of impaired psychomotor development at ten months of age (RR 5.8, 95 percent CI: 1.3-12.6). These studies indicate that even small decrements in maternal fT4 within the "normal" range during the first trimester may result in impaired neuropsychological development of the child. An exact level of fT4 at which this effect would not be seen has not been determined. Women with TPO-ab during pregnancy constitute a distinct subpopulation at dramatically increased risk for fT4<$10^{th}$ percentile and subsequent impairment of their children.

It has been shown that pregnancy itself puts stress on the thyroid (Crooks *et al*., 1967; Glinoer *et al*., 1990, 1992; Smyth *et al*., 1997; Caron *et al*., 1997; Brent, 1999; Kung *et al*., 2000). In areas of marginal iodine deficiency, there is an increased risk of abnormally low serum T4 and T3 levels, thyroid enlargement as well as goiter in pregnant women. For this reason, pregnant women with marginal or frank iodide deficiency and their fetuses are identified as sensitive sub-populations for perchlorate exposure.

## *Toxicological Effects in Animals*

In December 31, 1998, U.S. EPA released a draft document titled "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information." Then in January 18, 2002, U.S. EPA updated the toxicological information and risk assessment on perchlorate and released an external review draft titled "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization." A number of the animal toxicity data sets described in the two drafts are not published and until recently were not available to OEHHA. For this reason, interpretations of some of the animal toxicity data discussed in this section rely heavily on the work of U.S. EPA (1998a; 2002).

### 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 5, 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 (29 mg/kg to 1,500 mg/kg), so the cation appears to play a role in the toxicity of these salts.

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 significant.

**Table 5. 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 | Intraperitoneal (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 |

**Subchronic Toxicity**

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. They 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 sex, number of animals in each dose group, and dosing medium were not reported. In the two highest dose groups, the authors found a significant increase in the amount of iodide excreted from the thyroid. It is not clear if the reported effect was seen in one or both species tested (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.

U.S. EPA (2002) evaluated the thyroid hormone data and concluded that perchlorate exposure decreased circulating T3 and T4 and increased serum TSH. There is evidence that rT3 (formed mostly in extrathyroidal tissues) and thyroglobulin levels were also increased. The lowest dosage of 0.11/0.12 mg/kg-day was a LOAEL in both sexes for T4, T3, and hTg (Tg in rats was assayed with a human radioimmune assay kit, thus the notation "h"), and in females for TSH. Relative thyroid weights were significantly increased in the 11.44/11.47 and the 22.16/24.86 mg/kg-day dose groups compared to the controls. Evaluation of the thyroid follicular lumen size data U.S. EPA (2002) identified a NOAEL at the 0.44/0.47 mg/kg-day dosage.

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.

U.S. EPA (2002) evaluated the hormonal data and concluded that there was a dose- and time-dependent effect of perchlorate on thyroid hormones and TSH. The LOAEL, based on decreases in T3 and T4 at 90 days, is 0.01 mg/kg-day. The NOAEL identified for TSH is 0.05 mg/kg-day based on significant increases in both sexes on both days 14 and 90. Full recovery from the effects on T3 was observed at the 120-day evaluation (30 days after cessation of treatment), whereas only partial recovery was demonstrated for T4 and TSH.

Significant increases in the absolute and relative thyroid and parathyroid weights were observed in male rats at the highest dose after 14 days and in both sexes at the highest dose after 90 days of treatment. However, the absolute and relative thyroid weights of both males and females at the highest dose (10 mg/kg-day) were comparable to controls at the end of the 30-day recovery period, indicating at least some of the thyroid effects were reversible (Springborn Laboratories, 1998).

On day 14, female rats showed decreased colloid and follicular cell hypertrophy at 10 mg/kg-day. Male rats also showed a significant increase in follicular cell hypertrophy at this dose but also exhibited changes at lower doses and in addition had hyperplasia. By 90 days, all three measures (colloid depletion, follicular cell hypertrophy and follicular cell hyperplasia) in both sexes were significant at 10 mg/kg-day. Based on these histopathological results, U.S. EPA (2002) determined a LOAEL at 10 and a NOAEL at 1 mg/kg-day. Recovery of the histopathological changes was essentially complete by 30 days post exposure, although the males did have some indication of residual toxicity (U.S. EPA, 2002).

**Genetic Toxicity**

Ammonium perchlorate was tested in a battery of genotoxicity tests, and found to be negative in all tests (U.S. EPA, 1998a, 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 active 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 was 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, and 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 hrs 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 increase 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 (Springborn Laboratories, 1998). 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 cause any reduction of the polychromatic erythrocyte/normochromatic erythrocyte ratio 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.

**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) demonstrated that perchlorate crosses the placenta in guinea pigs and causes goiter in fetuses when administered at a level (one percent potassium perchlorate in distilled water) that does not cause goiter in the dam.

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 to 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.

A developmental neurotoxicity study of ammonium perchlorate in rats was conducted by Argus Research Laboratories (1998a). 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 (DG 0) to post-natal day 22 (PND22). Five dams per group were selected for sacrifice and blood collection on PND10. Pups (F1 generation) were counted and clinical signs recorded daily during pre- and postweaning. Some of the pups were assigned to four different subsets for additional evaluations: Subset 1 for brain weight and neurohistological examination on PND12; Subset 2 for passive avoidance testing, water maze testing, and finally 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. Details of the study design are described in a risk assessment of U.S. EPA (2002). 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 possibility 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 as cited in U.S. EPA, 2002) reported a two-generation reproductive toxicity study in Sprague-Dawley rats. Male and female rats (30 rats/sex/group) of the first generation (P1) 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. Day 1 of lactation (LD1, 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 P1 rats were sacrificed. Pups not selected for continued evaluation were also sacrificed on LD21. The selected F1 pups were dosed during the postweaning, cohabitation, and lactation periods. All F1 generation dams and their litters (F2 generation) were sacrificed on LD21. Details of the study design and findings have been reported by U.S. EPA (2002).

Increases in thyroid and brain weight were observed in the highest dose group for both male and female P1 rats. Thyroid colloid depletion and hypertrophy were also found in the P1 females at 3 and 30 mg/kg-day; hyperplasia was prominent at 30 mg/kg-day (U.S. EPA, 2002). Among the offspring, increases in thyroid colloid depletion, hypertrophy, and hyperplasia were observed in the F1-generation weanling rats, F1-generation parental rats, and the F2-generation weanling rats exposed to perchlorate at 3 and 30 mg/kg-day. Applying benchmark dose analysis on the thyroid histology data (P-1, F-1, and F-2 results) generated in the study, U.S. EPA (2002) estimated $BMDL_{10}$s (the lower limit of a benchmark response level of a 10 percent increase in incidence over controls) ranging from 0.11 to 0.9 mg/kg-day for colloid depletion, 0.057 to 0.32 mg/kg-day for hypertrophy, and 0.0004 to 2.44 mg/kg-day for hyperplasia.

U.S. EPA (2002) identified a NOAEL of 3 mg/kg-day for effects on T4 and TSH in the P1 males. Similarly, a significant increase in TSH was found in the adult F1 rats at 30 mg/kg-day.

U.S. EPA (2002) also 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. 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 significant.

In response to regulators' suggestion of the need of a better understanding of the neurodevelopmental effects of perchlorate, the United States Navy and Argus Research Laboratories, Inc. (2001; as cited in U.S. EPA, 2002) performed additional studies. The United States Navy evaluated the effects of perchlorate on motor activity in Sprague-Dawley rats of both sexes (Bekkedal *et al*., 2000; as cited in U.S. EPA, 2002). 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 PND10. As dosing was stopped on PND 10, it is likely that the pups were not directly exposed to perchlorate in drinking water. On PND14, 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 PND14, PND18, and PND22. Details of the study are described in a risk assessment of U.S. EPA (2002). U.S. EPA evaluated the data, finding evidence of a dose-response trend of increasing motor activity with a NOAEL of 1 mg/kg-day.

Argus Research Laboratories (2001; as cited in U.S. EPA, 2002) studied the effects of perchlorate on thyroid and brain development both during gestation and post-natally. 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. F-1 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 thyroid and brain from one male and one female pup per litter were selected for histological and morphometric evaluation, with one set evaluated on PND4, PND9, and PND21. Details of the study and findings are described in a risk assessment of U.S. EPA (2002), and a summary of the findings and evaluations are provided below.

The absolute thyroid weights of pups at all ages were significantly increased at 30 mg/kg-day. U.S. EPA (2002) found the effects of ammonium perchlorate on the pup's thyroid glands are largely limited to colloid depletion. The dams show additional dose-related effects on thyroid histopathology that were described as thyroid hypertrophy and hyperplasia. Based on the results of benchmark dose analyses, U.S. EPA (2002) noted that the $BMDL_{10}$ for colloid depletion is lowest in the gestation day 21 (GD21) pups, and is estimated at 0.12 mg/kg-day for the male and female pups combined or for male pups alone, and 0.04 mg/kg-day for female pups alone. The observation that $BMDL_{10}$ increases with age (GD21 to PND21) may suggest that the thyroid gland is most

susceptible to the effects of perchlorate during gestation or at the time of parturition. After 21 days of post-natal exposure, the male pups also show thyroid follicular cell hyperplasia.

As described in the perchlorate risk assessment of U.S. EPA (2002), a statistically significant effect on both T4 and TSH in the dams was observed on GD21 at all dose levels tested, indicating 0.01 mg/kg-day to be a LOAEL and the dams to be in a hypothyroid state. The fetuses were also affected, with clear dose-trends for all three hormones at all doses; statistical significance was not achieved for TSH until the 1 mg/kg-day dose and T4 at 30 mg/kg-day, whereas T3 was affected significantly at all doses. Dose-related trends in T3, T4, and TSH were also observed in PND4, PND9, and PND21 pups. Using benchmark dose analysis on the pup hormone data, U.S. EPA (2002) determined $BMD_{10}$s (a benchmark response level of a 10 percent change compared to the controls) ranged from 0.003 to 0.65 mg/kg-day and $BMDL_{10}$s ranged from $3\times10^{-7}$ to 0.49 mg/kg-day.

Size of various brain areas was also measured in brain sections from the PND9 and PND21 pups. Due to signs of disruption or damage found in the PND9 sections that might have compromised the measurements, U.S. EPA (2002) relied upon the PND21 measurements. In the PND21 brains, the striatum, cerebellum, and corpus callosum all showed significant differences from control with the lowest administered dose of ammonium perchlorate, 0.01 mg/kg-day. As shown in Figure 3, 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 PND21 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. U.S. EPA (2002) noted that this type of dose-response relationship is not uncommon in biological systems as compensatory or other mechanisms may be triggered at high doses. Based on the results reported by Argus Research Laboratories (2001; as cited in U.S. EPA, 2002), 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.

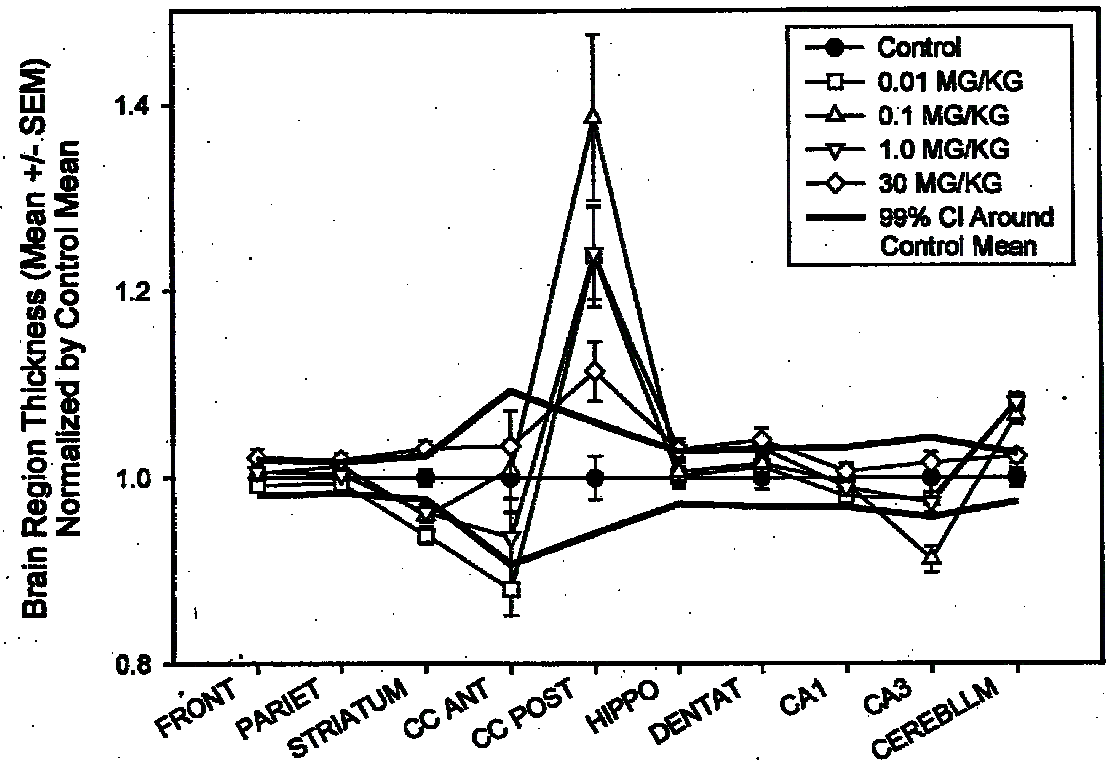


**Figure 3. Profile analysis of brain morphometry measurements for PND21 rat pup brain regions. The male and female data on linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis determines 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% 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).**

Reproductive toxicity

A number of animal reproductive studies have been reviewed by U.S. EPA (2002) and are summarized here. 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 (Brown-Grant and Sherwood, 1971 as cited in U.S. EPA, 2002). Postel (1957) administered one percent potassium perchlorate in drinking water to 16 pregnant guinea pigs during gestation day 21 through gestation day 48. The author reported that perchlorate treatment produced enlarged thyroids in the fetuses but not in dams. From the water intake and body weight data, the author calculated an average daily dose to the dams of 740 mg/kg-day. In a separate experiment, one percent potassium perchlorate was administered to nonpregnant female guinea pigs for 30, 60, or 90 days. Thyroid enlargement and hyperplasia were observed in animals following 60 or 90 days of treatment. Lampe *et al*. (1967, as cited in U.S. EPA, 2002) dosed pregnant rabbits with 100 mg potassium perchlorate/kg by weight daily mixed with feed from conception through gestation day 21 or gestation day 28. Maternal thyroid weights in treated animals were three times higher than control thyroids; fetal thyroids were nearly four times the control weights.

More recently, 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 Caesarean 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 placentae 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 at any dose of T3 or TSH.

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 hormones. A similar transport mechanism is not known to exist in human placenta (Hall *et al*., 1956 and Roti *et al*., 1983, as cited in Fisher, 1996).

In a study by Argus (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 the increase over control in this parameter was statistically or biologically significant. 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 groups. Ossification sites per litter for sternal centers and forelimb phalanges were significantly reduced at 30 mg/kg-day, as noted by U.S. EPA (2002).

**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 was conducted as part of the U.S. EPA's perchlorate testing strategy (U.S. EPA, 1998a, 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 as cited in U.S. EPA, 2002; Burleson Research Technologies, 2000 as cited in U.S. EPA, 2002).

In the hematological studies, there were no differences observed between control and dosed mice in 14- or 90-day experiments for erythrocyte cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, 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 (U.S. EPA, 2002).

Upon reviewing the immunotoxicological studies, U.S. EPA (2002) found three immune function parameters that 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) 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 as cited in U.S. EPA, 2002). However, in a 90-day perchlorate exposure followed by a 30-day recovery period study, similar effects were not observed. 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 (U.S. EPA, 2002). 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 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 as determined by the PFC assay. After reviewing the data, U.S. EPA (2002) determined that perchlorate does not suppress the immune response to SRBCs.

The data from Burleson Research Technologies (2000 as cited in U.S. EPA, 2002) 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, exposure to the same dose for 90 days suppressed the response. Exposure to a lower dose of 2 mg/kg-day did not affect the response in both the 14-day and 90-day studies. U.S. EPA (2002) noted the interpretation of the results was 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 interferes with development of the central nervous system. Thyroid hormone plays an essential role in the development

of the corpus callosum and other brain structures (U.S. EPA, 1998a, 2002). 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 described in detail in U.S. EPA (2002) and summarized in the "Developmental and Reproductive Toxicity" section.

**Endocrine Toxicity**

Many oral and injection studies documented the effects of perchlorate on the thyroid and pituitary hormones as well as the thyroid of the treated animals. The designs and findings of these studies have been described in detail in U.S. EPA (2002) and some of them are summarized in the "Subchronic Toxicity" and "Developmental and Reproductive Toxicity" sections.

Recently, 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 to groups of male Sprague-Dawley rats (6 animals per dose and time point). At 2 hr 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 2, 6, and 9 hr time points. In addition, significant inhibition was also observed in the 0.1 mg/kg dose group at the 9 hr time point (Table 6).

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 2 hr later. Blood and thyroid gland were collected for analyses. A dose-related inhibition was noted in the one-day treated group. The degree of inhibition was reduced over time and by day 14, no inhibitory effect was observed in the 1 and 3 mg/kg-day. 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*. (2000) suggested that the regulations of thyroidal iodide uptake and serum T4 are rapid in rats and can compensate for the anti-thyroid actions of perchlorate at low doses.

**Table 6. 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 * (%) |
|---|---|---|---|
| 2 hours | Control ** | 24.4 | - |
| | 0.01 | 21.3 | 13 |
| | 0.1 | 18.6 | 24 |
| | 1 | 7.4 [a] | 70 |
| | 3 | 3.0 [a] | 88 |
| 6 hours | Control ** | 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 ** | 55.0 | - |
| | 0.01 | 49.2 | 11 |
| | 0.1 [a] | 39.2 | 29 |
| | 1 | 24.7 [a] | 55 |
| | 3 | 10.0 [a] | 82 |

* Percent inhibition = (control mean – dose mean) x 100 / (control mean)
** Dosed with $^{125}$I with carrier only (33 µg/kg)
[a] Significantly different from control at $p<0.05$

**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 the 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 adenomatic 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 1000 ppm potassium perchlorate, 1000 ppm potassium iodide, or 1000 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 was not itself carcinogenic in this experiment.

Groups of male Wistar rats were exposed for two years to potassium perchlorate in drinking water at concentrations of 0 or one percent (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 at intervals of 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 interval of examination. At 40 days, the exposed rats developed 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 period of 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 tumors of the thyroid gland. Twenty untreated control rats had no thyroid gland tumors.

Pajer and Kalisnik (1991) treated groups of female BALB/c mice with 0 or 1.2 percent sodium perchlorate in drinking water for 46 weeks. One group of control and one group of perchlorate treated mice were irradiated with a total of 4 Gy of ionizing radiation (gamma rays) over a period of five days. The perchlorate dose to the treated mice is approximately 2,100 mg/kg-day based on standard default assumptions about body weight and water consumption. Perchlorate treatment alone caused hypothyroidism with hypertrophic and hyperplastic thyroid epithelial cells as well as pituitary thyrotropic cells. Irradiation alone caused no uniform changes in the structure or function of these 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 and after perchlorate with irradiation. The incidences of these carcinomas are shown in Table 7 below.

**Table 7. Carcinoma incidence in female BALB/c mice treated with sodium perchlorate in drinking water (Pajer and Kalisnik, 1991)**

| Treatment group | Thyroid follicular cell carcinoma incidence |
|---|---|
| Control (tap water), unirradiated | 0/22 |
| Control (tap water) + irradiation (4 Gy) | 0/22 |
| Perchlorate in water (1.2%) | 5/6 * |
| Perchlorate (1.2%) + irradiation (4 Gy) | 14/14 * |

* Statistically significant, Fisher exact test, $p<0.001$.
Gy = Gray, the SI unit for measuring absorbed ionizing radiation.

The data indicate perchlorate caused thyroid follicular cell carcinomas in the treated mice. Because of the small numbers of surviving mice, it is not possible to say whether irradiation enhanced this effect.

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) determined the increase in tumor incidence to be treatment related.

In a number of subchronic drinking water perchlorate studies, increased thyroid follicular cell hypertrophy and hyperplasia were observed in some of the treated animals (Caldwell *et al*., 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998a, 1999, 2001; Keil *et al*., 1998). Details of the study results have been presented and discussed in the U.S. EPA (2002) risk assessment. Summaries of these data sets are provided in sections on "Subchronic Toxicity" and "Developmental and Reproductive Toxicity." These 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.

## *Toxicological Effects in Humans*

The major adverse health effects of perchlorate at low dosages are associated with disruption of thyroid hormone balance. These effects are similar to those caused by iodine deficiency. Some of the adverse health effects of iodine deficiency are discussed in the "Sensitive Subpopulation" section under "Dose-Response Assessment: Noncarcinogenic Effects."

### Acute Toxicity

The acute lethal oral dose of perchlorate for an adult human is 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[3] in humans, and most of the prior data on perchlorate effects on humans are in patients with this disease. 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 as long as 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; it appears that one patient was followed for 22 weeks.

Morgans and Trotter (1960) reported that 3 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)

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[3] 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.

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.

**Chronic Toxicity**

Connell (1981) reported a case of a female Graves' disease patient who was treated with 200 mg/day perchlorate for 22 years with good control of the disease, and no apparent adverse effects.

**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 6 weeks, and no other abnormalities were observed. Several key parameters are not provided in the paper: detailed dosage information, what time during the gestation period perchlorate treatment was given, thyroid function of the newborns, and the neurological as well as behavioral development of the babies. Furthermore, interpretation of the result is made difficult by the fact that the women were suffering from thyrotoxicosis (excess quantities of thyroid hormones).

A preliminary health review of a potentially exposed area of Rancho Cordova, CA by the California Department of Health Services (DHS, 1997) analyzed several state databases for possible perchlorate-related adverse health effects during the suspected years of drinking water contamination within the area or region most likely exposed. The analysis of newborn thyroid hormone data for the period 1985 through 1996 did not indicate a positive correlation between residence in the potentially-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. U.S. EPA (2002) evaluated the data and suggested that due to the low prevalence rate of thyroid disorders in neonates (1:30,000 to 1:100,000), studies with larger sample sizes may be necessary to detect these subtle effects.

Using similar data from a statewide newborn screening registry, Schwartz (2001) evaluated the serum T4 and TSH levels of all newborns in California during 1996. All infants were screened by their serum T4 levels; the samples of those with a low T4 (i.e., less than or equal to 9 mg/dL and the next lowest 5 percent of the values in each tray of

samples) were further analyzed for TSH levels. Perchlorate exposure was estimated based on the following information: postal zip code, concentration of perchlorate measured in underground water sources sampled between February 1997 and June 2000, water source production, water purchases and sales, and characteristics of the water distribution system. After adjustment for gender, ethnicity, multiple birth, birth weight, and age at time of blood sample (in 6-hr increments), and separation of infants into groups whose mothers were presumably exposed to low (1-2 ppb), medium (3-12 ppb), and high (≥13 ppb) levels of perchlorate in their drinking water, the study found statistically significant associations between putative exposure and serum thyroid hormone levels (T4 and TSH) (Table 8). Substantial birth weight, gender, ethnicity, age at sampling, and birth multiplicity effects were observed for T4, and smaller effects for TSH. The author noted that 90 percent of the variability in T4 and TSH infant hormone levels remained unexplained after controlling for all the parameters described. Two variables not controlled, gestational age and laboratory measurement variability, may explain some of the variability not already accounted for in the model. Among the ecological studies described in this section, this study controlled for most of the important variables and found that even an extremely low level of perchlorate exposure through drinking water consumption was associated with a decreased serum T4 in newborns in California.

**Table 8. Perchlorate exposure and thyroid function in California neonates, 1996; infant characteristics versus perchlorate levels in drinking water (Schwartz, 2001).**

| | **Perchlorate concentration** | | | | **Test statistic p value (df)** |
|---|---|---|---|---|---|
| | **None (0 ppb)** | **Low (1-2 ppb)** | **Medium (3-12 ppb)** | **High (≥13 ppb)** | |
| Number of infants (%) | 255,382 (49.5) | 127,041 (24.6) | 131,483 (25.5) | 1,945 (0.4) | |
| Female, % | 48.8 | 48.8 | 49 | 49 | |
| T4, mg/dL Mean (SD) | 170.9 (50.9) | 162.1 (48.7) | 160.6 (48.3) | 150.5 (44.0) | F=1,649.6 p<0.0001 (3) |
| TSH, µU/mL Mean (SD) | 7.6 (18.8) | 7.6 (19.8) | 7.7 (19.4) | 7.9 (4.6) | F=0.05 p=0.9 (3) |

SD = standard deviation
df = degree of freedom

Crump *et al*. (2000) studied a total of 162 school-age children (6-8 years old) and 9,784 newborns in three proximate cities in northern Chile that have different concentrations of perchlorate in drinking water: Taltal (100 to 120 µg/L), Chañaral (5-7 µg/L), and Antofagasta (non-detectable: <4 µg/L). School-age children from all three cities demonstrated evidence of normal dietary iodine ingestion as reflected by their urinary

iodine concentrations. Approximately 25 separate water sources were sampled in each study city. Water samples were taken from portable water faucets at participating schools, homes of students, and public buildings located near the schools. Crump *et al*. (2000) reported that mean levels of TSH, T4, free T4, and T3 of the school-age children were very similar among 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). However, the high background goiter prevalence rate in Antofagasta represents an unexplained, confounding variable.

Crump *et al*. (2000) also found that schoolchildren with lifelong residence in Taltal were 5 times more likely to report a family history of thyroid disease compared with schoolchildren with lifelong residence in Antofagasta, adjusted for age, sex, and urinary iodine (Table 9). 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 as having 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. Reasons for these observations are yet unknown. Crump *et al*. (2000) speculated that the findings might reflect a change in exposure patterns over the past several generations. Because iodized salt was not introduced in the region until 1982, it is possible that a combination of low iodine intake and the relatively high perchlorate exposures in Taltal was sufficient to cause thyroid problems in past decades.

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 the study reported by 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 3 µg/kg-day. It must be emphasized that in addition to perchlorate exposure, a low dietary iodine intake might have also contributed to the increased thyroid problems reported in the residents of Taltal.

**Table 9. Odds ratios for association between self-reported family history of thyroid disease* among schoolchildren and city of residence** (from Crump *et al*., 2000).**

| | Schoolchildren with less than lifelong residence (n=162) | | Schoolchildren with lifelong residence (n=127) | |
|---|---|---|---|---|
| **City** | **Odds ratio** | **95% confidence interval** | **Odds ratio** | **95% confidence interval** |
| 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 |

* Direct relative (parent, sibling, grandparent, great-grandparent, aunt, uncle, or cousin) with history of goiter, hypothyroidism, or subtotal thyroidectomy.

** Adjusted for age, sex, and urinary iodine; excluded one child with autoimmune hypothyroidism.

No cases of congenital hypothyroidism were detected in cities with detectable perchlorate in drinking water (Taltal and Chañaral). Among newborns analyzed in the study, seven presumptive cases of congenital hypothyroidism (TSH $\geq$ 25 µU/mL) were detected, all originating in Antofagasta, corresponding to an incidence rate in Antofagasta of 1 per 1270 newborns. The national average rate of congenital hypothyroidism in Chile between 1992 and 1999 is 1 per 3484 newborns screened. Maximum TSH levels observed in Chañaral and Taltal were 17.1 µU/mL and 13.5 µU/mL, respectively. Adjusted for sex and age, linear regression comparisons of log TSH of the newborns by city showed that average log TSH in Taltal was significantly lower compared with those of the other two cities. However, for the group of newborns sampled on day 1-2, the mean and the median TSH levels of Taltal (4.2±1.2 µU/ml and 4.2 µU/ml) were higher than those of Chañaral (3.2±3.5 µU/ml and 1.9 µU/ml) and Antofagasta (3.2±1.9 µU/ml and 2.7 µU/ml).

Low levels of perchlorate (4 to 16 µg/L) have been detected in the drinking water supplies of six counties in California (Los Angeles, Orange, Riverside, Sacramento, San Bernardino, San Diego) and one in Nevada (Clark). Lamm and Doemland (1999) evaluated the congenital hypothyroidism incidence rates of the seven counties and compared them with the state rates. All infants were screened by their serum T4 levels; those with a low T4 (i.e., less than the 10 percentile) were further screened for high TSH levels. An infant is considered to be potentially congenitally hypothyroid if the serum TSH $\geq$ 25 µU/mL. Infants that are judged positive on this test are diagnosed by a physician to confirm whether they are suffering from congenital hypothyroidism.

County-specific, ethnicity-specific data for the two states were obtained for 1996 and 1997. Within the seven counties, nearly 700,000 newborns were screened. In all, 249 cases were identified, where 243 were expected based on the state incidence rate, for an overall risk ratio of 1.0 (95 percent confidence interval, 0.9 to 1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. Based on these results, Lamm and Doemland (1999) concluded that the study did not indicate an increase in the incidence of congenital hypothyroidism with the reported perchlorate levels. Although Clark County of Nevada obtains nearly all its water from a source (Lake Mead) that is known to be contaminated with perchlorate, the six California counties obtain their water from multiple sources and many of them are not contaminated with perchlorate. Because of this, there is a significant uncertainty in the estimation of the level of perchlorate exposure in the California counties. Nevertheless, it is noted that out of the 36,016 newborns screened in Clark County between 1996 and 1997, seven cases were identified, where 8.3 cases were expected. The risk ratio was 0.8; 95 percent confidence limits ranged from 0.34 to 1.74.

In a related study, Lamm and his colleagues compared serum T4 levels of newborns (1 to 4 days of life) from the city of Las Vegas, Clark County, Nevada, which has perchlorate in its drinking water, and those from the city of Reno, Nevada, which does not (detection limit, 4 µg/L) (Li *et al*., 2000a). A total of 17,308 newborns from Las Vegas and 5,882

newborns from Reno during the period April 1998 through June 1999 were included in the study. Drinking water perchlorate levels measured monthly for Las Vegas during the study period ranged from non-detectable to 15 µg/L. Li *et al*. (2000a) reported that they observed no differences in the mean blood T4 levels (approximately 17 µg/dL) of the newborns from the two cities. It is important to note that variables, such as age at time of blood sampling, birth weight, and ethnicity, that have been shown to be associated with serum T4 levels in the study reported by Schwartz (2001), were not controlled for in this study.

Based on the perchlorate concentration data of Las Vegas for the 9 months preceding the study period, Li *et al*. (2000a) also estimated the cumulative prenatal perchlorate exposure of the newborns. The Las Vegas newborns during the study period would have had maternal perchlorate exposures that ranged between 9 ppb-months and 83 ppb-months; the Reno newborns during this period were presumed to have had 0 maternal ppb-months. For each month, the difference between mean neonatal T4 levels of the two cities was examined, along with the cumulative maternal perchlorate exposure. Linear regression analysis showed no evidence of an association (slope = -0.0003; $R^2$ = 0.002). However, it is not clear how the cumulative exposures were calculated, especially the statistical treatment of non-detects.. As discussed earlier, serum T4 and TSH levels of neonates may not be the most sensitive end-points or indicators of perchlorate exposure. For women with iodine deficiency or marginal iodine deficiency, the additional stress due to pregnancy can cause low maternal T4, which may in turn impair the brain development of the fetus.

In another study, Li *et al*. (2000b) studied neonatal blood TSH levels sampled between December 1998 and October 1999 in Las Vegas (with up to 15 ppb perchlorate in drinking water) and in Reno (with no perchlorate in drinking water). Serum TSH levels were determined on screening samples that were below the $10^{th}$ percentile on T4 in each daily batch of samples collected throughout the state. The study samples were from newborns in their first month of life (excluding the first day of life, when TSH levels are unstable) with birth weights of 2.5 – 4.5 kg. The authors found neonatal TSH levels were not associated with perchlorate exposure of less than or equal to 15 ppb (p=0.97). However, the lack of control of a number of variables, such as age at sampling, birth weight, and ethnic origin, that are known to affect serum T4 and TSH levels in infants, makes interpretation of the results difficult.

Brechner *et al*. (2000) recently reported a study that showed an association between low-level perchlorate exposure and serum TSH levels in newborns. They compared serum TSH levels of newborns in Yuma, a city that obtains its public drinking water entirely from the Colorado River below Lake Mead, with newborns in Flagstaff, a city that obtains none of its public drinking water from the Colorado River below Lake Mead. Lake Mead is known to have perchlorate contamination. However, no useful water monitoring data were available for Yuma and Flagstaff during the study period (between 1994 and 1997). This is because the detection limit of perchlorate in water was 400 ppb during that period of time. Since March 1997, the detection limit of perchlorate has been improved to approximately 4 ppb. In August 1999, U.S. EPA reported that perchlorate

levels in Yuma were 6 ppb in both raw water and finished drinking water (Brechner *et al*., 2000).

Brechner *et al*. (2000) found that median newborn TSH levels in Yuma were significantly higher than in Flagstaff. According to Brechner *et al*., this remained true after adjusting for factors known or suspected to affect serum TSH levels in newborns, including age in days at measurement of the first TSH level and race/ethnicity.

However, the major factor influencing the TSH levels was the time after birth, which was significantly different in the two cities. Also, only about 10 percent of all the blood samples were analyzed for TSH and the selection procedure was not random; the statistical biases thus introduced might have affected the results.

As described above, several published and unpublished ecological studies report investigations of the relationship between perchlorate in drinking water and thyroid function in newborns and children. There are several challenges regarding the interpretation of these study results. First, there are considerable uncertainties in the estimation of perchlorate exposure in all the studies. The level of contamination of an area or region was often estimated based on only a few samples. It was assumed that the concentration of perchlorate in the water in an area or region could serve as a proxy for individual exposure. Second, some confounding factors were either not controlled or only partially controlled for in the studies. For example, in the analyses of thyroid hormone levels in Las Vegas and Reno newborns (Li *et al*., 2000), ethnic differences were not accounted for. In studies reported by DHS (1997) and Brechner *et al*. (2000), the control on the age at sampling was not adequate. As serum T4 and TSH levels are known to change significantly during the first 24 hr after birth, knowing the age at sampling (in hours) is very important in this type of study. Finally, small sample size and relatively low prevalence rate of the event of interest might have severely limited the sensitivity of the studies.

There is a concern about the way the neonate blood samples were selected for TSH determination in the studies reported by Schwartz (2001), Brechner *et al*. (2000), and Li *et al*. (2000b). In all these studies, TSH levels were only determined on screening samples that were below the $5^{th}$ or the $10^{th}$ percentile on T4 in each daily batch of samples collected. Certain bias might have been introduced by this sampling procedure as the selection was not random and serum T4 level is not well correlated with serum TSH level, especially during the first 48 hr of life (Figure 4).

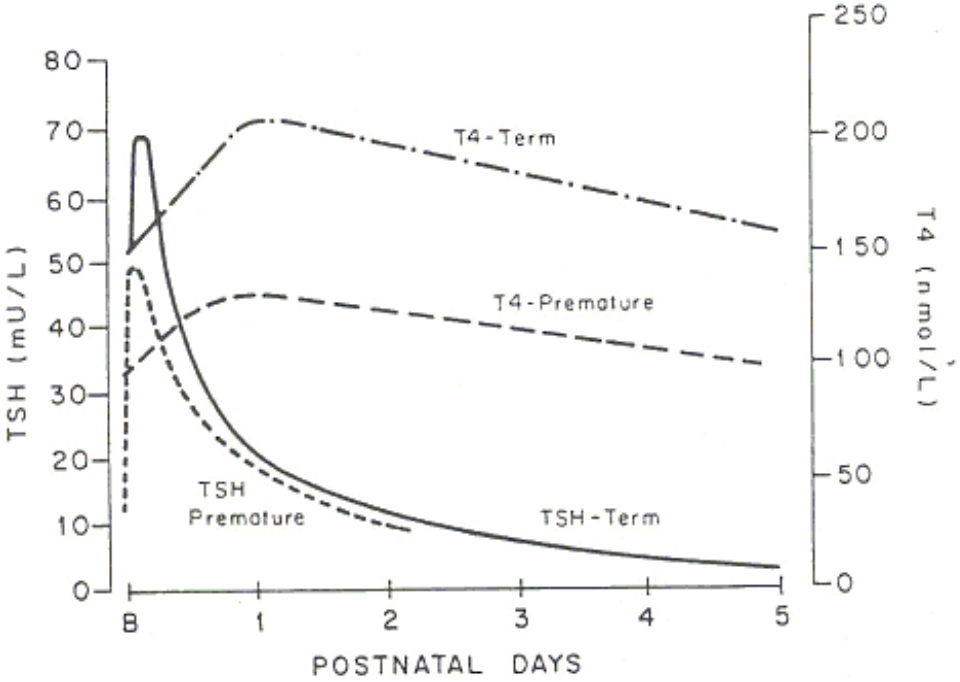


Figure 4. Changes in serum TSH and T4 concentrations in full-term and premature infants during the first five days of life. The neonatal TSH surge peaks at 30 minutes in both preterm and term infants and is followed by a progressive increase in serum T4 concentrations, peaking at 24 to 36 hours (from Fisher and Klein, 1981).

**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 IgG and IgM were measured by enzyme-linked immunoassays after culture 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).

**Endocrine Toxicity**

Stanbury and Wyngaarden (1952) 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 iodine, 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 $I^{131}$ was given. The accumulation of this isotope in the neck was recorded at frequent intervals until it was leveling off. At this point, quantities of potassium perchlorate 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 $I^{131}$ was incomplete, but doses of 100 mg caused a fall in counting rates nearly 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 iodine. 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, it is equivalent to an oral dose of 31 μg/kg.

A LOAEL is not identified for this experiment because of the following reasons: (a) the number of patients per dose group is not known, (b) 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 $I^{131}$. 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 a period of observation of only five hours was possible after the tracer. For the two patients with 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 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 $I^{131}$ 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 of the following reasons: (a) there were only two subjects that completed the experiment, (b) acute exposure, and (c) the patients suffered from a thyroid disease which might have affected iodide uptake by the thyroid.

Godley and Stanbury (1954) 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 iodine 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.

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 one of the 12 infants born to the mothers. They also reported that the enlarged thyroid returned to normal size in 6 weeks, and no other abnormalities were observed.

Bürgi *et al*. (1974) studied the effects of perchlorate treatment on the release of endogenous iodine 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) studied five healthy male volunteers 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 potassium 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 free T4 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. It is interesting to note that Brabant *et al*. (1992) found the perchlorate treatment significantly reduced intrathyroidal iodine concentration 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. The LOAEL for thyroid enlargement in humans is estimated to be 1.3 mg/kg-day.

Lawrence *et al*. (2000) 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 during waking hours for 14 days. Baseline serum TSH, total T4, total T3, 24-hour thyroid $I^{123}$ 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 $I^{123}$ 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 10). 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 11). Because iodide and perchlorate compete for the same receptor site on the sodium-iodide symporter (Wolff, 1998), a high dietary iodide intake is likely to reduce the impact

of perchlorate on the thyroid. It is also interesting to note that there was no statistical difference in serum perchlorate levels measured after 7 days and 14 days of exposure, indicating that there was no apparent accumulation of perchlorate in the subjects over that period of time.

**Table 10. 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 | $T_4$ (µg/dL) | $T_3$ (ng/dL) | TSH (µU/mL) |
|---|---|---|---|
| Baseline | 6.6±0.4 | 136±6 | 1.05±0.14 |
| Day 7 during perchlorate ingestion | 6.7±0.4 | 140±8 | 1.00±0.17 |
| Day 14 during perchlorate ingestion | 6.6±0.5 | 151±6 | 0.96±0.12 |
| 14 days after perchlorate was discontinued | 6.5±0.5 | 157±9 | 1.23±0.17 |

values are mean±standard error

**Table 11. 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 during perchlorate ingestion | 7.7±0.8 | 0.61±0.02 | 233±49 | 6.2±0.34 |
| Day 14 during perchlorate ingestion | 7.5±1.0 | 0.59±0.02 | 385±123 | 6.4±0.37 |
| 14 days after perchlorate was discontinued | <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 $I^{123}$ uptakes at all three time points. In each instance, 150 µCi $I^{123}$ was administered to a subject and thyroid iodide uptake was measured at 4, 8, and 24 hours. They reported that the average decrease below baseline values over all three time points was 38 percent. Two weeks after perchlorate was discontinued, the 24-hour thyroid $I^{123}$ uptakes were significantly higher than baseline at 4, 8, and 24 hours (Table 12).

**Table 12. Thyroid $I^{123}$ 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 $I^{123}$ uptake -baseline (% of dose) | Thyroid $I^{123}$ uptake 14 days on perchlorate (% of dose) | Thyroid $I^{123}$ 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.

Using the data reported by Lawrence *et al*. (2000) and assuming an adult body weight of 70 kg, a LOAEL of 0.14 mg/kg-day can be identified. Using an uncertainty factor of 10, a NOAEL of 14 µg/kg-day is estimated.

In a follow-up study, Lawrence *et al*. (2001) administered a daily oral dose of 3 mg to a group of eight healthy male volunteers for 14 days. They reported that the mean 8-hr thyroid radioactive iodine uptake decreased from 13.1 percent to 11.8 percent, during perchlorate ingestion. Similarly, the 24-hr thyroid radioactive iodine uptake decreased from 16.1 percent to 14.5 percent, a small but insignificant 10 percent decrease. Assuming a default body weight of 70 kg, the dose used in this study is estimated to be 0.043 mg/kg-day. Thus, a NOAEL of 0.043 mg/kg-day can be derived from the study.

Stanbury and Wyngaarden (1952) showed that potassium perchlorate doses as low as 3 mg (equivalent to 2.2 mg perchlorate) caused detectable, but incomplete, release of iodide from the thyroid of patients suffering from Graves' disease. According to these data, an oral dose as low as 31 µg/kg perchlorate still caused iodine release from the thyroid.

Greer *et al*. (2000, 2002) described two studies in which daily oral doses of perchlorate ($ClO_4^-$) dissolved in 400 ml 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 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 $I^{123}$ thyroid uptakes was performed prior to perchlorate exposure (baseline), on exposure days 2 and 14, and on post-exposure day 15. Expressed as a percentage of baseline, mean (±SE) 24-hour $I^{123}$ uptakes in the 0.02, 0.1, and 0.5 mg/kg dose groups (two study results combined) were 83 (5.6), 59 (3.5), 31 (2.6) on exposure day 2; 84 (4.1), 55 (3.9), 33 (3.8) on exposure day 14; and 105 (5.5), 107 (9.1), 105 (9.4) 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. Greer et al. (2002) found no change in serum levels of thyroid hormones (T3, T4, and free T4) in all dose groups throughout the 2-week exposure period. They found decreased serum TSH levels in volunteers exposed at 0.5 mg/kg-day for 2 weeks.

$I^{123}$ thyroid uptake measurements were taken prior to perchlorate exposure (baseline), on exposure day 14, and on post-exposure day 15 for the lowest dose group, 0.007 mg/kg-day. Expressed as a percentage of baseline, mean (±SE) 24-hour $I^{123}$ uptakes in this group were 98 (8.3) on exposure day 14 and 100 (8.4) on post-exposure day 15. Greer *et al*. (2002) also estimated that a perchlorate dose of 0.16 mg/kg-day and 3.9 mg/kg-day would inhibit thyroidal iodide uptake by 50 percent and 100 percent, respectively. Based on this study, a LOAEL of 0.02 mg/kg-day and a NOAEL of 0.007 mg/kg-day can be identified for the inhibition of thyroidal iodide uptake in humans.

Gibbs *et al*. (1998) monitored triiodothyronine resin uptake (T3U), total serum T4, the free T4 index (FTI) and serum TSH levels of 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, Gibbs *et al*. (1998) 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 humans (5-9 days) (U.S. EPA, 1998b), it would be very unlikely that T4 would exhibit a change in serum 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 measures of thyroid function (T3U, T4, FTI, and TSH levels). However, the tenure of the workers ranged from 1 to 27 years (Gibbs *et al*., 1998), while thyroid hormone levels are most likely to be affected by relatively recent perchlorate exposures (probably in the range of 1-3 months). 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) conducted 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. Lamm *et al*. (1999) 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 13). Based on these data, a NOAEL of 0.48 mg/kg-day (33.6 mg/day divided by 70 kg) can be estimated. However, it should be noted that 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 hr), 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.

**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). Mechanism of this blood disorder is not known. The use of perchlorate to treat Grave's 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 end-point.

**Table 13. Perchlorate exposures and thyroid-function parameters, by plant and exposure groups (adapted from Lamm *et al.*, 1999).**

| Group | Total airborne perchlorate exposure (mg/day) | Respirable airborne perchlorate exposure (mg/day) | Absorbed dose, derived from urinary perchlorate levels (mg/shift) | 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) |

Values are mean±standard deviation
n = sample number

**Carcinogenicity**

A search of the literature found no carcinogenicity studies on humans exposed to perchlorate.

## DOSE-RESPONSE ASSESSMENT

### *Noncarcinogenic Effects*

Animal data

As discussed earlier in this document, one of the main effects of perchlorate exposure, especially at low doses, is the disruption of thyroid hormone regulation. This mode of action is supported by a number of animal study results that showed perchlorate inhibits thyroidal iodide uptake, changes serum T3, T4, and TSH levels, causes thyroid enlargement, induces thyroid follicular cell hypertrophy and hyperplasia, and increases thyroid tumors. U.S. EPA (2002) in the draft perchlorate risk assessment has performed a comprehensive review and evaluation of the animal toxicity data. A brief summary of U.S. EPA's evaluation is given below.

Rodents are found to be highly sensitive to the anti-thyroid effects of perchlorate, significant changes in thyroid and pituitary hormone levels were observed even at the 0.01 to 0.1 mg/kg-day dose range. Results from two developmental toxicity studies showed that the thyroid and the brain of rat pups are susceptible to perchlorate exposure. Increased colloid depletion of the thyroid and thyroid hypertrophy were found in rat pups exposed to perchlorate in utero and after birth. Using benchmark dose analyses, U.S. EPA estimated that the $BMDL_{10}$s of these two end-points ranged from 0.008 to 0.28 mg/kg-day. In a similar study, morphometric changes of several brain regions were noticed in the neonatal rats exposed to perchlorate in utero and after birth. Though no simple dose-response relationships can be identified, brain morphometric changes are evident at ammonium perchlorate doses as low as 0.01 mg/kg-day. U.S. EPA (2002) took a weight-of-evidence approach to the entire animal toxicity data base and determined a LOAEL of 0.01 mg/kg-day (ammonium perchlorate). This is equivalent to a LOAEL of 0.0085 mg/kg-day, based on perchlorate anion alone.

Human data

According to the California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), in the development of PHGs OEHHA is required to consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult. There are data showing that

pregnancy can induce hypothyroidism in normal women (Crooks *et al*., 1967; Glinoer *et al*., 1990, 1992; Smyth *et al*., 1997; Caron *et al*., 1997; Brent, 1999), and pregnant women with insufficient iodide intake may be particularly susceptible to the adverse effects of perchlorate exposure.

Sensitive subpopulations

The most important and early effect of perchlorate exposure is its reduction of iodide uptake by the thyroid. For this reason, adverse health effects associated with iodide deficiency are discussed in this section, in addition to the perchlorate toxicity information, which is discussed in the sections under "Toxicological Effects in Humans."

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 iodine deficiency is influenced by age and sex, with maximal frequency in females during puberty and childbearing age (Figure 5).

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) as in non-pregnant women (19 percent). Crooks *et al*. (1967) suggested that the results can be explained by the fact that Icelandic diet is based on fish and contains a lot 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) suggested that in conditions of marginally low iodide intake, pregnancy constituted a goitrogenic stimulus. They followed prospectively a group of 606 healthy pregnant women in Brussels, Belgium, an area of marginally low iodide 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. Glinoer *et al*. (1990) 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, 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 14). They found that the average thyroid volume increased by 18 percent of the initial size determined at the beginning of the pregnancy. The increase was significant and thyroid size increased in a majority of women (73 percent). Goiter, defined as thyroid volume greater than 23 mL, was found in 9 percent of the cohort at delivery.

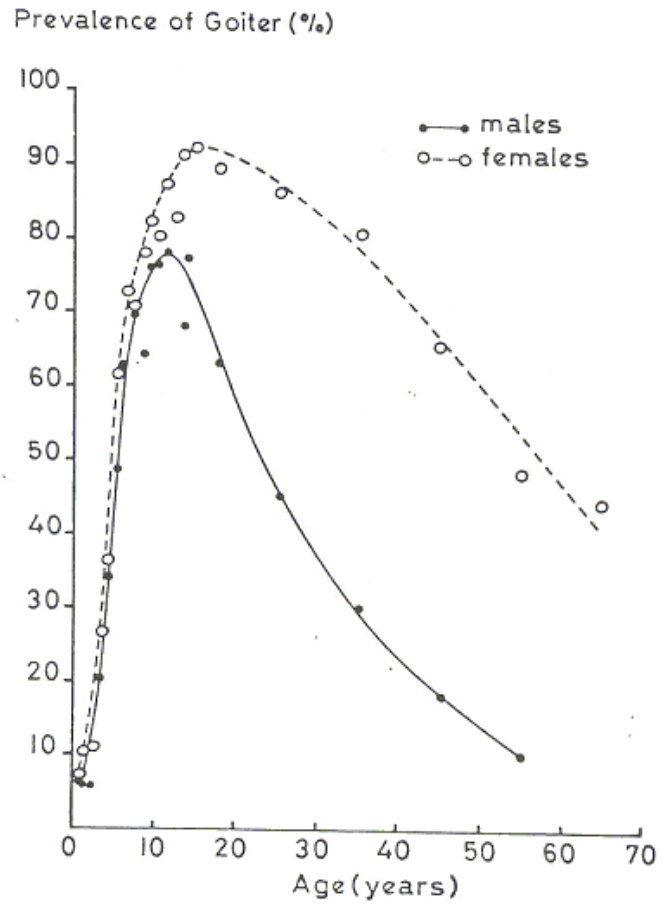


Figure 5. Changes in the prevalence of goiter as a function of age and sex in severe endemic goiter (Idjwi Island, Zaire) (from Delange, 1994).

**Table 14. 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 * |
| Delivery | 179 | 15.0±6.8 ** |

* $p < 0.03$
** $p < 0.001$

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 iodine deficiency state.

In a related study, Glinoer *et al*. (1992) monitored thyroid condition of pregnant women in an area without overt iodine deficiency, but with a marginal iodide supply (less than 100 µg/day in 80 percent of women). They found that maternal thyroid function was characterized at delivery by 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 also goiter formation in almost 10 percent of women.

Klein *et al*. (1991) measured TSH concentration in 2,000 sera obtained from two cohorts of consecutively examined pregnant women in Maine. The sera were obtained during the 15$^{th}$ to 18$^{th}$ week of gestation. Sera with TSH concentration 6 mU/L or more and control sera were subjected to further thyroid hormone analyses. The control sera were those with TSH concentrations below 6 mU/L that were in the test sequence immediately before and after the sera with TSH concentrations of 6 mU/L or over. Klein *et al*. (1991) reported that TSH concentrations above 6 mU/L or over were found in the sera of 49 women, 2.5 percent of the pregnant women. They can be considered as having compensated thyroid disease, although some may have been hypothyroid. Results of thyroid-related measurements of both groups are shown in Table 15. Six women with elevated TSH concentrations (range 6.9-54 mU/L) had both a free T4 concentration and a T4/TBG ratio and/or a T4 concentration more than two standard deviations below the respective control means, meeting the study criteria for thyroid deficiency, and thus giving a prevalence of 0.3 percent.

**Table 15. Mean thyroid related measurements in pregnant women, and age and gestational duration (from Klein *et al*., 1991).**

| | Age (years) | Gestation (weeks) | TSH (mU/L) | T4 (nmol/L) | Free T4 (pmol/L) | TBG (mg/L) | T4/TBG (nmol/mg) |
|---|---|---|---|---|---|---|---|
| Control women (TSH<6 mU/L) (n=99) | 26.9±0.5 | 16.8±0.06 | 2.34 | 145±2.8 | 13.4±0.28 | 35.6±0.68 | 4.2±0.08 |
| High-TSH group (TSH>6 mU/L) n=49 | 29.1±0.8* | 16.8±0.12 | 10.02 | 137±5.1 | 11.5±0.44* | 37.2±1.03 | 3.7±0.12* |

* Statistically significant, p<0.05.

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 114 pregnant women during one of the three trimesters. Control values for thyroid volume were obtained from 95 pre-menopausal females. All subjects were from Dublin, Ireland, an area of moderate dietary iodide intake (median urinary iodine was 82 µg/day). All pregnant women studied delivered live-born, normally formed, singleton infants and received no iodide-containing supplements during their pregnancy. Smyth *et al*. (1997) 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 prospective study, Smyth *et al*. (1997) studied a group of 38 pregnant women. Ultrasound thyroid volume and urinary iodine excretion of 20 of the subjects were measured 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. The increase of thyroid volume, which occurred as early as the first trimester, was paralleled by increased urinary iodine excretion. Smyth *et al*. (1997) suggested that in an area of moderate dietary iodide intake, urinary loss during pregnancy may result in maternal thyroid enlargement.

In a prospective study, Caron *et al*. (1997) evaluated 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 free T4 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 (more than 10 µg/dL) urinary iodine (Figure 6). Goiter (thyroid volume greater than 22 mL) was present in 11 percent of the mothers. They concluded that in areas with a marginally low iodide supply, pregnancy constitutes a goitrogenic stimulus.

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 16. 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 of 30 percent increase (range 3 percent – 230 percent) in thyroid volume, with some subjects having more than twofold increase in their thyroid volume. This thyroid enlargement persisted and failed to revert completely even 3 months after delivery.

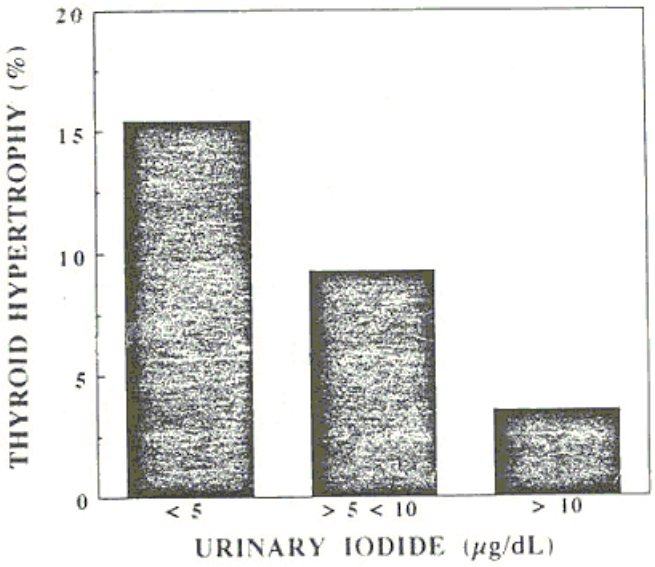


Figure 6. Percentage of maternal thyroid hypertrophy (thyroid volume > 18 ml) in relation to urinary iodine concentration during the first trimester of pregnancy (from Caron *et al*., 1997).

**Table 16. 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) * | 125 (106-142) * | 89 (81-98) § | 92 (82-101) § |
| Free T3 (pmol/L) | 3.9 (3.6-4.3) | 3.4 (3.1-3.7) * | 3.3 (3.0-3.7) * | 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) * | 11.7 (10.1-13.0) * | 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) * | 0.95 (0.60-1.36) § | 1.15 (0.74-1.58) § | 1.14 (0.81-1.61) § |
| Urine iodine (µmol/L) | 0.84 (0.60-1.09) | 0.91 (0.65-1.14) * | 0.98 (0.72-1.24) * | 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) * | 11.2 (8.9-13.8) * | 11.0 (8.3-14.2) * | 10.6 (8.6-13.7) * |

Results are median, * $p < 0.05$, § $p < 0.01$, 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 17).

**Table 17. Thyroid hormone dose requirement in pregnancy (from 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 |

There are two prospective studies showing that in an area of marginal iodide intake, iodide supplement can reduce the stress on the thyroid during pregnancy (Romano *et al*., 1991; Pedersen *et al*., 1993). 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 them had a normal pregnancy and no personal history of thyroid disease. They had a mean age of 27.1±3.8 years and a mean body weight of 61.6±4.9 kg 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 iodide supplement 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 18) only in group A, treated with iodide salt.

**Table 18. 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 * | 100.0±39.0 ** |
| Group B | 30.5±42.0 | 55.0±35.0 | 50.0±37.0 |

* $p<0.0001$.
** $p<0.01$ vs first trimester.

Thyroid volume did not change significantly throughout pregnancy in the group treated with iodide salt, whereas in the control group (Group B) it increased significantly ($p<0.0001$), with a mean increase of 1.6±0.6 ml (16.25 percent ± 6.29 percent) between the first and third trimester. Romano *et al*. (1991) concluded that an adequate dietary iodide intake is necessary to prevent the development of a gestational goiter, and iodine deficiency is the main causative cofactor 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 treated (28 subjects) and control groups (26 subjects). Before iodine supplementation was initiated, the measured variables were nearly identical in the two groups. Treated subjects received

200 µg iodide/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 ameliorated by iodide supplementation (Figures 7, 8, and 9). Iodine did not induce significant variations in serum T4, T3 or free T4. Pedersen *et al*. (1993) concluded that a relatively low iodide intake during pregnancy leads to thyroid stress, with increases in Tg release and thyroid size. It is important to note that even in the iodide-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 iodide supplement. 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[4] in elderly subjects. It was suggested that iodine deficiency during pregnancy or even during fetal life could be a factor of importance for the late development of thyroid autonomy.

However, a survey of the scientific literature shows that not all researchers found an association between pregnancy and enlarged thyroids. Long *et al*. (1985) 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 having 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.

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[4] Multinodular toxic goiter is usually found in older persons who had a goiter for a long time. Histologically, the nodules are follicular adenomas. The illness is characterized by suppressed TSH levels and marked elevation of T3 levels, with T4 levels showing a lesser increase. Antibodies against the TSH receptor and thyroid peroxidase are absent, in contrast to patients with Graves' disease.

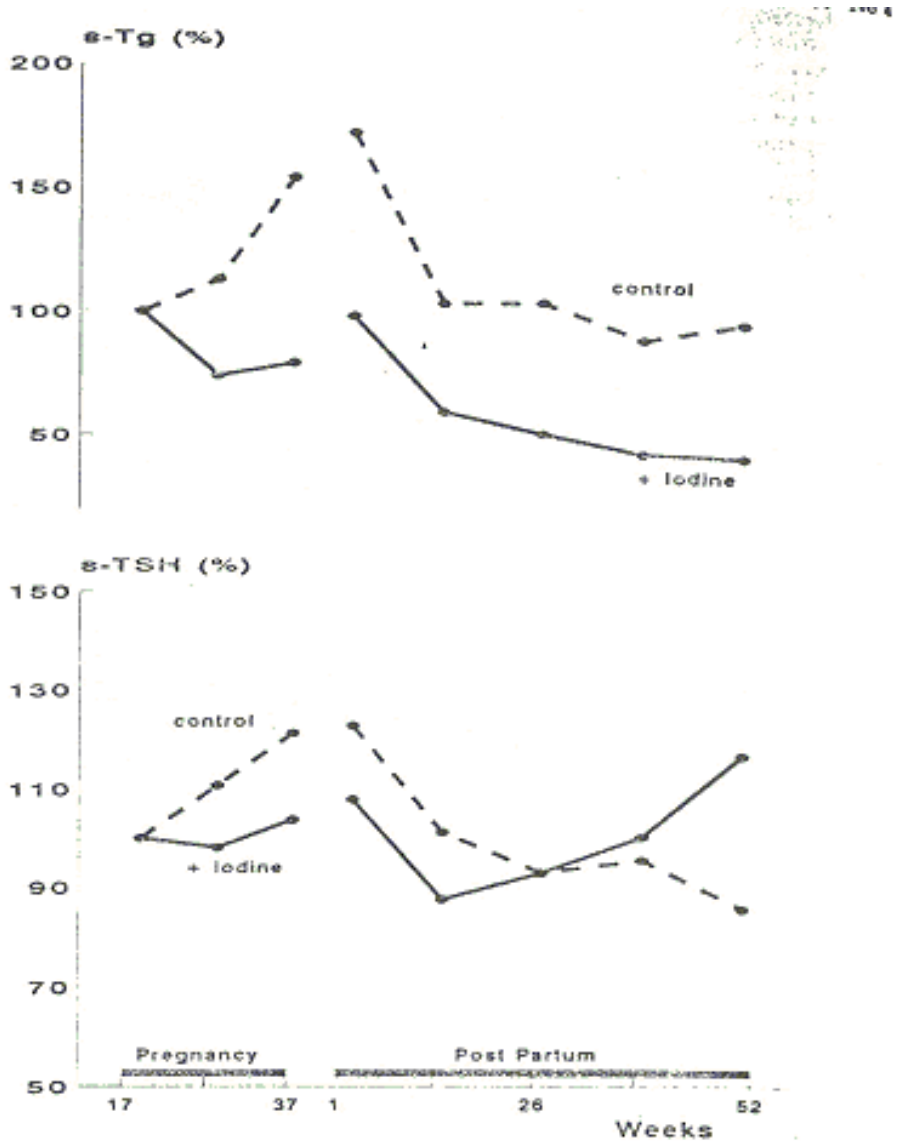


Figure 7. Serum Tg and TSH during pregnancy and for 52 weeks postpartum in women receiving iodide supplementation and control women, as a percentage of the initial values. Median values are shown. The increase in serum Tg during pregnancy in the control group was statistically significant ($p<0.01$), 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 iodide 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 iodide supplemented group ($p=0.29$, by Friedman's test). During the postpartum period, no significant TSH differences between the groups were found (from Pedersen *et al*., 1993).

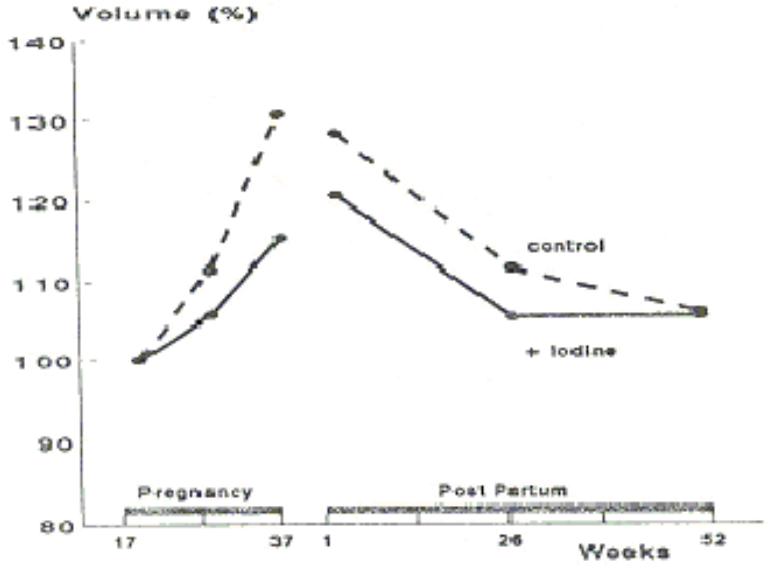


Figure 8. Median thyroid volume during pregnancy and 52 weeks postpartum in women receiving iodide supplementation and control women, as percentage of initial values. In both groups, significant increases during pregnancy and decreases during the postpartum period were found ($p<0.05$). The increase during pregnancy in controls was higher than that in the iodide-supplemented group ($p<0.05$) (from Pedersen *et al*., 1993).

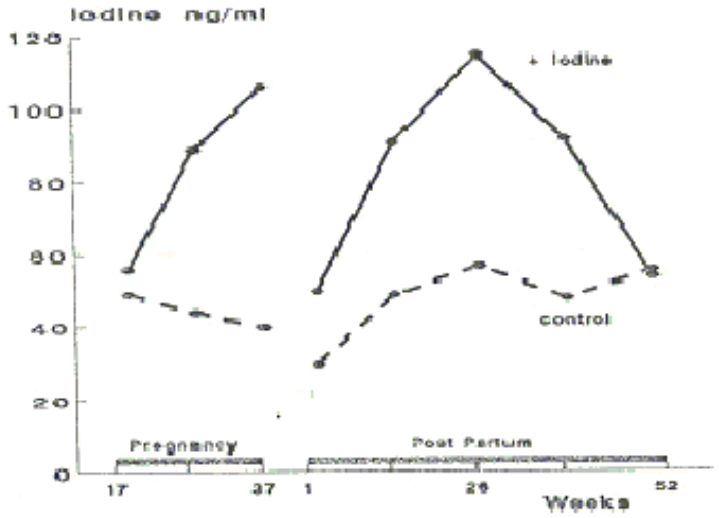


Figure 9. Iodine concentration in spot urine samples during pregnancy and for 52 weeks postpartum in women receiving iodide supplementation and control women. The last sample was obtained after iodide supplementation was stopped (Pedersen *et al*., 1993).

Levy *et al*. (1980) 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. They suggested that goiter in pregnancy should be considered to be a pathologic condition in an iodide-replete population. 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 iodide intake was adequate.

Almost 15 percent of women of childbearing age in U.S. have relatively low iodide intake

Urinary iodine (UI) concentrations are an indicator of the adequacy of iodide intake for a population. The median urinary iodine concentrations in iodine-sufficient populations should be greater than 10 µg/dL, and no more than 20 percent of the population should have urinary iodine concentration less than 5 µg/dL (WHO, 1994; as cited in Hollowell *et al*., 1998). Median urinary iodine concentrations (spot urine samples) from both the National Health and Nutrition Examination Surveys [NHANES I (1971-1974) and NHANES III (1988-1994)] indicate adequate iodide intake for the overall U.S. population, but the median (±standard error) concentration decreased more than 50 percent between 1971-1974 (32.0±0.6 µg/dL) and 1988-1994 (14.5±0.3 µg/dL) (Hollowell *et al*., 1998). Low urinary iodine concentrations (<5 µg/dL) were found in 11.7 percent of the 1988-1994 population, a 4.5-fold increase over the population in the 1971-1974 population. The percentage of people excreting low concentrations of iodine (urinary iodine <5 µg/dL) increased in all age groups.

In pregnant women, 6.7 percent, and in women of childbearing age, 14.9 percent had UI concentration below 5 µg/dL (Table 19). Because urinary iodine excretion reflects dietary iodide intake, 5 µg/dL is equivalent to an iodide intake of 68 µg/day (daily iodine intake [µg] = urinary iodine [µg/L] x 0.0235 x body weight [58 kg]) (NAS, 2001). This level of iodide intake is less than half of the 175-200 µg/day range recommended for pregnant women by the World Health Organization (Delange and Bürgi, 1989; as cited in Caron *et al*., 1997). It is also less than the estimated average requirement of 160 µg/day determined by the National Academy of Sciences (NAS, 2001).

Daily iodine intake is most closely estimated by the amount of iodine excreted in the urine in 24 hr. To compensate for the use of spot urine samples, the creatinine concentration was used to adjust for factors that might have affected the iodine

concentration in urine. An iodine/creatinine ratio of less than 50 µg/g is often used to indicate dietary iodine deficiency. If this approach is used, Hollowell *et al*. (1998) 1988-1994 survey data indicate 8.2 percent of women of childbearing age had inadequate dietary iodine intake.

In addition, there are data to indicate that there may be seasonal variations in dietary iodide intake. Hetherton *et al*. (1991) measured monthly urinary iodine excretion from consecutive patients (n=448) in a medical center in Dublin, Ireland for a year. They found a monthly variation in mean urinary iodine excretion, being lowest at 53 µg/g in July compared to a high of 104 µg/g in April. Similar differences were also observed in a group of schoolchildren (n=131) sampled both in summer (74±27 µg/g) and winter (138±78 µg/g) ($p<0.01$). Hetherton *et al*. (1991) reasoned that since milk consumption was the major source of dietary iodide, seasonal variation in milk iodide was also examined in the study area of Ireland. They found that the monthly variations in dietary milk paralleled those in urinary iodine excretion, being lowest at 44 µg/L in June and highest at 222 µg/L in February. If a similar seasonal variation in dietary iodide intake exists in the U.S., women pregnant during the months when dietary iodide intake is relatively low could be more susceptible to perchlorate exposure compared with those who are pregnant during the other months of the year.

**Table 19. Median concentrations of urinary iodine in U.S. women of child-bearing age (15-44 year) in 1988-1994, and percentage who had urinary iodine levels below 5 µg/dL or iodine/creatinine levels below 50 µg/g creatinine (Hollowell *et al*., 1998; NHANES III survey results).**

| | | Urinary iodine | | Iodine/creatinine | |
|---|---|---|---|---|---|
| | Sample number | median value | % < 5 µg/dL | median value | % < 50 µg/g |
| Total | 5405 | 12.8±0.4 | 14.9±1.1 | 113.1±3.2 | 8.2±0.9 |
| Known pregnant | 348 | 14.1±1.4 | 6.9±1.9 | 132.2±11.9 | 5.1±1.9 |
| Not pregnant | 5057 | 12.7±0.4 | 15.3±1.2 | 111.9±3.2 | 8.4±0.9 |

Mean±standard error

Adverse neurological development in infants born to mothers with iodine deficiency

The classical recommendation on dietary allowance of iodide is 100 µg/day for adolescents and adults (150 µg/day in pregnant and lactating women). It is 60-100 µg/day for children aged 1-10 years and 40 µg/day for infants aged 6-12 months (Delange, 1994). Iodine deficiency disorders range from the most severe form, endemic

cretinism, which is characterized by mental and growth retardation, rigid spastic motor disorders, and deaf mutism; to endemic goiter and less severe forms of brain damage. The impact of iodine deficiency differs depending on the age and life stage of the individual affected.

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 hypothyroidism during pregnancy risk causing neurologic damage in their 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 does reach the fetus (Vulsma *et al*., 1989) made it understandable that thyroid hormones are necessary for brain development during its early developmental period, 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. 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. Harakawa *et al*. (1989) reported that the administration of serum from rats with either hypothyroidism or hyperthyroidism induced malformations in rat-embryo cultures *in vitro*. 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 *et al*., 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.

In two animal developmental studies, ammonium perchlorate was administered to female Sprague-Dawley rats via drinking water at target doses between 0.01 and 30 mg/kg-day (Argus Research Laboratories, 1998a; 2001). 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 is not observed in any of the changes (Figure 3).

Many human studies have been published that demonstrate maternal thyroid deficiency during pregnancy affects neuropsychological development of the child. Man and Jones (1969) first reported that mild maternal hypothyroidism was associated with lower intelligence quotient scores (IQs) in 8-month-old infants. Hypothyroidism was defined 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 butanol extractable 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 butanol extractable iodine values who were not given adequate thyroid replacement therapy were "normal."

Glorieux *et al*. (1985) 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. In a later study, Glorieux *et al*. (1988) 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 20).

**Table 20. Mental outcome in infants with congenital hypothyroidism relative to newborn risk criteria (from Glorieux *et al*., 1988).**

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

* $p < 0.01$
** $p < 0.001$

Similar findings have been reported by Rovet *et al*. (1987), 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 on the basis of 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 21. 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 (Rovet *et al*., 1987).

**Table 21. Hormone levels at diagnosis in children with delayed and nondelayed skeletal maturity (from 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.
T4 level: 5.1 µg/dL is equivalent to 65.6 nmol/L.

Tillotson *et al*. (1994) 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. Severity of congenital hypothyroidism was assessed from the first quantitative T4 measurement after the positive screening test and before treatment (median age 17 days; range 0-114). The study showed that in children with congenital hypothyroidism and given early treatment there was a sharp threshold in intellectual outcome that divided them into two distinct groups – those with plasma T4 concentrations of less than 42.8 nmol/L (3.3 µg/dL) at diagnosis, who showed a global deficit in mean IQ of 10 points, and those with less severe congenital hypothyroidism, who showed no deficit.

Vermiglio *et al*. (1990) demonstrated that normal euthyroid children conceived and born to mothers exposed in a severe (area A) and less severe (area B) iodine deficiency region in northeastern Sicily showed a defective visual perceptual integrative motor ability. They studied 719 primary schoolchildren (366 males and 353 females) ranging from 6 to 12 years old (i.e., they had been conceived and born between 1975 and 1981). The prevalence of goiter in the schoolchildren and the daily urinary iodine excretion in the general population between 1976 and 1984 are given in Table 22.

**Table 22. Prevalence of goiter in the schoolchildren and daily urinary iodine excretion in the adults (measured between 1976 and 1984) residing in the study areas (from Vermiglio *et al*., 1990).**

| Study area | Total population | Prevalence of goiter in the schoolchildren (%) | Daily urinary iodine excretion (µg/day)* |
|---|---|---|---|
| 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) |

* Mean±standard deviation
The prevalences and daily urinary excretion were established between 1976 and 1979. The number of the observations is given in parentheses.

Area A vs area B: $\chi^2$ =112; p<0.001, t =2.43; p<0.05.
Area A vs area C: $\chi^2$ =111; p<0.000005, t =8.98; p=0.
Area B vs area C: $\chi^2$ =78; p<0.000005, t =10.55; p=0.

Variable degrees of thyroid enlargement were found in 205 of 719 (28.5 percent) children from both area A and area 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 area C (3.5 percent) (Table 23). The control group consisted of 370 age-matched schoolchildren from an iodine-sufficient goiter-free area (area C).

**Table 23. Number of defective, borderline, and nondefective schoolchildren assessed by the Bender Gestalt test (from Vermiglio *et al*., 1990).**

| Performance on Bender | 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) |

The percentage is given in parentheses. Performance score: defective, below –1 standard deviation from the average score of normal children of the same age; borderline, equal to –1 standard deviation from the average score; nondefective, higher than –1 standard deviation from the average score.

Statistical comparisons:

Defective

Area A vs area B: $\chi^2$ =2.75; p=0.87 (NS);

Areas A+B vs area C: $\chi^2$ =36.25; p<0.000001

Borderline

Area A vs area B: $\chi^2$ =1.22; p=0.27 (NS);

Areas A+B vs area C: $\chi^2$ =77.55; p<0.000001

In addition, 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). The Terman Merrill test of general intellectual aptitude was administered to 96 of 99 "defective" children and 62 of 124 borderline children from both areas A and B (Table 23). Ninety-one of 96 "defective" children (94.8 percent) had IQs lower than 90, as did 35 of 62 borderline (56.4 percent) children (Table 24).

**Table 24. Performance at the scale test (Terman Merrill) administered to schoolchildren with defective or borderline performance at the Bender Gestalt test (from Vermiglio *et al*., 1990).**

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

Statistical analysis: $\chi^2$ =52.1; p<0.0000005.

Despite the adverse effects observed, Vermiglio *et al*. (1990) found serum T3 and T4 levels of the children from area A and area B were within the normal range. These data suggest serum T3 and T4 are not good indicators of neurological damages caused by iodine deficiency. Vermiglio *et al*. (1990) hypothesized that fetal and postnatal hypothyroidism, maternal hypothyroxinemia, and iodine deficiency are the likely underlying causes of the observed defective neuromotor and cognitive abilities in schoolchildren.

Pop *et al*. (1999) reported that low maternal free T4 concentrations in apparently healthy women during early gestation implicate a significantly increased risk (RR=5.8) 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 and/or thyroid hormones. Maternal thyroid determinants (free T4, 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. Pop *et al*. (1999) found that children of women with free T4 levels below the $5^{th}$ (<9.8 pmol/L, n=11) and $10^{th}$ (<10.4 pmol/L, n=22) percentiles at 12 weeks' gestation had significantly lower scores on the Bayley Psychomotor Developmental Index scale at 10 months of age, compared to children of mothers with higher free T4 values (t-test, mean difference: 14.1, 95 percent confidence interval: 5.9-22 and 7.4, 95 percent confidence interval: 1.1-13.9, respectively). At 32 weeks' gestation, no significant correlations were found between thyroid hormone levels and test scores. Haddow *et al*. (1999) measured thyrotropin in stored serum samples collected from 25,216 pregnant women (during the second trimester) in Maine between January 1987 and March 1990. They then located 47 women with serum thyrotropin concentrations at or above the $99.7^{th}$ percentile of the values for all the pregnant women, 15 women with values between the $98^{th}$ and $99.6^{th}$ percentile, inclusive, in combination with low T4 levels. They used 124 matched women with normal thyrotropin levels as controls. Measurements of thyroid function of the women in the study are shown in Table 25. Haddow *et al*. (1999) then administered 15 tests to their seven-to-nine-year-old children, none of whom had hypothyroidism as newborns. The neuropsychological 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 children of the 62 women with high serum thyrotropin concentrations performed slightly less well on all 15 tests. Of the 62 women with thyroid deficiency, 48 were not treated for the condition during the pregnancy under study. The full-scale IQ scores of their children average 7 points lower than those of the 124 matched control children (P=0.005). Haddow *et al*. (1999) concluded that that even mild and probably asymptomatic hypothyroidism in pregnant women can adversely affect their children's subsequent performance on neuropsychological tests.

**Table 25. Measurements of thyroid function in the study women during pregnancy (from Haddow *et al*., 1999)*.**

| Variable | Women with hypothyroidism (N=62) | Control women (N=124) |
|---|---|---|
| Serum thyrotropin (TSH) level (mU/L) | 13.2±0.3 ** | 1.4±0.2 |
| Serum thyroxine (T4) level (µg/dL) | 7.4±0.1 ** (95.2 nmol/L) | 10.6±0.1 (136.4 nmol/L) |
| Serum free thyroxine (T4) level (ng/dL) | 0.71±0.1 ** (9.1 pmol/L) | 0.97±0.07 (12.5 pmol/L) |

* Values are geometric means ± the logarithmic standard deviation.

** <0.001 for the comparison with the control women.

To convert values of serum T4 from µg/dL to nmol/L or free T4 from ng/dL to pmol/L, multiply by 12.87.

Not all researchers have found an association between fetal hypothyroidism and impaired brain development. Several studies examined children exposed to antithyroid drugs such as carbimazole, propylthiouracil, or thiamazole[5] 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 powers of these studies are limited as they have relatively small sample size 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 where maternal T4 levels during the first and second trimesters were not known. It is possible that the treated women had normal T4 levels during 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 moderate iodine deficiency (Tuscany, Italy). Another group of 352 sex- and age-matched schoolchildren of a control iodine sufficient area was used as control. Goiter prevalence in the endemic and control areas was 51.9 percent and 5.6 percent, respectively. No significant differences in serum total T4, total T3, TSH levels between the endemic and control areas were found. Serum thyroglobulin values were significantly 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

[5] Thiouracils and imidazoles are two groups of antithyroid drugs that inhibit thyroid hormones production by interferring the iodination of tyrosine.

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.

New England Congenital Hypothyroidism Collaborative Program (1981) found that there was no correlation of eventual IQs with the severity of the 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. 336,000 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 IQs 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. Serum T4 levels at birth may not be a good indicator for neurodevelopment in early gestation.

Liu *et al*. (1994) examined IQs of eight children (Group 1) who were born to eight mothers that were hypothyroid during the first trimester of pregnancy. Maternal free T4 values at the fifth to 10$^{th}$ 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 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.

Liberman *et al.* (1998) 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. It is noted that the daily iodide intakes of the subjects were high, indicated by the relatively high average urinary iodine excretion (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. However, they also acknowledged that in iodine-deficient regions, maternal thyroid hormone deficiency is aggravated during pregnancy.

To summarize, study results of Pop *et al*. (1999) and Haddow *et al*. (1999) showed that even a relatively small decrease in maternal serum T4 and free T4 levels can have a negative impact on the neurodevelopment of the infant. Knowing that (1) women with marginally adequate iodide intake are susceptible to hypothyroxinemia and hypothyroidism during pregnancy; (2) a significant fraction (15 percent) of women in the child-bearing age in the U.S. can be considered iodine deficient (urinary iodine concentrations <5 µg/dL); and (3) exposure to perchlorate is likely to further reduce iodide uptake by the thyroid, OEHHA recommends that perchlorate exposure should be kept at a level that does not inhibit iodide absorption by the thyroid nor cause release of iodide from the thyroid.

A number of human studies (Lawrence *et al*., 2000, 2001; Greer *et al*., 2000, 2002) have documented the inhibitory effect of perchlorate on iodide uptake by the thyroid in adults. Greer *et al*. (2000, 2002) administered a daily oral dose of perchlorate in drinking water to groups of volunteers at 0.007, 0.02, 0.1, or 0.5 mg/kg-day for 2 weeks. At the end of the study, they found a significant reduction in thyroidal iodide uptake in the three highest exposure groups. Based on this study, a NOAEL of 0.007 mg/kg-day can be identified.

In two similarly designed studies, Lawrence *et al*. (2000, 2001) administered a daily dose of 10 mg or 3 mg of perchlorate in water to groups of male volunteers for 2 weeks. They reported that the reduction in thyroidal iodide uptake in the high-dose study was statistically significant while the reduction in the low-dose study was not. Details of the low-dose-study (Lawrence *et al*., 2001) are not reported. Based on this study a NOAEL of 0.043 mg/kg-day can be determined. This NOAEL is slightly higher than the LOAEL identified in the Greer *et al*. study (2002) of 0.02 mg/kg-day. Given the inherent variability in the measurements, we assume an actual NOAEL for human uptake in the lower range of these doses. Rounding up the lowest estimate from the Greer et al. studies (2000, 2002), OEHHA estimated a NOAEL of 0.01 mg/kg-day for the inhibition of thyroidal iodide uptake by perchlorate through the oral route.

This identified NOAEL is also supported by another study in humans. Stanbury and Wyngaarden (1952) reported that perchlorate doses as low as 2.2 mg (LOAEL = 0.031 mg/kg) caused detectable, but incomplete, release of iodide from the thyroid of patients with Graves' disease.

### *Carcinogenic Effects*

A search of the literature found no carcinogenicity studies on humans exposed to perchlorate.

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; 1999; 2001, and 1998d; Keil *et al*., 1998). In two chronic oral studies, perchlorate at relatively high concentrations (over 1,000 mg/kg-day) was shown to be tumorigenic 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 there were only six exposed mice that survived the whole study period in the study reported by Pajer and Kalisnik (1991). In a developmental study reported by Argus Research Laboratories (1999), thyroid follicular cell adenomas were 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 significant (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 data presented in the analysis, there are reasons to believe that perchlorate is a potential carcinogen in animals, causing thyroid tumors.

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 deals with the influence of protein carriers of thyroid hormones in the blood. In humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin, which 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 26, the estimated serum half-life of T4 is much shorter in rats (<1 day) than in humans (5-9 days). The much 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 more frequently develop thyroid cancer (U.S. EPA, 1998b).

**Table 26. 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 ≤ 2 × Female |
| Sensitivity to thyroid cancer | Female = 2.5 × Male | Male > Female |

The quantitative difference in the thyroid responses of humans and rodents to perchlorate is also evident in the data provided in this document. 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 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). Similar results have been reported at lower doses. 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 day 1, 2, 3, 4, 8, and 14. 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.

U.S. EPA (1998b) described in the "Assessment of Thyroid Follicular Cell Tumors" in that (a) it is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid and (b) in the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action, because rodents appear to be more sensitive to this carcinogenic mode of action than humans.

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 indicate 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 (see "Subchronic Toxicity" and "Developmental and Reproductive Toxicity".

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 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 indicate 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, no thyroid follicular cell hypertrophy and hyperplasia, then there would be no thyroid tumors. For this reason, the NOAEL determined for the inhibition of thyroidal iodide uptake in humans (non-carcinogenic effect) is reasoned to be protective against thyroid tumors as well.

## CALCULATION OF PHG

### *Noncarcinogenic Effects*

As perchlorate competitively blocks iodide from entering the thyroid gland, many of the adverse effects of perchlorate exposure in the low dose range are similar to those of iodine deficiency. In the evaluation of adverse effects of perchlorate, thyroid enlargement in women during pregnancy and impaired brain development in fetuses during the first half of gestation are identified as the most sensitive toxic end-points. Two other possible sensitive sub-populations are the infants and elder people with existing thyroid problems. OEHHA recommends that perchlorate exposure should be kept at a level that does not inhibit iodide absorption by the thyroid nor cause release of iodide from the thyroid. Based on three human studies (Lawrence *et al*., 2000; 2001; Greer *et al*., 2002), OEHHA estimates a human NOAEL of 0.01 mg/kg-day (10 µg/kg-day).

The human data presented in the section on hematological effects suggest that humans may be more sensitive to these effects than animals thus far studied. However, it is important to note that these data were mostly derived from clinical studies, where high doses were used (6-14 mg/kg-day). Compared with the NOAEL (10 µg/kg-day) determined for inhibition of iodide uptake into the thyroid, an oral dose of 6 mg/kg-day is approximately 600 times higher. It is therefore believed that the identified NOAEL is adequate to protect against the known high-dose hematological effects of perchlorate.

The calculation of a proposed PHG for non-carcinogenic health effects is as follows.

$$C = \frac{NOAEL \times BW \times RSC}{UF \times WC}$$

$$C = \frac{10\ \mu g/kg\text{-}day \times 65\ kg \times 0.6}{30 \times 2\ L/day} = 6\ \mu g/L = 6\ ppb$$

Where:

| | | |
|---|---|---|
| NOAEL | = | no-observed-adverse-effect-level, 10 µg /kg-day; |
| BW | = | body weight of a pregnant woman, a value of 65 kg is assumed[6]; |
| RSC | = | relative source contribution of 60 percent is used because of the potential exposure to perchlorate through food sources (e.g., lettuce); |
| UF | = | an overall uncertainty factor of 30 is used, which includes a factor of 3 for the quality of the data upon which the NOAEL is derived and a factor of 10 for inter-individual variability; and |
| WC | = | adult daily water consumption rate, 2 L/day. |

An uncertainty factor of 10 is used to account for inter-individual variability. Either due to genetic makeup or behavioral characteristics, some individuals appear to be more susceptible to the effect of perchlorate than others. In the study results reported by Greer *et al*. (2002), they found an apparent inhibition of thyroidal iodide uptake in some volunteers exposed to the lowest dose tested, 0.007 mg/kg-day. There is an ecological study indicating that a low level of perchlorate in drinking water was correlated with thyroid hormone perturbation in newborns (Schwartz, 2001).

Because women during pregnancy are considered one of the sensitive sub-populations, the "critical period" or the period of vulnerability is expected to be short, ranging from a few weeks to several months. As a result, even a temporary disruption of the maternal thyroid-pituitary homeostasis can increase the risk of goiter in pregnant woman and improper brain development in the fetus. Below are a number of factors that may alter the thyroid hormone balance:

- Some vegetarian diets may have low iodide content. Remer *et al*. (1999) monitored the urinary iodine excretion of six healthy adult volunteers in four separate five-day diet periods. The study diets, normal, protein-rich, lactovegetarian, and repeat of the initial normal diet, were almost isoenergetic and contained no fish, sea food, iodized salt or processed foods fortified with iodine. During the last 48 hours of each diet period, two 24-hour urine samples were obtained from each subject. They found urinary excretion was significantly lower with the lactovegetarian diet (36.6±8.8 µg/day) than with the normal (50.2±14.0 µg/day) and the protein-rich diets (61.0±8.0 µg/day).
- Goitrogens are found in certain food such as millet, kelp, maize, bamboo shoots, sweet potatoes, lima beans, cabbages, turnips, and mustard. These chemicals, such as

[6] It is assumed that the average body weight of an adult female is 58 kg and the average total weight gain during pregnancy is 12 kg (ICRP, 1974). Thus the average body weight of a pregnant woman is estimated to be between 58 kg and 70 kg, depending on the time into the pregnancy. An average body weight of a pregnant woman of 65 kg is calculated here as the average of the two values, rounded to the nearest 5 kg.

the isothiocyanates found in cruciferous vegetables, can compete for iodine uptake in a manner analogous to perchlorate.

- Exposure to thyroid-disrupting environmental contaminants, such as nitrate, polychlorinated biphenyls (PCBs) and dioxins. When administered at high doses via drinking water, nitrate appeared to competitively inhibit uptake of iodine by the thyroid in rats and sheep (Wijngaarden *et al*., 1953 and Bloomfield *et al*., 1960). Van Maanen *et al*. (1994) reported an association between high nitrate concentration in drinking water and increased thyroid volume. There are data from animal and human studies showing that PCBs and dioxins can compete for binding to the serum carrier proteins (e.g., transthyretin) for the thyroid hormones (Brouwer, 1989; Heussen *et al*., 1992). This may lead to an increased thyroid hormone turnover, increased biliary excretion of thyroxine, hypothyroidism, and thyroid enlargement (Porterfield, 1994; Bastomsky, 1976). Certain PCBs and dioxin congeners are structurally similar to the thyroid hormones. These structural similarities could theoretically lead to endocrine disruption (Porterfield, 2000).
- Selenium deficiency has been linked to hypothyroidism in three clinical cases (Pizzulli and Ranjbar, 2000). Selenium is an indispensable part of the type I 5'-deiodinase. Selenium deficiency can lead to TSH elevation and decrease of type I 5'-deiodinase and possibly type II 5'-deiodinase. Individuals who are suffering from selenium deficiency may be more susceptible to the thyroid-disrupting effects of perchlorate exposure than those who are not (Lee *et al*., 1999).

There are preliminary results showing that some vegetables (e.g., lettuce) may bioaccumulate perchlorate. In a recent greenhouse study, U.S. EPA (2001) reported that lettuce irrigated with 10.0 µg/ml perchlorate exhibits a perchlorate content of about 300 µg/g on a wet mass basis. Assuming this transfer ratio can be applied to low levels of perchlorate contamination, an average consumption rate of lettuce of 40 g/day (U.S. EPA, 1989), and a water consumption rate of 2 L/day, it can be shown that drinking water contributes approximately 62 percent of the total dose while lettuce consumption contributes the remaining 38 percent. In this estimation, it is assumed that all the lettuce consumed is contaminated and the contamination is confined to lettuce only (i.e., no other produce is contaminated). Until more specific data are available on the uptake of perchlorate in produce, it is reasonable to assume a portion of perchlorate exposure may come from this source.

Based on the calculations shown above, the proposed PHG for non-carcinogenic health effects is 6 ppb. It is derived from three studies showing inhibition of iodide uptake by the thyroid gland in human volunteers orally exposed to perchlorate for 14 days (Lawrence *et al*., 2000, 2001; Greer *et al*., 2002).

### *Carcinogenic Effects*

Serum thyroid and pituitary hormones of rodents are highly sensitive to perchlorate exposure. When exposed to perchlorate in drinking water for 14 or 90 days, serum T3,

T4 and TSH levels were significantly changed at doses as low as 0.01 mg/kg-day (Springborn Laboratories, 1998). In contrast, human subjects that are not iodide deficient appear to be less sensitive to the anti-thyroid effects of perchlorate. Greer et al. (2002) and Lawrence et al. (2000) showed that human T3, T4, and TSH levels were not affected by short-term perchlorate exposure (1-2 weeks) at doses as high as 0.14 mg/kg-day. Brabant et al. (1992) pretreated a group of male volunteers with iodine (200 µg/day) for 4 weeks and followed with an oral daily perchlorate dose of 13 mg/kg-day for another 4 weeks. At the end of the study, they found the subjects' serum T3 and T4 levels were not changed, although the serum free T4 levels were significantly depressed. The serum TSH levels were not elevated. Based on these data, it appears that humans are not as sensitive as rodents to the perturbation of thyroid hormones caused by perchlorate that may ultimately lead to thyroid tumors.

Furthermore, there are difficulties in estimating cancer potency of perchlorate based on animal cancer data because of differences in iodine deficiency and thyroid disease status (background rates) in control animals and the human population. For the reasons described, a quantitative dose-response evaluation is not performed for the carcinogenic effects of perchlorate. It is reasoned that by setting the perchlorate PHG low enough to avoid impacts on thyroid iodine status, all other potential adverse thyroid effects, including benign and malignant thyroid tumors, will be prevented.

## RISK CHARACTERIZATION

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, perchlorate must be periodically replaced. As a consequence of this use, large volumes of perchlorate have been disposed of since the 1950s and some of them have found their way into soil and aquifers that are needed as drinking water sources. Perchlorate is highly mobile in aqueous systems and can persist for many decades under typical groundwater and surface water conditions.

Since early 1997, DHS has sampled several hundred drinking water systems and drinking water wells throughout California. It was found that 23 out of the 273 public water systems and 46 out of the 1,589 drinking water sources sampled had perchlorate concentrations exceeding 18 µg/L (ppb) (DHS, 2000). As there is no existing drinking water regulation for perchlorate, DHS established an action level of 18 µg/L for perchlorate (DHS, 2000). In 2002, U.S. EPA (2002) determined an oral reference dose of 0.03 µg/kg-day and a drinking water equivalent level of 1 ppb (1 µg/L), based on the effect levels of perchlorates in rats. Because of this new development, California Department of Health Services revised the action level of perchlorate to 4 ppb in 2002 (DHS, 2002).

Data from human studies indicate that an oral dose of perchlorate is almost completely absorbed from the gastrointestinal tract and is excreted primarily unchanged via the urine. In an occupational study where the urinary perchlorate levels of two workers during and

after work shifts were monitored, it was found that the elimination of perchlorate appears to follow a first-order kinetic pattern with an elimination half-life of 8 hours.

Potassium perchlorate has been used to treat Graves' disease in humans, and most of the high-dose toxicity data on humans are obtained from clinical studies. At the 10-20 mg/kg-day range, some patients reported gastrointestinal irritation, skin rash, and nausea. In a few occasions, agranulocytosis and aplastic anemia have also been reported.

Perchlorate is known to compete with iodide for a transport protein called NIS. NIS can be found in salivary gland, stomach, lactating mammary gland, and thyroid tissues. In several animal as well as human studies (Yu *et al*., 2000; Lawrence *et al*., 2000; 2001; Greer *et al*. 2002), perchlorate even at relatively low doses has been shown to significantly reduce or inhibit thyroidal iodide uptake in acute and sub-chronic exposures. Since iodide is a key ingredient of thyroid hormones and perchlorate inhibits iodide absorption by the thyroid, perchlorate exposure reduces the production of thyroid hormones and disrupts thyroid-pituitary homeostasis.

This has been confirmed in animal studies. At relatively low doses (0.01-0.1 mg/kg-day) perchlorate exposure causes depression of serum thyroid hormones (T3 and T4) and elevation of serum TSH. At higher doses (3-30 mg/kg-day), perchlorate induces thyroid follicular cell hypertrophy, thyroid follicular cell hyperplasia, or thyroid adenoma in the exposed animals. In several developmental studies, perchlorate exposure was found to be associated with a decrease in thyroid gland follicular lumen size, depression in serum T3 and T4 levels, elevation in serum TSH, and motor activity changes in rat pups (U.S. EPA, 2002). In a neurodevelopmental study (Argus Research Laboratories, Inc., 2001), morphometric changes were noted in several brain regions of pups exposed to perchlorate in utero and after birth. These changes are significant even at the lowest ammonium perchlorate dose tested of 0.01 mg/kg-day (or 0.0085 mg/kg-day, based on perchlorate anion alone). However, the dose-response relationships of the brain morphometric measurements are not monotonic, they resemble either an inverted U or U-shape curve (U.S. EPA, 2002).

The sensitivity of human and rodent thyroidal NIS towards the inhibitory effect of perchlorate appears to be similar. In an injection study reported by Yu *et al*. (2000), inhibition of thyroidal iodide uptake was observed in male rats at doses as low as 0.1 mg/kg. In several drinking water studies, human volunteers exposed to perchlorate for up to 14 days showed reduced thyroidal iodide uptake. The LOAEL of this effect appears to be in the 0.02 to 0.14 mg/kg-day range.

However, there are short-term exposure data to indicate adult humans that are not iodide deficient are better than rodents in maintaining serum T3, T4 levels when exposed to perchlorate. For instance, human volunteers exposed to perchlorate in drinking water at targeted doses between 0.14 and 0.5 mg/kg-day for 7 or 14 days showed no significant changes in serum T3, T4, and TSH (Lawrence *et al*., 2000; Greer *et al*., 2002). Even at doses as high as 12 mg/kg-day (4-week exposure), human volunteers showed no significant changes in serum T3 and T4 levels. Serum TSH levels were depressed and intrathyroidal iodine concentrations were reduced in the volunteers. When human volunteers were dosed at 12 mg/kg-day for more than 4 weeks, thyroid enlargement was

observed (Brabant *et al*., 1994, as cited in U.S. EPA, 2002). By contrast, several 14-day drinking water studies showed depression in serum T3, T4, and elevation in serum TSH 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). This conclusion is weakened by the high dietary iodide intake of the volunteers tested, and in light of the findings of decreased serum T4 in newborns with parental exposure to low levels of perchlorate in drinking water (Schwartz, 2001).

Ammonium perchlorate was tested in a battery of genotoxicity tests, and found to be negative in all tests (U.S. EPA, 1998a, 2002). This is consistent with the fact that perchlorate is relatively inert at physiological conditions and does not appear to be metabolized to mutagenic or clastogenic metabolites in humans as well as test animals.

There are no carcinogenic data of perchlorate in humans. Carcinogenicity data in animals are limited. In a two-generation reproductive toxicity study (Argus Research Laboratories, 1999), thyroid follicular cell adenomas were observed in two male Sprague-Dawley rats (2/30) exposed to 30 mg/kg-day ammonium perchlorate in utero and after birth. No such tumors were found in the vehicle control (0/30). Though the incidence is not significant by 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 make the finding significant (U.S. EPA, 2002).

The proposed PHG of 6 ppb is based on a human NOAEL of 0.01 mg/kg-day (10 µg/kg-day), with an overall uncertainty factor of 30 and assuming a body weight of 65 kg for a pregnant woman, a daily water consumption rate of 2 L/day and a relative source contribution of 60 percent.

A number of human studies (Lawrence *et al*., 2000, 2001; Greer *et al*., 2000, 2002) documented the inhibitory effect of perchlorate on iodide uptake by the thyroid. Greer *et al*. (2000, 2002) administered a daily oral dose of perchlorate to groups of volunteers (5 of each sex per dose) at 0.5, 0.1, or 0.02 mg/kg-day for 14 days. Another group of 6 females and 1 male received 0.007 mg/kg-day of perchlorate. The reduction in thyroidal iodide uptake was statistically significant in all groups with the exception of the low-dosed group. Based on this study, a No Observed Adverse Effect Level (NOAEL) of 0.007 mg/kg-day can be identified. Lawrence *et al*. (2000, 2001) administered a daily dose of 10 mg or 3 mg of perchlorate in water to groups of male volunteers for 14 days. The reduction in thyroidal iodide uptake in the high-dose study is statistically significant while the reduction in the low-dose study is not. Details of the low-dose-study (Lawrence *et al*., 2001) are not reported. The low dose (3 mg/person or approximately 0.043 mg/kg-day) administered in this study is higher than the NOAEL identified in the Greer *et al*. study (2002), further evidence that the 0.02 mg/kg-day LOAEL in the Greer et al. (2002) study is close to a population NOAEL. Thus the NOAEL estimated in the human study of Greer *et al*. (2002), is rounded up from 0.007 mg/kg-day to 0.01 mg/kg-day for calculation of the proposed PHG value.

An uncertainty factor of 10 is used to account for individual variability in the target populations due to (a) small number of subjects in each dose group of the studies

selected; (b) relatively high iodine intake levels of the subjects studied compared to the sub-populations at greatest risk; (c) variation in exposure, such as seasonal variation in dietary iodide intake or exposure to goitrogens in certain foods, and (d) variation in genetic makeup. Individual variability is evident in the Greer *et al*. study (2002); apparent decreases in thyroidal iodide uptake were noticed in some volunteers exposed to the lowest dose tested, 0.007 mg/kg-day. There is also an ecological study indicating a low level of perchlorate in drinking water was correlated with a reduction of thyroid hormone in newborns in California (Schwartz, 2001).

An uncertainty factor of 3 is used to compensate for the quality of the database and concerns about extrapolating the 14-day study results to lifetime exposure. The standard default uncertainty factor for extrapolating from short-term data to chronic exposure is 10. While confidence in the study results reported by Lawrence *et al*. (2000, 2001) and Greer *et al*. (2002) is limited because of the short exposure duration, the inhibitory effect of perchlorate most likely precedes other adverse effects of perchlorate. As perchlorate competes with iodide for absorption sites on thyroid cells, high dietary iodide intake might have elevated the LOAEL and NOAEL for perchlorate inhibition of thyroidal iodide uptake. Furthermore, there are some concerns for the immunotoxicity and developmental neurotoxicity observed in rodents that have not been adequately studied in humans.

There are preliminary results showing that some vegetables (e.g., lettuce) may bioaccumulate perchlorate. In a recent greenhouse study, U.S. EPA (2001) reported that lettuce irrigated with 10 µg/ml perchlorate exhibits a perchlorate content of about 300 µg/g on a wet mass basis. Using this study result and some simple assumptions, it is estimated that the relative source contribution of drinking water to the overall perchlorate dose is about 60 percent.

The proposed PHG is developed to protect the general population as well as several sensitive subgroups identified in this evaluation: (i) pregnant women that are marginally iodide deficient, (ii) their fetuses, (iii) patients suffering from hypothyroidism, and (iv) infants and small children.

There are reports showing that pregnancy itself constitutes a form of stress on the thyroid. For women with marginally deficient dietary iodide intake (<100 µg/day), pregnancy increases the risk of thyroid enlargement and goiter. Two prospective studies (Romano *et al*., 1991; Pedersen *et al*., 1993), showed that the pregnancy-related thyroid enlargement could be prevented by administering iodide salts to pregnant women, confirming that iodine deficiency is a cause of thyroid enlargement during pregnancies. There are also data indicating that fetal brain tissues need a certain level of maternal T4 to ensure proper development (Morreale de Escobar *et al*., 2000). Severe iodine deficiency during pregnancy can cause prenatal death and cretinism. Even moderate to mild iodine deficiency or hypothyroidism during pregnancy has been linked to impaired neurological development and lowered IQs in offspring (Man and Jones, 1969; Glorieux *et al*., 1988; Rovet *et al*., 1987; Tillotson *et al*., 1994; Vermiglio *et al*., 1990; Bleichrodt and Born, 1994; Haddow *et al*., 1999). There is a concern that perchlorate exposure of pregnant women with marginal iodine deficiency may permanently impair the neurological development of the fetal brain.

According to the most recent national-wide dietary survey, NHANES III (1988-1994) (Hollowell *et al*., 1998), 14.9 percent of the women of childbearing age had urinary iodine concentration below 5 µg/dL. This is equivalent to a dietary iodide intake of approximately 68 µg/day (daily iodine intake [µg] = urinary iodine [µg/L] x 0.0235 x body weight [58 kg]) (NAS, 2001).. This level of iodide intake is less than half of the 175-200 µg/day range recommended for pregnant women by the World Health Organization (Delange and Bürgi, 1989; as cited in Caron *et al*., 1997). Recently, National Academy of Sciences determines an estimated average requirement of 160 µg/day and a recommended dietary allowance of 220 µg/day for pregnant women (NAS, 2001). The recommended dietary allowance is defined as equal to the estimated average requirement plus twice the coefficient of variation to cover the needs of 97 to 98 percent of individuals in the group. Based on these data, a substantial fraction of women of childbearing in the U.S. can be defined as marginally iodine deficient.

## OTHER REGULATORY STANDARDS

Currently, there is no Federal or State MCL for perchlorate. There is a State action level of 4 ppb (DHS, 2002). U.S. EPA (2002) recently released a draft risk assessment on perchlorate and determined an oral RfD of 0.03 µg/kg-day based on the Argus Research Laboratories rat data. Applying the default body weight of 70 kg and daily drinking water consumption of 2 L/day, U.S. EPA (2002) estimated a health-protective drinking water equivalent level of 1 ppb or 1 µg/L.

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