mother. Radioactivity of the blood and thyroids of the mothers and fetuses were analyzed.

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

Postel (1957) also reported that the thyroid/serum radioiodide concentration ratio was approximately 175 in fetus and 50 in mothers in the control group. It was suggested that the data support the concept that the normal fetal thyroid is in a relatively hyperplastic state. The thyroid/serum radioiodide concentration ratios of both the perchlorate-treated mothers and their fetuses were significantly depressed, compared to the controls.

Lampe *et al.* (1967) gave perchlorate-treated food to 12 rabbits from the beginning of pregnancy through gestation day 21 or 28. The rabbits were dosed at 100 mg/kg-day. No concurrent controls were used. Ingestion of perchlorate was found to cause an increase in maternal and fetal thyroid weights. On the 21st day of treatment, the maternal thyroid weights in treated animals were nearly three times higher than control thyroids; fetal thyroids were nearly four times the control weights. Continued intake of perchlorate further enhanced the increase in thyroid weight, particularly in the fetus. On the 28th day of treatment, the maternal thyroid weights in treated animals were nearly four times higher than control thyroids; fetal thyroids were nearly nine times the control weights. The researchers noted that the placenta is permeable to perchlorate, which caused intensive hyperplasia as a sign of hyperfunction, and a loss or complete disappearance of colloid in the maternal and fetal thyroids. They also noted the increased sensitivity of fetal thyroid to the treatment, compared with the maternal thyroid.

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. Study results reported by Postel (1957), Lampe *et al*. (1967) and Sztanyik and Turai (1988) suggest that perchlorate can pass through the placenta from the mother to the fetus in the test animals.

A developmental neurotoxicity study of ammonium perchlorate in rats was conducted by Argus Research Laboratories (1998a; 1998b; 1998c). Ammonium perchlorate was administered to groups of 25 female Sprague-Dawley rats via drinking water at target doses of 0, 0.1, 1.0, 3.0, and 10 mg/kg-day. The dosing period was from the beginning of gestation (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. 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; York *et al.*, 2001) reported a two-generation reproductive toxicity study in Sprague-Dawley rats. Male and female rats (30 rats/sex/group) of the first generation (P) were exposed to ammonium perchlorate in drinking water at 0, 0.3, 3, and 30 mg/kg-day. One male and one female were allowed a cohabitation period of a maximum of 14 days. 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. York *et al.* (2001) reported that perchlorate is not a reproductive toxicant in the doses tested. In both the P and F1 adult rats, there were no deaths, abortions, or premature deliveries. No changes were reported in any sperm parameters in either P or F1 adult male rats nor on mating or fertility parameters in either P or F1 adult female rats (estrous cyclicity, fertility index, number of days in cohabitation, and number of rats mated). Natural delivery and litter observations for both F1 and F2 generation pups were comparable among the treated and control groups. Treatment-related effects were not observed on the gestation index, the number of dams delivering litters, the duration of gestation, the average number of implantations, the average number of live pups, the viability and lactation indices, the sex ratios, or the pup body weights.

York *et al.* (2001) found that perchlorate exposure caused statistically significant, dose-dependent changes in thyroid weight, histopathology, and hormone levels in P, F1, and F2 generation rats. Relative thyroid weights were significantly increased in the 30 mg/kg-day dose group for both sexes in the P generation and for F2 generation pups. However, in the F1 generation adult rats, relative thyroid weights were significantly increased in all dose groups for females and in the 3 and 30 mg/kg-day dose groups for male rats. All three generations developed hypertrophy and hyperplasia of thyroid follicular epithelium that increased in incidence and severity in a dose-related manner. Dose-related changes in TSH, T3, and T4 were also observed in the treated rats. However, these changes were inconsistent among the different generations, sexes, and ages of animals.

U.S. EPA (2002) noted that two male rats from the high dose group (30 mg/kg-day) in the F1 generation (second parental generation) in the study had adenomas of the thyroid. These males were dosed from conception to 19 weeks of age. Without incorporating historical data, the difference between 0/30 in the control and 2/30 in the 30 mg/kg-day is non-significant by standard tests (e.g., Fisher's exact). However, using two earlier reported background incidence rates of 3.6 percent and 3.9 percent for thyroid follicular

cell adenomas in male Sprague-Dawley rats in 2-year studies and Bayesian analysis, U.S. EPA (2002) determined the increase in thyroid follicular cell adenoma at 19 weeks in male Sprague-Dawley rats exposed to 30 mg/kg-day to be significant.

Effects of perchlorate on motor activity in Sprague-Dawley rats were studied by Bekkedal *et al*.(2000)). The females were dosed with ammonium perchlorate in drinking water for two weeks at 0, 0.1, 1, 3, or 10 mg/kg-day prior to mating with the breeder males and through 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. Pups were individually tested in automated Opto-Varimex Activity boxes where 9 different measures of activity were recorded for 90 minutes on each test day. Data were analyzed in 9, 10-minute blocks using a repeated measures ANOVA.

Bekkedal *et al*. (2000) reported no statistically significant differences for any of the 9 measures of motor activity, and there were no reliable interactions related to treatment. A general pattern in the results was noticed. The authors suggested that there was a divergence in activity between the control and treated groups which emerged late in the 90-minute testing sessions.

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

Argus Research Laboratories (2001) studied the effects of perchlorate on thyroid and brain development both during gestation and postnatally. Perchlorate was administered in drinking water to female rats two weeks prior to cohabitation at 0, 0.01, 0.1, 1, or 30 mg/kg-day and continued through the day of sacrifice. F1 generation rats were not directly dosed but might have been exposed in utero during gestation and via maternal milk and maternal water during the postpartum period. The rats were selected only from female rats that had litters of at least 12 live offspring at the time of Caesarean-sectioning (Part A) or at the time of the first tissue collection (Parts B and C). P generation rats assigned to Parts B and C that delivered a litter were sacrificed on either PND 9 (Part B) or PND 21 (Part C). The thyroid and brain from one male and one female pup per litter were selected for histological and morphometric evaluation, with one set evaluated on PND4, PND9, and PND21. Details of the study and findings are described in the study reported prepared by the Argus Research Laboratories (2001), and a summary of the findings and evaluations are provided below.

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

The absolute and relative thyroid weights of dams at the highest dose were increased. The absolute thyroid weights of some pups exposed at 1 and 30 mg/kg-day were increased. Furthermore, the absolute thyroid weights of the PND9 male pups in the 0.01, 0.1, 1 and 30 mg/kg-day dose groups were significantly increased over the controls.

An exposure-related increase in the incidence and severity of decreased colloid was noted in dams in the 1 or 30 mg/kg-day groups. Similar observations were also made on the fetuses at birth and pups at PND4 and PND9. An increased incidence of follicular cell hypertrophy and/or hyperplasia was found in dams in the 30 mg/kg-day dose group. An increased incidence of follicular cell hyperplasia was also found in the 1 mg/kg-day dams sacrificed on PND21.

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

Maternal and fetal thyroid and pituitary hormone levels were also affected by various doses of perchlorate in Parts B and C. Most changes occurred in an exposure-dependent manner. In the PND21 male pups, TSH levels were significantly increased and T4 levels were significantly decreased at all exposure levels. T3 levels were also significantly decreased in the 1 and 30 mg/kg-day groups. In the PND21 female pups, TSH levels were increased at all exposure levels, reaching significance in the 0.1, 1, and 30 mg/kg-day groups. T4 levels were decreased with increased exposure but did not reach statistical significance.

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. Using this data, U.S. EPA (2002) identified a LOAEL of 0.01 mg/kg-day for the adverse effects of ammonium perchlorate on the developing brain in rats. This is equivalent to 0.0085 mg/kg-day for the perchlorate anion alone.

The design and implementation of this study have been criticized, and U.S. EPA's interpretation of the study data has been challenged (TERA, 2003). It was noted that the

way the brain sections were prepared and the method used to measure different regions of the brain are susceptible to experimental artifacts. The study has no positive control and it is not clear if the observed neurodevelopmental changes are related to thyroid hormone disruption. The association of some of the brain measurements with perchlorate exposure has also been questioned as there are no clear dose-response relationships. There are also concerns about the statistical method used in the U.S. EPA analysis.

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

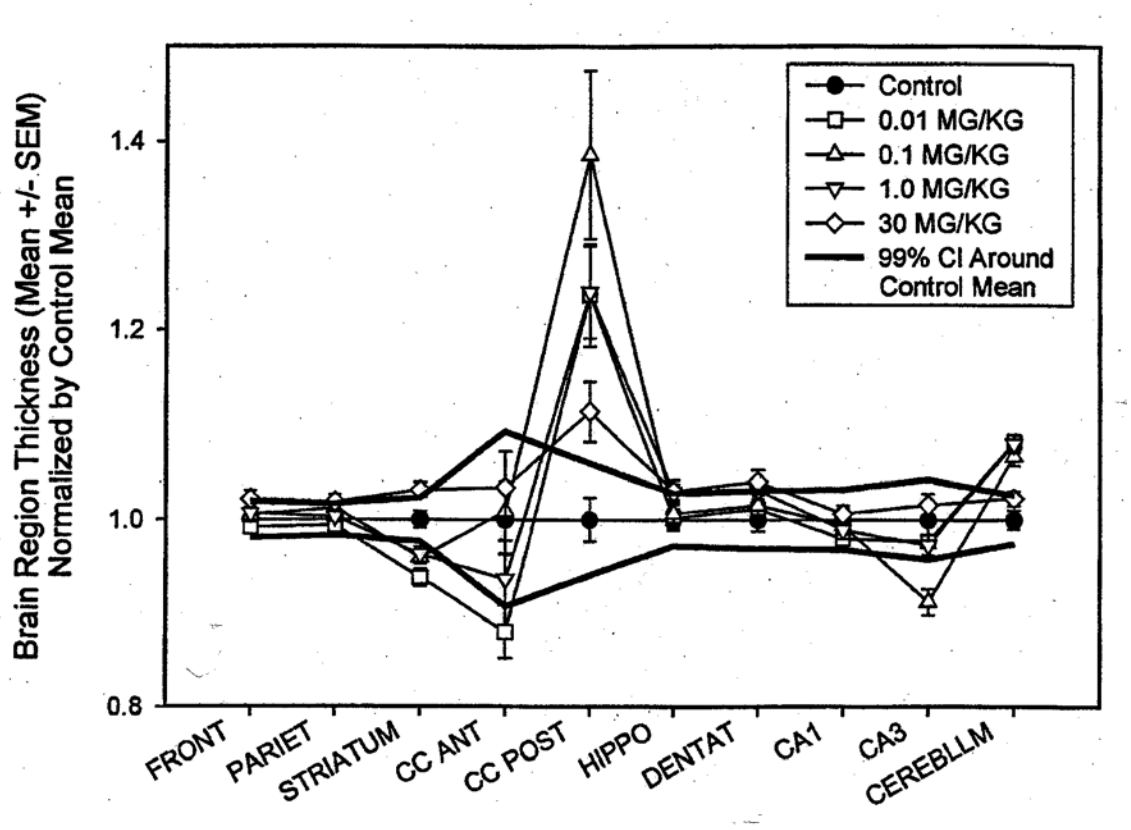

Figure 3. Profile analysis of brain morphometry measurements for PND21 rat pup brain regions.

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 percent confidence interval around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values (from U.S. EPA, 2002).

Reproductive toxicity

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

A developmental toxicity study was performed on New Zealand White rabbits (Argus Research Laboratories, 1998d). It involved 25 naturally-mated does per group exposed to ammonium perchlorate in drinking water at 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day from gestation day 6 to gestation day 28. Observations were based on 22, 24, 23, 24, 24, and 23 pregnant does that survived to gestation day 29 in the 0, 0.1, 1, 10, 30, and 100 mg/kg-day dosage groups, respectively. Fetuses were delivered by 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 and Myant, 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.

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; Burleson Research Technologies).

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. 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). 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. 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. The data from Burleson Research Technologies (2000) indicate that exposure to perchlorate enhances the LLNA response to DNCB. While a dose of 50 mg/kg-day for 14 days enhanced the response, exposure to the same dose for 90 days suppressed the response. Exposure to lower doses of 0.06 and 0.2 mg/kg-day also increased the response; however, interpretation of these data is made difficult by the observation that exposure to 2 mg/kg-day did not affect the response, in the 14-day study. OEHHA agrees with U.S. EPA (2002) that interpretation of the results is made difficult by (a) some technical problems encountered in the studies, (b) the apparent inconsistency of the high-dose study results, and (c) the unknown biological significance of the response enhancement.

Neurotoxicity

By interfering with the thyroid-pituitary axis, perchlorate 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 . As part of the U.S. EPA's program to evaluate the toxicity of perchlorate, neurodevelopmental tests on Sprague-Dawley rats were conducted by Argus Research Laboratories (1998a, 2001). The study design and findings have been summarized in the "Developmental and Reproductive Toxicity" section.

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 are summarized in the "Subchronic Toxicity" and "Developmental and Reproductive Toxicity" sections.

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).

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 $p<0.05$

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 treatment group. The degree of inhibition was reduced over time and by exposure day 14, no inhibitory effect was observed in the 1 and 3 mg/kg-day groups. In a similar study, thyroid hormone profile of rats exposed to perchlorate was investigated. Male rats in groups of 8 were exposed to perchlorate in drinking water at 0, 0.1, 1, 3, and 10 mg/kg-day continually for 1, 5, or 14 days. In all treated groups, regardless of dose or exposure duration, TSH levels were increased compared to the control. The serum T4 levels were initially decreased in all dose groups except the lowest, 0.1 mg/kg-day. By 14 days, the 1 mg/kg-day dose group returned to control T4 values while T4 levels of the 3 and 10 mg/kg-day dose groups were still significantly depressed. Yu *et al*. (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.

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

Clewell *et al.* (2001) developed and validated rat and human physiologically-based pharmacokinetic (PBPK) models for the estimation of the effect of life stage and species on perchlorate and iodide inhibition kinetics. In a later paper, Clewell *et al.* (2003) used the models to estimate perchlorate distribution in the male, pregnant, fetal, lactating and neonatal rat, and predict resulting inhibitory effects on thyroidal iodide uptake. They reported that the PBPK model predicted that while the lactating dam has the greatest internal perchlorate exposure, the fetal rat thyroid is most vulnerable to inhibition.

Carcinogenicity

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

Gauss (1972) treated female NMRI mice with one percent potassium perchlorate in the diet or the control diet for 160 days. The one percent dose is equivalent to approximately 2,000 mg/kg-day based on standard assumptions. The investigator noted progressive changes in the thyroids of treated mice beginning with colloid loss, progressing to increases in size of nuclei and increased epithelial height, followed by appearance of hyperplasia and hypertrophy of the thyroid parenchyma. Later in the treatment period, hyperplastic follicles, areas of 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 the chemical itself was not 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 time. Diffuse degenerative changes with fibrosis and increased colloid were observed after 200 days. Four of 11 rats treated with one percent potassium perchlorate for two years developed benign thyroid tumors. The twenty untreated controls had no thyroid tumors.

Pajer and Kalisnik (1991) divided 72 female BALB/c mice into 6 groups. Three groups were treated with 1.2 percent sodium perchlorate, while three groups were controls. Eight or 32 weeks after the beginning of the study, one perchlorate and one control group of animals were irradiated with a total of 4 Gy of ionizing radiation (gamma rays) over a period of five days. Forty-six weeks after the beginning of the experiment, 42 animals were sacrificed, while 30 had died during the experiment. The perchlorate dose to the treated mice 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. 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)	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. The study result is limited by the small number of animals in the perchlorate-only treated group, the design of the study, and the deaths of over 40 percent of the mice before the end of the experiment.

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

In a number of subchronic perchlorate studies, increased thyroid follicular cell hypertrophy and hyperplasia were observed in some of the treated animals (Lampe *et al*., 1967; Caldwell *et al*., 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998a, 1999, 2001; Keil *et al*., 1998). Details of the study results have been presented. 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. At high doses, perchlorate exposure is known to cause other adverse health effects such as blood disorder. 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

[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.

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) 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

Clinical Studies

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 were not provided in the paper: detailed dosage information, time of perchlorate treatment in relation to the gestation

period, thyroid function of the newborns, and the neurological as well as behavioral development of the offspring. Furthermore, interpretation of the result is made difficult by the fact that the women were suffering from thyrotoxicosis (excess quantities of thyroid hormones).

Ecological and Epidemiological Studies

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.

Using similar data from a statewide newborn screening registry (California Newborn Screening data), 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 ($\geq$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 that were 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. There are uncertainties associated with the exposure estimates and this difficulty is alleviated to a degree by categorizing the infants into three dose groups (low, medium, and high). Another source of uncertainty is that the serum T4 and TSH data were from 1996, but the perchlorate in water source data were collected later, between February 1997 and June 2000.

Kelsh *et al.* (2003) criticized the TSH results because more than 50 percent of the data had the value recorded as the minimum, 5 µU/mL. Thus the true variance of TSH is reduced, which inflates the level of significance.

Table 8. Perchlorate Exposure and Thyroid Function in California Neonates; 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

Kelsh *et al.* (2003) used the California Newborn Screening database to study the thyroid health of newborns whose mother resided in Redlands during the period 1983 through 1997. Perchlorate has been detected in groundwater wells in Redlands. Two parameters, primary congenital hypothyroidism and elevated concentrations of TSH, were used to represent the thyroid health. Newborns of San Bernardino and Riverside counties excluding newborns from Redlands and communities where perchlorate has been detected were used as the comparison group. All residents in the Redlands community were assumed to be uniformly exposed to perchlorate. However, there is a lack of information about perchlorate levels in drinking water of the study area over the 15-year study period. The detection limit of perchlorate in water before 1997 was about 400 ppb. In the 2001 and 2002 Consumer Confidence Reports, the City of Redlands reported that the concentrations of perchlorate in its water system ranged from non-detect to 9 ppb, with an average concentration below 1 ppb.

Kelsh *et al.* found no excess prevalence of primary congenital hypothyroidism in Redlands newborns over the 15-year study period. They reported an adjusted standardized prevalence ratio of 0.45 and a 95 percent confidence limit of 0.06-1.64. Kelsh *et al.* also did not find evidence of elevated TSH for Redlands newborns either using San Bernardino and Riverside (excluding Redlands) newborns or using only San Bernardino newborns as the comparison group. The Colorado River is one of the water sources of Riverside, so the water serving this community, the "unexposed group", is also likely to have been contaminated with perchlorate. The researchers found that Hispanic ethnicity, low and high birth weights, and female sex are risk factors for primary

congenital hypothyroidism. High TSH levels are mostly influenced by the age (in hours) when the blood samples are collected. Immediately after birth, a normal surge in TSH occurs so that early collection of blood samples (<24 hours) can produce high TSH results. The study results are limited by the small number of observations for the Redlands community, due to the rarity of primary congenital hypothyroidism and elevated TSH status. Like many other ecological studies, this study is also limited by the lack of detailed exposure information for the residents of Redlands.

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). 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.

It should be noted that Crump *et al*. (2000) also reported relatively high urinary iodine excretion of the schoolchildren in all three cities. They reported mean iodine excretion levels of 76.6 µg/dL, 61.4 µg/dL, and 75.6 µg/dL for Taltal, Chañaral, and Antofagasta, respectively. These levels are much higher than those in the NHANES III database, which found a mean urinary iodine level of 30.5 µg/dL in the 6-11 years old age group of children in the U.S. The high urinary iodine levels and high goiter prevalence among the schoolchildren in the Chile study make the interpretation of thyroid function data difficult, and together they indicate there may be other confounding factors.

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). However, interpretation of these data is complicated by the high variability of TSH levels in newborns (1-2 days).

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 Lake Mead, which 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, with 95 percent confidence limits of 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 were 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 marginal iodine deficiency, additional stresses to the thyroid due to pregnancy and perchlorate exposure 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 (up to 15 ppb perchlorate in drinking water) and Reno (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. 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, lack of control of several 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) 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) at which blood samples were collected, which was significantly different in the two cities. In addition, 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*., 2000b), 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 a certain cut-off value or below a certain (e.g., 10^{th}) percentile on T4 in each daily batch of samples collected. 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).

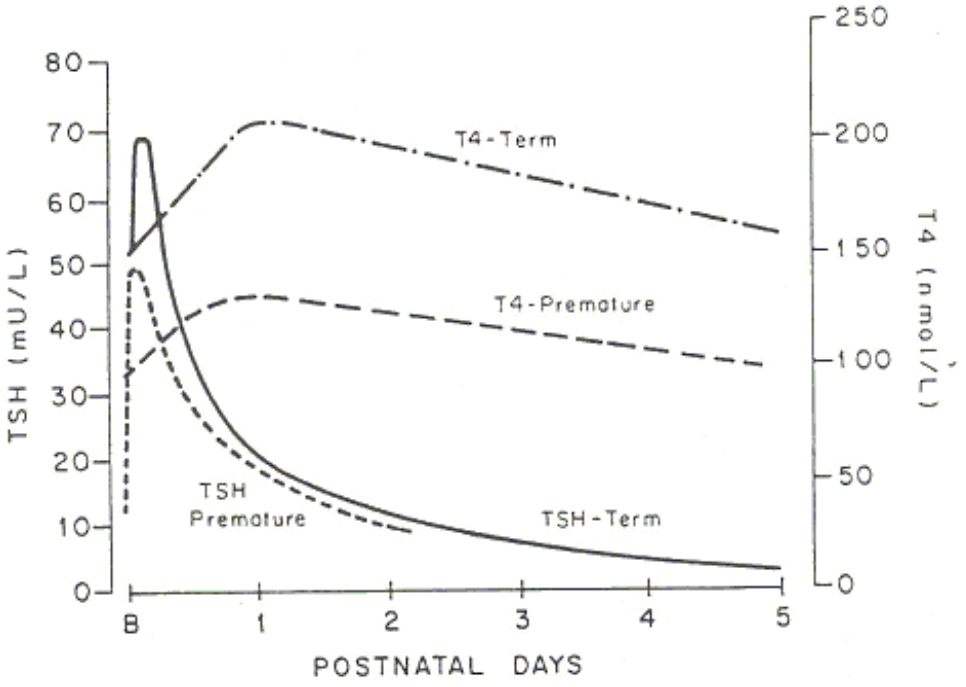

Figure 4. Changes in serum TSH and T4 concentrations in full-term and premature infants during the first five days of life. The neonatal TSH surge peaks at 30 minutes in both preterm and term infants and is followed by a progressive increase in serum T4 concentrations, peaking at 24 to 36 hours (from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

Clinical Studies

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: (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.

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.

Other Human Studies

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 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.

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 may reduce the impact of perchlorate on the thyroid. It is also interesting that there was no statistical difference in serum perchlorate levels after 7 days and 14 days of exposure, indicating no apparent accumulation of perchlorate in the systemic circulation over that period 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

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 statistically insignificant 10 percent decrease. Details of the study were not reported. Assuming a default body weight of 70 kg, the dose used in this study is estimated to be 0.043 mg/kg-day.

Greer *et al*. (2002) 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. There was a strong correlation between the 8- and 24-hr uptakes over all dose groups and measurement days. There was no difference between exposure days 2 and 14 in the inhibition of uptake produced by a given perchlorate dose. There was no sex difference. Uptakes measured on post-exposure day 15 were not significantly different from baseline. Table 13 provides the 24-hr thyroid radioiodine uptake data by dose. Greer *et al*. (2002) measured total T4, free T4, total T3, and TSH in blood sampled throughout the study, and found them to be in the normal range for all subjects. One woman in the lowest dose group had abnormally high TSH on both the screening visit and on exposure day 14. Because of the limited data in the follow-up study, analysis of treatment effects on hormonal levels was confined to the 24 volunteers in the first study. Greer *et al*. (2002) reported no association of thyroid hormone levels with blood-draw events in any dose group except a marginally significant association of TSH with blood-draw in the 0.5 mg/kg-day dose group.

Table 13. Descriptive Statistics for the 24-hr Thyroid Radioiodine Uptake Data (from Greer *et al*., 2002)

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

Occupational Studies

Gibbs *et al*. (1998) monitored triiodothyronine resin uptake (T3U), total serum T4, 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 14). 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 endpoint.

Table 14. 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

Li *et al.* (2001) studied the prevalence of eight thyroid diseases (including malignant neoplasm of the thyroid gland) in Clark County, Nevada compared to that in Washoe County, Nevada and in other Nevada counties using Nevada Medicaid data for 1997 and 1998. Eighty-six measurements were taken of the perchlorate levels in the water supply of Clark County (Las Vegas) between July 1997 and January 2001. These values ranged from non-detect to 24 μg/L (ppb), with a mean of 11.5 μg/L. The researchers reported that none of the eight thyroid disease prevalences was significantly higher in Clark County than in the rest of the state, and for acquired hypothyroidism the prevalence in Clark County was significantly lower in the rest of the state. They also found none of the eight thyroid diseases significantly differed in prevalence between Clark County and Washoe County, with all relative risks being close to one.

Morgan and Cassady (2002) assessed observed and expected number of new invasive cancer cases for all sites combined and 16 cancer types among residents of the greater Redlands area between 1988 and 1998. The community is known to have drinking water contaminated with perchlorate (levels as high as 98 ppb measured in 2001) and trichloroethylene (0.09-97 ppb measured in 1980). They reported no significant differences between observed and expected numbers for all cancers, thyroid cancer, or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus and the colon and rectum. More cases were observed for uterine cancer (standardized incidence ratio = 1.35; 99 percent CI = 1.06 to 1.70) and skin melanoma (standardized incidence ratio = 1.42; 99 percent CI = 1.13 to 1.77).

Adverse Health Effects Associated with Iodide Deficiency or Low Thyroid Hormone Levels

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.

Thyroid problems in pregnant women with low iodide intake

A number of human studies have shown that pregnancy stresses the thyroid (Crooks *et al*., 1967; Glinoer *et al*., 1990, 1992, 1995; Smyth *et al*., 1997; Caron *et al*., 1997; Brent, 1999; Kung *et al*., 2000). In areas of iodine deficiency (intake level <100 μg/day), there is an increased risk of abnormally low serum T3 and T4 levels, thyroid enlargement as well as goiter in pregnant women. The nature and severity of changes in thyroid functions are related to the severity of iodine deficiency. In an epidemiologic survey reported by Delange and Ermans (1991; as cited in Delange, 1994), the investigators found the prevalence of goiter in an area with severe iodine deficiency is influenced by age and sex, with maximal frequency in females during puberty and childbearing age (Figure 5).

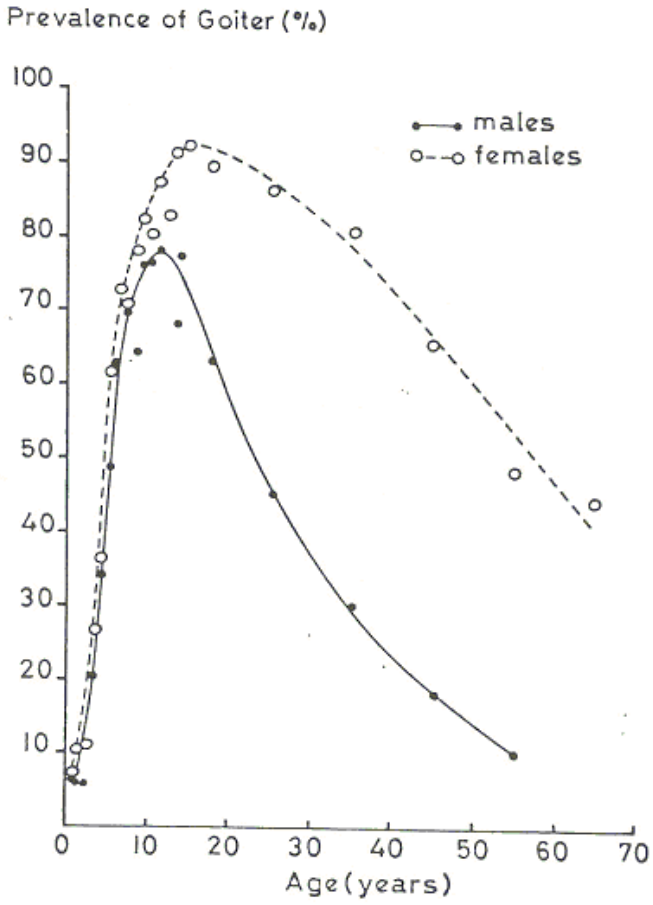

Figure 5. Changes in the prevalence of goiter as a function of age and sex in severe endemic goiter (Idjwi Island, Zaire) (from Delange, 1994).

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 15). 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.

Table 15. 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. In the newborns, they found free T4 levels were significantly higher than in the respective mothers. However, mean neonatal TSH and Tg levels were significantly higher than maternal values. Furthermore, these values were highly correlated with maternal data, suggesting the limited availability of iodine supply was the common link.

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

Supportive results were reported in two other studies, one in Ireland and the other in France. Smyth *et al*. (1997) evaluated ultrasound-measured thyroid volume of 115 pregnant women during one of the three trimesters. These women (Group A) were enrolled based on availability, and each trimester's study group comprised different individuals. Control values for thyroid volume were obtained from 95 pre-menopausal females. All subjects were from Dublin, Ireland, an area of 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 related study, Smyth *et al*. (1997) studied a group of 38 pregnant women (Group B), prospectively. Casual urine samples were collected sequentially during the 3 trimesters of pregnancy and at approximately 6 weeks postpartum. Of those 38 subjects, 20 had thyroid ultrasound scans during each trimester of pregnancy and at 6 weeks postpartum. Thyroid volumes greater than 18.0 mL were defined as enlarged. The number of enlarged thyroids increased from the non-pregnant control value of 6.3 percent, through 19.5 percent in the first trimester, to reach a plateau of approximately 32 percent in the second and the third trimesters, which was maintained up to 40 days postpartum.

Urinary iodine of the women in Group A and Group B were also measured. Urinary iodine measurements from 1063 premenopausal women over a one-year period served as controls. Smyth *et al*. (1997) found that urinary iodine levels measured throughout the pregnancies of the women in Group A and Group B (Table 16) were higher than in the controls (median 70 μg/L). Smyth *et al*. (1997) suggested that in an area of moderate dietary iodide intake, urinary loss during pregnancy may result in maternal thyroid enlargement.

Table 16. Median Urinary Iodine Excretion (μg/L) in Pregnancy (from Smyth *et al*., 1997) *

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

* Some of the values were estimated from a graph.

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 17. 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.

They also reported that 14 women with excessive thyroidal stimulation in the second trimester had lower urine iodine concentrations and larger thyroid volumes throughout pregnancy. Furthermore, their neonates had higher cord TSH, Tg, and slightly higher thyroid volumes as compared to the findings in 216 pregnant women without evidence of thyroid stimulation. Seven neonates (50 percent) born to these women had subnormal free T4 levels at birth.

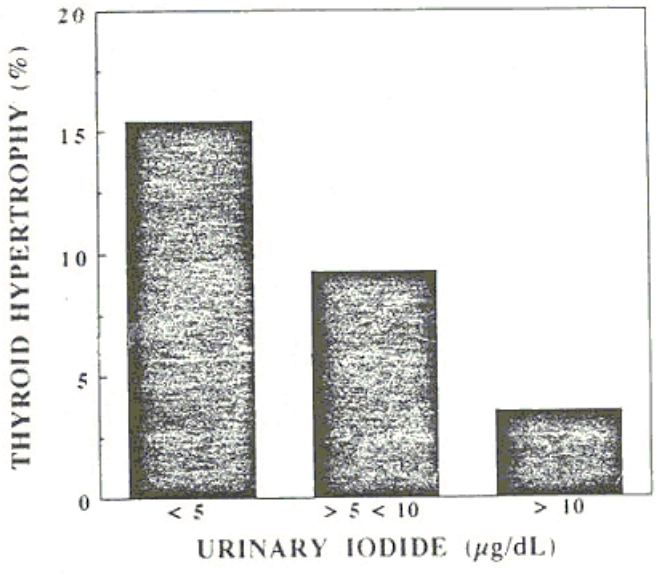

Figure 6. Percentage of maternal thyroid hypertrophy (thyroid volume > 18 ml) in relation to urinary iodine concentration during the first trimester of pregnancy (from Caron *et al.*, 1997).

Table 17. 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 18).

Table 18. Thyroid Hormone 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 three prospective studies showing that in an area with marginal or moderate iodide deficiency, iodide supplement often can reduce the stress on the thyroid during pregnancy (Romano *et al*., 1991; Pedersen *et al*., 1993; Glinoer *et al*., 1995). 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 19) only in group A, treated with iodide salt.

Table 19. 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).

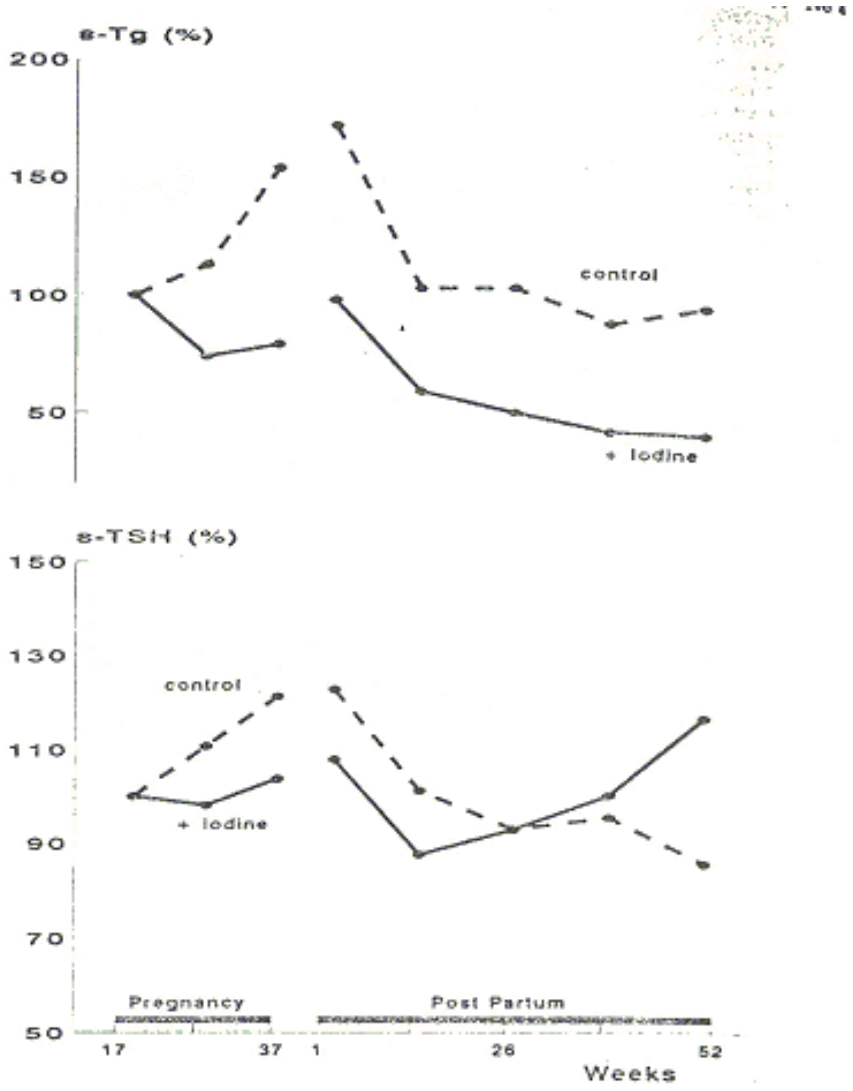

Figure 7. Serum Tg and TSH during pregnancy and for 52 weeks postpartum in women receiving iodide supplementation and control women, as a percentage of the initial values. Median values are shown. The increase in serum Tg during pregnancy in the control group was statistically significant ($p<0.01$), 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).

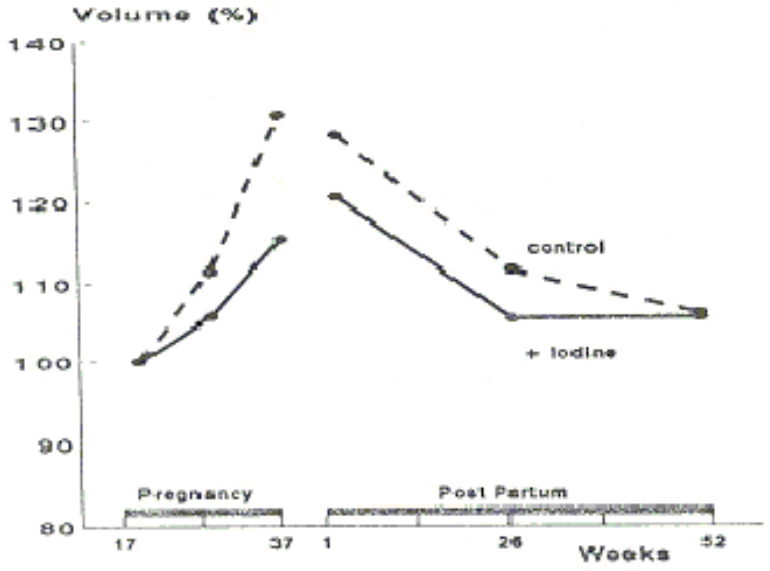

Figure 8. Median thyroid volume during pregnancy and 52 weeks postpartum in women receiving iodide supplementation and control women, as percent of initial values. In both groups, significant increases during pregnancy and decreases during the postpartum period were found ($p<0.05$). The increase during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

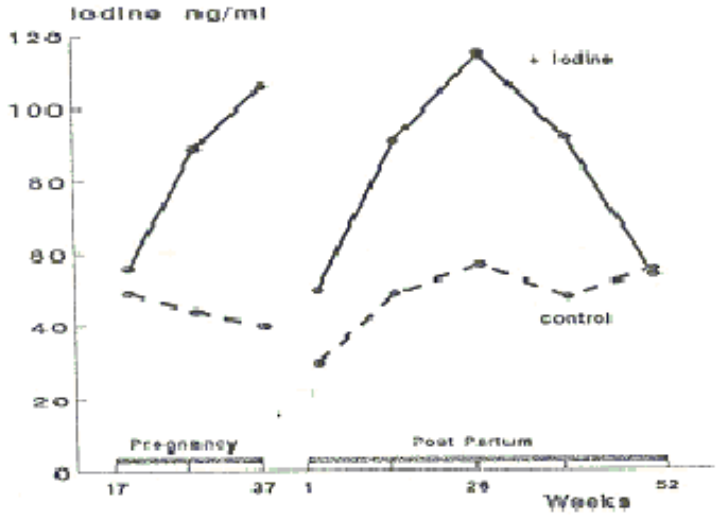

Figure 9. Iodine concentration in spot urine samples during pregnancy and for 52 weeks postpartum in women receiving iodide supplementation and control women. Last sample obtained after iodide supplementation was stopped (Pedersen *et al*., 1993).

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 an important factor for the late development of thyroid autonomy.

Glinoer *et al*. (1995) studied a group of euthyroid pregnant women with mild to moderate iodine deficiency and found pregnancy stresses on the thyroid could be prevented by the administration of potassium iodine or potassium iodine plus L-T4. They selected 180 pregnant women at the end of the first trimester on the basis of biochemical criteria of excessive thyroid stimulation, defined as serum thyroglobin > 20 μg/L associated with a low normal free T4 index (<1.23) and/or an increased T3/T4 ratio (>$25x10^{-3}$). The subjects were randomized in a double blind protocol into three groups and treated until term with a placebo (Group A), potassium iodine (100 μg/day) (Group B) or potassium iodine (100 μg/day) plus L-T4 (100 μg/day) (Group C). At the beginning of the study, all the subjects were mildly or moderately iodine deficient as indicated by a median urinary iodine concentration of 36 μg/L, with 56 percent below 40 μg/L, 34 percent between 41-80 μg/L, and only 10 percent between 81-160 μg/L. After therapy was instituted, urinary iodine concentration of Groups B and C rose significantly (to approximately 75-130 μg/L) in the second and third trimesters; while urinary iodine of Group A remained low during gestation and at delivery.

Study results showed that total T4 levels of all groups increased during the second and third trimesters compared to those measured during the first trimester. However, the increases observed in Group A (4 percent and 7 percent for the second and third trimesters, respectively) were much smaller than those observed in Group B (9 percent and 11 percent) and Group C (19 percent and 15 percent). Glinoer *et al*. (1995) also reported that in Groups A and B, the ratios of T3/T4 were higher than normal at the start of the therapy and remained elevated during gestation. In contrast, the ratios decreased rapidly toward normal and were maintained at an level of approximately $22x10^{-3}$ in Group C. These results indicated that thyroid stimulation associated with pregnancy and leading to preferential T3 secretion by the thyroid was suppressed after potassium iodide plus L-T4 administration. Glinoer *et al*. (1995) found an average increase of 30 percent in thyroid volume in Group A. Sixteen percent of the women in this group developed a

[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.

goiter during gestation, with thyroid volume up to 34 mL at delivery. The increment in thyroid volume was much less in Group B (mean increase of 15 percent) and in Group C (mean increase of 8 percent). Furthermore, goiter formation in Groups B and C was less frequent than that in Group A, as it was observed in only 10 percent and 3 percent of the cases, respectively. In the same study, Glinoer *et al*. (1995) also evaluated the thyroid status of the newborns, 3-6 days after delivery. They found the mean thyroid volume of newborns in Group A (1.05±0.05 mL) was significantly larger than those in Groups B (0.76±0.05 mL) and Group C (0.75±0.05 mL). Furthermore, glandular hyperplasia (thyroid volume >1.4 mL) was found in 10 percent of newborns in Group A (range 1.5-2.2 mL) compared to none in Groups B and C ($p=0.01$, by χ^2 test). Glinoer *et al*. (1995) found the study results in agreement with other investigations on goitrogenesis during pregnancy in areas with less than adequate iodine supply. They attributed the significantly study findings on the goitrogenic role of pregnancy to the selection of pregnant women at the extreme fringe of the population.

Rotondi *et al*. (2000) reported that in areas of moderate iodine deficiency, there is a significant association between a larger thyroid size, in healthy women, with the number of their previous pregnancies. They studied the size of thyroids of 208 nongoitrous healthy females by ultrasound examination. All subjects lived in a region (Naples, Italy) that is known to have moderate iodine deficiency, with usual urinary levels ranging from 40-100 µg/day. All subjects underwent serum free T3, free T4, and TSH determinations, as well as thyroglobulin antibody and thyroid peroxidase antibody detection. All subjects were clinically and biochemically euthyroid and had no detectable thyroid autoantibodies. The subjects were divided into five groups, according to the number of completed pregnancies (0, 1, 2, 3, 4 or more term pregnancies). The researchers found mean thyroid volume increased progressively among the groups; group 0 (14.8±0.7 ml); group I (16.0±0.9 ml); group II (17.1±0.6 ml); group III (18.2±0.6 ml); group IV (20.3±0.9 ml). The increase in thyroid volume was statistically significant between group 0 and groups III ($p<0.01$) and IV ($p<0.001$), and also between group I and group IV ($p<0.05$). No independent effect of body weight and age on thyroid volume was seen. Based on the results, Rotondi *et al*. (2000) suggested that, in an area with moderate iodine deficiency, there is a cumulative goitrogenic effect of successive pregnancies and the goitrogenic effect of pregnancy is not fully reversible.

However, a survey of the scientific literature shows that not all researchers found an association between pregnancy and enlarged thyroids. Gerghout *et al*. (1994) studied 10 healthy women before and during a normal pregnancy in an iodine replete area of Amsterdam, the Netherlands. They found no change in thyroid volume during pregnancy (data given before pregnancy and during first, second, and third trimesters, respectively: 10.3±5.1, 10.6±4.4, 9.6±3.8, and 9.4±3.0 mL. Urinary iodine levels were not measured and dietary iodide intake levels were not estimated by the authors.

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.

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.

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.

Adverse neurological development in infants born to mothers with iodine deficiency or low thyroid hormone levels

The changes in thyroid function associated with pregnancy are related to increased hormone requirements. The need can only be met by proportional increased hormone production and is dependent upon the availability of iodine in the diet (Glinoer, 2001). For this reason, the 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). These values are approximately 50 percent higher than the Estimated Average Requirement of 95 µg/day and the Recommended Dietary Allowance of 150 µg/day determined for adults (age 19 years and older).

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 as well as the degree of iodine deficiency. The most severe problems caused by iodine deficiency are among fetuses, neonates, and infants because of the irreversible changes that can occur during this period of rapid structural and behavioral development. Cognitive impairment is the most common finding seen with iodine deficiency and thyroid disorders during pregnancy 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. Several researchers also reported that nuclear T3 receptors in brain tissues obtained from rat and human fetuses early in gestation (before the development of the fetal thyroid) were relatively saturated with T3 (Bernal and Pekonen, 1984; Perez Castillo *et al*., 1985; Ferreiro *et al*., 1988; as cited in Burrow *et al*., 1994). The presence of occupied T3 nuclear receptors in brain tissues early in fetal development supports a role for maternal thyroid hormones in the maturation of the brain.

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).

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

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 maternal hypothyroidism was associated with lower intelligence quotient scores (IQs) in 8-month-old infants. Hypothyroidism was defined in this study by two low serum butanol extractable iodine test values during pregnancy or by one low serum butanol extractable iodine value with clinical hypothyroidism. They found that 81 percent of 26 infants of women given thyroid replacement therapy after two low serum 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)

Age in years	T4 < 2 µg/dL and bone surface measures < 0.05 cm^2			T4 > 2 µg/dL and/or bone surface measures > 0.05 cm^2		
	n	Mean IQ	IQ distribution	n	Mean IQ	IQ distribution
3	17	91 ± 4 *	(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 based on degree of skeletal maturity at the time of diagnosis. Forty-five children with bone age <36 weeks were assigned to the delayed group; 35 with bone age 37 to term were assigned to the nondelayed group. Both groups were treated for congenital hypothyroidism and the initial starting dosages of L-thyroxine for the delayed and nondelayed were similar, 8.1 mg/kg and 7.8 mg/kg, respectively. Although most children with athyrosis were found in the delayed group, the group did not differ in birth weight, hormone levels, or family background. Hormone levels at diagnosis of both groups are shown in Table 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 Schoolchildren and Daily Urinary Iodine Excretion in Adults (1976-1984) 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 number of observations is given in parentheses.
The prevalences and daily urinary excretion were established between 1976 and 1979.
Area A vs. area B: χ^2 =112; p<0.001, t =2.43; p<0.05.
Area A vs. area C: χ^2 =111; p<0.000005, t =8.98; p=0.
Area B vs. area C: χ^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 as 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)

Percent in parentheses. Performance score: defective, below –1 standard deviation from average score of normal children of the same age; borderline, equal to –1 standard deviation from average score; nondefective, higher than –1 standard deviation from average score.
Statistical comparisons:
Defective
Area A vs. area B: χ^2 =2.75; p=0.87 (NS);
Areas A+B vs. area C: χ^2 =36.25; p<0.000001
Borderline
Area A vs. area B: χ^2 =1.22; p=0.27 (NS);
Areas A+B vs. area C: χ^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: χ^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.

Bleichrodt and Born (1994) performed a meta-analysis on the data from 21 iodine and mental development studies. A study was selected if it contained information on the general cognitive functioning of children and adults living in iodine-deficient areas and if it gave the necessary statistical data. Three of the studies were excluded from the analysis because the composition of the groups studied was different (they were composed exclusively of school children). The remaining 18 studies formed a homogeneous group. In the meta-analysis of the effects of iodine deficiency on cognitive development, a large effect size was found with a d-value of 0.90. This means that the mean scores for the two groups studied, the iodine-deficient group and the noniodine-deficient group, are 0.90 of a standard deviation (or 13.5 IQ points), apart. In other words, a typical child with an average score in the noniodine-deficient group scores higher than 82 percent of the children from the iodine-deficient group, assuming the IQ scores of the two groups are normally distributed.

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 5th (<9.8 pmol/L, n=11) and 10th (<10.4 pmol/L, n=22) percentiles at 12 weeks' gestation had significantly lower scores on the Bayley Psychomotor Developmental Index 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.

In another study, Pop *et al.* (2003) reported that maternal hypothyroxinemia during early pregnancy was associated with a delay in infant neurodevelopment. They followed 115 children and their mothers for two years. Maternal hypothyroxinemia was defined as having free T4 below the lowest tenth percentile and TSH within the reference range. All children had normal Apgar scores at birth and normal screening results for congenital hypothyroidism on the seventh postpartum day. Pop *et al.* observed that children of women who had hypothyroxinemia during early gestation and who exhibited a further decrease of free T4 during gestation had the lowest mental/motor scores. In contrast, children whose mother showed early hypothyroxinemia, but whose free T4 levels increased during later gestation, did not show any delay in development.

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 99.7th percentile of the values for all the pregnant women, 15 women with values between the 98th and 99.6th 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	Hypothyroidism (N=62)	Controls (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 serum T4 from μg/dL to nmol/L or free T4 from ng/dL to pmol/L, multiply by 12.87.

In a follow-up study, Klein *et al.* (2001) studied serum TSH concentrations of pregnant mothers at a mean of 17 weeks gestation and the standard neuropsychological testing results of their offspring at a mean age of 8 years. They found there was an inverse correlation between the severity of maternal hypothyroidism and IQs in the offspring and suggested that the result supports a causal association of maternal hypothyroidism and poor cognitive development of offspring. Klein *et al.* (2001) divided the mothers and their offspring into three groups: group 1, 124 control mothers with TSH concentrations <98th percentile; group 2, 28 hypothyroid mothers with TSH concentrations between the 98th and 99.85th percentile; group 3, 20 hypothyroid mothers with TSH concentrations ≥99.85th percentile. Mothers treated for hypothyroidism during pregnancy were excluded from the study. They found the mean (standard deviation) for the children of the 124 control mothers was 107 (12). Means (standard deviation) for the children in groups 2 and 3 were 102 (15) and 97 (14), respectively. The difference between the children in group 3 mothers differed significantly from those of group 1 mothers (p=0.003). The mean for group 2 children was intermediate between those for the group 1 and group 3 children but not statistically significantly different from either. The incidences of IQs greater than one standard deviation below the control mean were 15 percent, 21 percent, and 50 percent for the children in group 1, group 2, and group 3, respectively. In a related study, the same authors also reported spontaneous abortions and intra-uterine fetal deaths were more than five times as common in the mothers with TSH concentrations above the 98th percentile than in control mothers with TSH concentrations below the 98th percentile.

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

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

(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 the early part of their pregnancies.

Fenzi *et al*. (1990) conducted neuropsychological assessments on a group of 384 school children (aged 6-14 years) residing in an area of known iodine deficiency (Tuscany, Italy). 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 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 10th gestation weeks ranged from 2.3 to 6.3 pmol/L (normal range,

11.6 to 24.5 pmol/L) in six of the eight cases. In the other two cases, maternal total T4 values were 52.8 and 30.9 nmol/L (normal range, 92.7 to 218.8 nmol/L). TSH levels of the eight mothers at that time ranged from 25 to 190 mU/L (normal range < 5 mU/L). Maternal T4 and TSH levels became normal after T4 supplementation by 13 to 28 weeks of gestation. Seven of the eight children had nine siblings who had not been exposed to maternal hypothyroidism throughout gestation (Group 2); they were used as controls. Ages of the children in groups 1 and 2 at the time of IQ examination were 4 to 10 years in group 1 and 4 to 15 years in group 2. The investigators reported that all children in group 1 showed normal IQs. There was no 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.

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 results from a number of animal studies 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 the risk of thyroid tumors.

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

Using data derived from animal studies, Clewell *et al.* (2003) developed a PBPK model to predict the distribution and the NIS inhibition effect of perchlorate in rats of different life stages (e.g., adult male, pregnant female, fetus, lactating female, and neonate). The model predicted that the fetal rat thyroid is most vulnerable to the inhibitory effect of perchlorate on the uptake of iodine by the thyroid.

Human data

According to the California Safe Drinking Water Act of 1996 (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.

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

Results of a prospective study reported by Kung *et al.* (2000) showed that in a borderline iodine-sufficient area (median urinary iodine level = 9.8 µg/dL), pregnancy can pose a stress on the thyroid, resulting in higher rates of maternal goitrogenesis as well as neonatal hypothyroxinemia and hyperthyrotrophinemia. It was also noted that thyroid enlargement in these women persisted and failed to revert completely even 3 months after delivery.

There are several epidemiological studies indicating that iodine deficiency during pregnancy may adversely affect brain development and cause neurointellectual deficits in the offspring. These effects are not limited to areas with severe iodine deficiency and endemic cretinism. The severity of effects appears to depend on the timing and the severity of iodine deficiency and thyroid disorder. In several studies conducted in areas with moderate or even mild iodine deficiency, mainly from southern Europe, it was shown that developmental abnormalities may also occur in clinically euthyroid schoolchildren. Even borderline iodine deficiency, as observed in some European countries, may be accompanied by impaired school achievements in apparently normal children (Glinoer, 2001).

These studies suggest pregnant women and their fetuses are more sensitive to the anti-thyroid effects of perchlorate, especially when the supply of iodine is less than ideal.

Potential low iodide intakes in women of childbearing age

Urinary iodine concentration is 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 percent 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 26). Focusing on the Western region of the U.S., the results are about the same; median urinary iodine concentration was 15.3±1.0 µg/dL and the fraction of people excreting low concentrations of iodine (urinary iodine <5 µg/dL) was estimated to be approximately 12.7 percent.

It is important to realize that variability of iodine concentration in spot urine samples is likely to be larger than variability of the average annual urinary iodine. This has been illustrated by a study reported by Andersen *et al.* (2001). Serum T4, T3, and TSH as well as urinary iodine excretion were measured longitudinally for a year in a group of 15 healthy men living in an area of mild to moderate iodine deficiency. Andersen *et al.* (2001) found that the variation around the mean urinary iodine concentration was 2.4 times larger when calculated for the 180 individual urine samples than when calculated for the 15 average annual values. Therefore, the fact that 14.9 percent of the spot urine samples collected from women of childbearing age had iodine concentration less than 5 µg/dL does not necessarily mean that 14.9 percent of them were iodine deficient.

Table 26. 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)

	Sample number	Urinary iodine		Iodine/creatinine	
		median	% < 5 µg/dL	median	% < 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

According to the NHANES III data, the median urinary iodide in women of childbearing age (15-44 years) was 12.8 µg/dL. Using an equation linking urinary iodine and daily iodine intake and assuming a body weight of 58 kg for an adult female (ICRP, 1974), a median dietary iodide intake[6] of 174 µg/day can be estimated. Alternatively, if one assumes the 24-hr urine volume for adults is approximately 1.5 L (NAS, 2001), the median urinary iodine of 12.8 µg/dL is roughly equivalent to a median dietary iodide of

[6] Daily iodine intake [µg] = urinary iodine [µg/L] x 0.0235 x body weight [58 kg]) (NAS, 2001).

192 µg/day. These median estimates are very close to or within 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). The National Academy of Sciences determined an Estimated Average Requirement of 160 µg/day and a Recommended Dietary Allowance of 220 µg/day for pregnant women (NAS, 2001). The comparison shown here indicates that while most of the women in the survey received enough iodine to meet the average requirement of 160 µg/day, many were receiving less than the recommended dietary allowance of 220 µg/day.

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 appropriate to examine in the study area of Ireland. They found that the monthly variations in iodine 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.

In consideration that: (1) women with marginally adequate iodide intake are susceptible to hypothyroxinemia and hypothyroidism during pregnancy; (2) there is a possibility that some women in the child-bearing age in the U.S are getting less than the recommended daily iodide intake level; (3) iodide deficiency during early pregnancy may cause neurointellectual deficits in offspring, and (4) exposure to perchlorate is likely to further reduce iodide uptake by the thyroid, OEHHA recommends that perchlorate in drinking water should be kept at a level that does not inhibit iodide absorption by the thyroid.

A number of rat and human studies (Yu *et al*., 2000; Lawrence *et al*., 2000, 2001; Greer *et al*., 2002) have documented the inhibitory effect of perchlorate on iodide uptake by the thyroid; they have been described in this document. To determine a level of perchlorate exposure that would not inhibit thyroidal iodide uptake, OEHHA chose the Greer *et al*. (2002) study as the critical study and applied the benchmark dose approach for the identification of the point of departure. OEHHA used the BenchMark Dose Software, version 1.3.1 provided by U.S. EPA (2002) to perform the analyses based on the human data reported by Greer *et al*. (2002) shown in Table 27. A detailed discussion of the application of the software is provided in a draft U.S. EPA (2000) document, "Benchmark Dose Technical Guidance Document, External Review Draft."

OEHHA tried several curve fitting models provided by the software and found the Hill model[7] adequately describes the data (goodness of fit test, p=0.46), shown plotted in Figure 10. The fit is generally considered adequate when the p-value is greater than 0.05.

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

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

where:

intercept = 0
v = -73.4469
n = 1.15067
k = 0.0663651.

Table 27. Benchmark Dose Modeling of the Human Data of Greer *et al*. (2002)

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

[7] The Hill model was run with the following settings: intercept = zero, power parameter restricted to be greater than one, a constant variance model assumed.

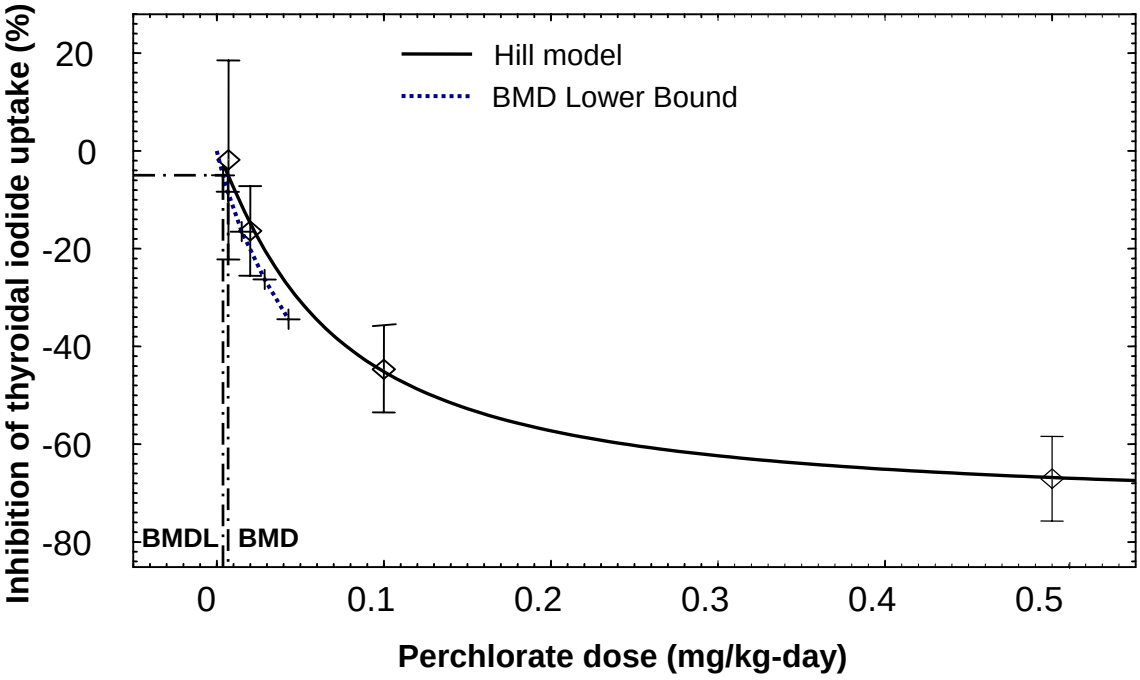

Figure 10. Analysis of the Greer *et al*. (2002) data by the benchmark dose approach.

Two approaches have been suggested by U.S. EPA (2000) for identification of the point of departure for continuous data:

(a) a minimal level of change in the response that is generally considered to be biologically significant; and

(b) a change in the mean equal to one control standard deviation from the control mean.

For this analysis, a five percent decrease of mean radioactive iodine uptake by the thyroid is defined as the point of departure or the benchmark dose (BMD). Approach (a) is selected over approach (b) for this data set because there is no control group in the Greer *et al.* (2002) study. The standard deviation estimated from the lowest exposed group, 0.007 mg/kg-day, is relatively large because it includes both interindividual variability and day-to-day intraindividual variability.

The lower limit of a one-sided 95 percent confidence interval on the BMD can be defined as the BMDL. The estimated BMD and BMDL corresponding to a five percent reduction of the mean thyroidal iodide uptake are 0.0068 mg/kg-day and 0.0037 mg/kg-day, respectively. It should be noted that the BMDL of 0.0037 mg/kg-day is lower than the lowest dose tested, 0.007 mg/kg-day, in the Greer *et al*. (2002) study.

U.S. EPA recommends benchmark dose methods to estimate reference doses (RfDs), which are used along with other scientific information to set criteria and standards for noncancer human health effects. Until recently, RfDs have been determined from NOAELs, which represent the highest experimental dose for which no adverse health effects have been documented. Using the NOAEL in determining RfDs has long been recognized as having limitations in that it 1) is limited to one of the doses in the study and is dependent on study design; 2) does not account for variability in the estimate of the dose-response; 3) does not account for the slope of the dose-response curve; and 4) cannot be applied when there is no NOAEL, except through the application of an uncertainty factor. A goal of the benchmark dose approach is to define a starting point of departure for the computation of a reference value (RfD) or slope factor that is more independent of study design. OEHHA agrees with U.S. EPA on this issue and uses a BMDL of 0.0037 mg/kg-day as the point of departure in the quantitative human health risk assessment of perchlorate.

Carcinogenic Effects

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

Several subchronic oral studies in rodents showed that perchlorate induced hypertrophy and hyperplasia in the thyroid gland (Caldwell *et al*., 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998b,d, 1999, 2001; Keil *et al*., 1998). In two

chronic oral studies, perchlorate at relatively high concentrations (over 1,000 mg/kg-day) was shown to produce tumors in rats (Kessler and Kruskemper, 1966) and mice (Pajer and Kalisnik, 1991). However, only benign tumors were observed in the study reported by Kessler and Kruskemper (1966), and inadequate reporting and low survival of the control and exposed animals lowered confidence in the results reported by Pajer and Kalisnik (1991). 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 noteworthy (U.S. EPA, 2002).

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

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

One factor that may play a role in interspecies quantitative sensitivity to thyroid stimulation 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 28, 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).

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). 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.

Table 28. Inter- and Intraspecies Differences of T3, T4, and TSH Levels and Sensitivity to Thyroid Cancer (Modified from U.S. EPA, 1998b)

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

As U.S. EPA (1998b) described in the "Assessment of Thyroid Follicular Cell Tumors," it is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid, and 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 indicates perchlorate is a genotoxic agent. Perchlorate is known to inhibit the uptake of iodide in the thyroid, thereby causing a reduction in the hormones T3 and T4. Subchronic and chronic drinking water studies showed that perchlorate exposure depressed serum T3 and T4 but elevated serum TSH levels in rodents and rabbits. At higher exposure levels, thyroid follicular cell hypertrophy, thyroid follicular cell hyperplasia, and increased thyroid weights were also observed in adults as well as postnatal rats (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 days as well as 90 days. However, no significant increases in both absolute and relative thyroid/parathyroid weights were observed in male rats exposed to 10 mg/kg-day for 90 days, followed by a 30-day recovery period. Similarly, absolute and relative thyroid/parathyroid weights were significantly increased in female rats exposed to 10 mg/kg-day for 90 days, but no significant increases in terms of both absolute and relative thyroid/parathyroid weights were observed in female rats exposed to 10 mg/kg-day for 90 days, followed by a 30-day recovery period.

The available data 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, there would be no thyroid tumors. For this reason, the perchlorate dose determined for prevention of 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. The nature and severity of the effects are related to the extent of exposure and the iodine status of the individual.

As discussed in the previous section, pregnant women and their fetuses are considered sensitive subpopulations, especially those who are not getting the required amount of iodine. OEHHA also identified three additional sensitive subpopulations, (i) lactating women, especially those who are getting less than the sufficient amount of iodine, (ii) infants, and (iii) individuals with thyroid problems.

Lactating mothers are considered a sensitive subpopulation because their need of iodine is greater and thus they are more at risk of getting an insufficient amount of iodine from the diet. NAS (2001) suggests an Estimated Average Requirement and a Recommended Dietary Allowance of iodine of 209 µg/day and 290 µg/day, respectively, for lactating mothers. These levels are almost two-fold higher than the Estimated Average Requirement of 95 µg/day and the Recommended Dietary Allowance of 150 µg/day determined for adults (age 19 years and older).

At sufficiently high doses, perchlorate can inhibit the NIS in the mammary gland and reduce the secretion of iodide into the breast milk. Since breast milk is the sole source of iodine for some infants and iodine is necessary for normal brain development, an adequate level of iodine in breast milk is vital to the well-being of breast-fed infants. Laurberg *et al.* (2004) reported that smoking by nursing mothers was associated with a reduction in iodine in breast milk and a decrease in urinary iodine in neonates. Tobacco smoke is known to contain thiocyanate, which is also a NIS inhibitor.

Perchlorate has been detected in human breast milk, showing that breast milk is a viable exposure pathway. There is information indicating that neonates are less capable of maintaining normal thyroid hormone production by using iodide stored in the thyroid, if there is a reduction in thyroidal iodide uptake. Van de Hove *et al.* (1999) demonstrated that newborns have less thyroid hormone stored in the thyroid gland and the turnover rates of the intrathyroidal pool of T4 in the preterm and term newborns are much higher than that of adults.

Finally, individuals with thyroid problems or impaired thyroid functions are also believed to be more sensitive to the anti-thyroid effects of perchlorate.

The following equation was used to estimate health-protective water concentrations (C, in mg/L) for pregnant women:

State and Tribal Advisory Levels for Perchlorate (U.S. EPA, 2003; DHS, personal communication)

California	6 ppb
New York	5 ppb and 18 ppb
Texas	4 ppb, 7 ppb or 10 ppb
Arizona	14 ppb
Massachusetts	1 ppb
Maryland	1 ppb
New Mexico	1 ppb
Nevada	18 ppb

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