Studies Identified by EPA Office of Water for the SAB Perchlorate Advisory Panel Meeting (as of June 13, 2012)

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Huber, D.R., B.C. Blount, D.T. Mage, F.J. Letkiewicz, A. Kumar, and R.H. Allen. 2011. Estimating perchlorate exposure from food and tap water based on U.S. biomonitoring and occurrence data. J. Expo. Sci. Environ. Epidemiol. 21(4):395-407.
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Gestational Exposure to High Perchlorate Concentrations in Drinking Water and Neonatal Thyroxine Levels

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Objective: To assess the effect of gestational perchlorate exposure through drinking water on neonatal thyroxine (T_4). *Design*: T_4 values were compared among newborns in Ramat Hasharon, Israel, whose mothers resided in suburbs where drinking water contained perchlorate ≤340 μg/L (very high exposure, $n = 97$), 42–94 μg/L (high exposure, $n = 216$), and <3 μg/L (low exposure, $n = 843$). In the very high and high exposure areas, T_4 values in newborns whose mothers drank tap water exclusively (as determined by a telephone interview) were analyzed as a subset. Serum perchlorate levels in blood from donors residing in the area were used as proxy indicators of exposure. *Main outcome*: Neonatal T_4 values (mean ± SD) in the very high, high, and low exposure groups were 13.9 ± 3.8, 13.9 ± 3.4, and 14.0 ± 3.5 μg/dL, respectively (p = NS). Serum perchlorate concentrations in blood from donors residing in areas corresponding to these groups were 5.99 ± 3.89, 1.19 ± 1.37, and 0.44 ± 0.55 μg/L, respectively. T_4 levels of neonates with putative gestational exposure to perchlorate in drinking water were not statistically different from controls. *Conclusion*: This study finds no change in neonatal T_4 levels despite maternal consumption of drinking water that contains perchlorate at levels in excess of the Environmental Protection Agency (EPA) drinking water equivalent level (24.5 μg/L) based on the National Research Council reference dose (RfD) [0.7 μg/(kg · day)]. Therefore the perchlorate RfD is likely to be protective of thyroid function in neonates of mothers with adequate iodide intake.

Introduction

Perchlorate can affect thyroid function by competitive inhibition of iodide uptake into the thyroid. Potassium perchlorate was used in the 1950s and 1960s for treatment of hyperthyroidism at doses of 400–2000 mg/day for many weeks or months. This use of perchlorate ceased by the late 1960s because it was found to cause aplastic anemia and agranulocytosis, including seven fatalities (1,2). Interest in perchlorate has resurged due to its widespread contamination of drinking water from military and other industrial uses. In Arizona, California, and Nevada alone, the drinking water supply for about 20 million people is contaminated to some degree by perchlorate (3). The range of proposed regulation of perchlorate in drinking water varied between 2 μg/L (4) and 220 μg/L, as suggested by the Department of Defense (5). A too stringent drinking water standard for perchlorate would necessitate an expensive cleanup as 35 states have more than 4 μg/L of perchlorate in drinking water. Drinking water standards are preferably based on risk assessment of the critical effect of a chemical in humans when data are available (6). To account for the intraspecies variability, studies should consider the most sensitive population—in the case of perchlorate, fetuses of pregnant women (2). The National Research Council (NRC) of the National Academy of Sciences recommended a reference dose (RfD) of 0.7 μg/(kg · day) based on a clinical study of 37 adult volunteers by Greer *et al.* (1). In this study, the no-observed-effect level (NOEL) for inhibition of iodide uptake by the thyroid was 7 μg/(kg · day) perchlorate. An additional uncertainty factor of 10 was introduced to protect the most sensitive population, that is, fetuses of pregnant women. A drinking water equivalent level (DWEL) of 24.5 μg/L can be extrapolated from the RfD based on a 70-kg person drinking 2 L water/day, and no perchlorate exposure from other sources.

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Data on the effect of perchlorate exposure on thyroid function in susceptible populations would provide a stronger basis for risk assessment. Several epidemiological studies have assessed the association between exposure to perchlorate in pregnant women and thyroid function of their offspring (3,7–11). Perchlorate concentrations in drinking water were up to 115–120 μg/L in two studies from Chile (8,12), and <16 μg/L in five reports from the United States. All studies except that of Brechner *et al*. (3) showed no association between exposure to perchlorate in pregnant women and effect on thyroid functions in their newborns. The methodology of the Brechner study has been severely criticized (13,14). These U.S. studies, as well as the present study (see "Discussion"), were conducted in iodine-sufficient areas and did not include individual iodine status measures. These studies are insufficient because, with the exception of Tellez *et al*. (12), they were ecological studies, which did not ascertain actual exposure of the individual pregnant mother to perchlorate. The Tellez *et al*. (12) study from Chile reported direct measurements of perchlorate intake and showed that gestational exposure to perchlorate at 114 μg/L in drinking water did not cause changes in neonatal thyroid function or fetal growth retardation. This study had not been peer reviewed at the time of the NRC assessment. Direct evidence on the effect of gestational perchlorate exposure on thyroid functions of newborns in humans was judged inadequate; therefore, experimental data in adults were reviewed as a second best alternative. Since the critical effect of perchlorate is impaired thyroid function in human fetuses, data on the effect of high gestational exposure to perchlorate on the thyroid function of newborns would contribute to a more reliable basis for its regulation in drinking water.

Herein we tested the null hypothesis that there would be no significant differences in neonatal thyroxine (T_4) levels following high gestational exposure to perchlorate in drinking water, in iodine-sufficient pregnant women. Our study addressed perchlorate concentrations in drinking water that were substantially above the recommended DWEL of the EPA as derived from the RfD established by the NRC (2).

Materials and Methods

Population and water perchlorate data

Ramat Hasharon is a city located in central Israel close to the Mediterranean coast with 35,600 inhabitants in 2004. Perchlorate contamination of groundwater was found in local wells that had been in the vicinity of a military plant that was active for decades (Fig. 1). Until October 2004, drinking water was supplied to this city exclusively from local wells (mainly wells A–E), whereas well H mainly supplied the suburb Morasha with 15,000 inhabitants, located in close proximity to the military plant.

In August 2004, water samples (in duplicates) were sent for assessment of perchlorate levels to two laboratories in the United States, Knoxville, Tennessee, and Savannah, Georgia. The following results were reported: the well that supplied Morasha had a perchlorate concentration of 684 μg/L as reported by the laboratory in Savannah and 1100 μg/L as reported by the laboratory in Knoxville. The wells supplying the other neighborhoods in Ramat Hasharon had perchlorate water concentrations in the range 63–88 μg/L (Savannah laboratory) and 50–62 μg/L (Knoxville laboratory). Upon receiving these results, the Israeli Ministry of Health ordered an immediate closure of the well in Morasha, whereas the other wells remained active until July 2005. A second set of water samples was taken from these wells on October 4, 2004, and the perchlorate

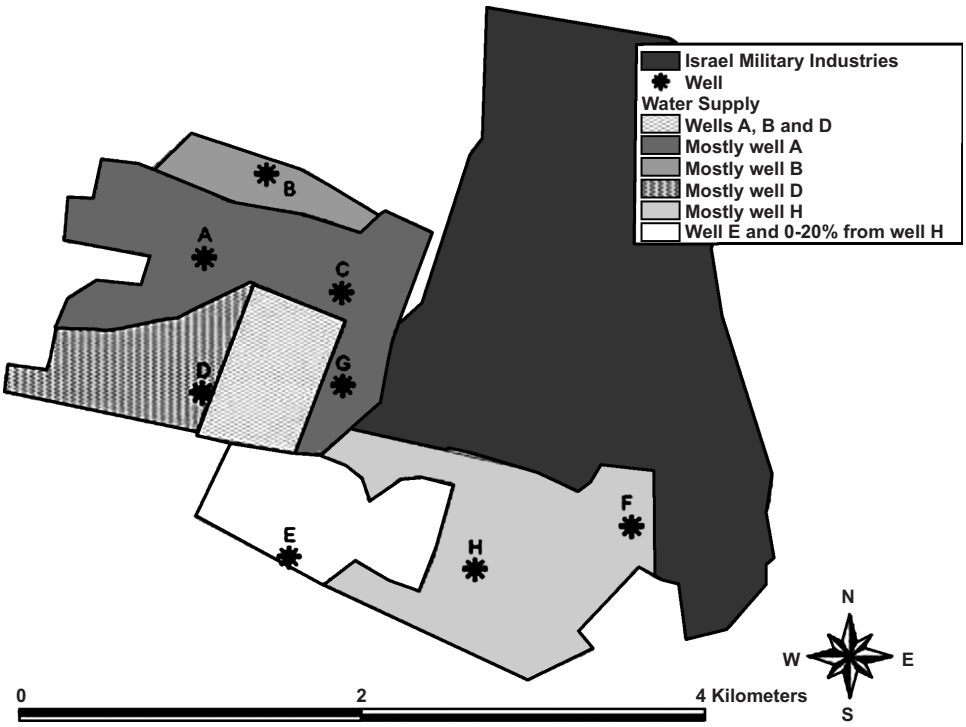

FIG. 1. Map showing well H, which was the primary drinking water source for Morasha (light gray area at the bottom). Wells A through E (remaining area in the figure) supplied the remaining area of Ramat Hasharon.

concentration in the well at Morasha remained 1100 μg/L. The wells supplying the other neighborhoods had perchlorate concentrations of 49–68 μg/L in October 2004, and 42–94 μg/L in March and April 2005. Four tap water samples taken from homes in these neighborhoods in April 2005 had similar concentrations of perchlorate (42–80 μg/L) as determined by the Savannah laboratory. By contrast, only one residential tap water sample in the highest exposure area was assayed for perchlorate before its closure on October 4, 2004, and the concentration was 340 μg/L.

The study population consisted of all singleton newborns born during the period January 1–September 30, 2004, whose mothers resided in Morasha (very high exposure group), the other neighborhoods of Ramat Hasharon (high exposure group). Only newborns who were born in hospitals and were screened by the Israeli National Newborn Screening (NBS) Program for congenital hypothyroidism (>99%) were considered. Newborns from a neighboring city, Hertzlia (with 83,500 inhabitants), with water perchlorate concentrations <2 μg/L in six samples and 2–2.7 μg/L in five samples, served as a control.

Newborn screening data

The Israeli NBS Program is a nationwide screening program for congenital hypothyroidism and phenylketonuria administered by the Ministry of Health. All newborns undergo blood sampling drawn by heel stick for T_4 and phenylalanine predischarge, at the age of 36–48 hours for >90% of newborns. In premature infants, the sampling is done at the age of 1 week. The same routine is held in all hospitals in Israel. All T_4 blood levels were assayed as previously described (15–17) at the central laboratory of the Sheba Medical Center, Tel Hashomer.

Initial exposure assessment

Figure 1 shows the water supply of Ramat Hasharon with respect to the local wells. A computerized, geo-referenced file of this information was then created (shape-file). All births in Ramat Hasharon during the study period were geo-coded according to maternal residential address. Those infants not geocoded were located manually. This file of geo-referenced births was then linked to the hospital T_4 data using identity number as a unique identifier. Thus T_4 data were superimposed onto the polygonal water supply map using Arcview GIS 3.2. This enabled linking of the two data sources, allowing us to add an estimated gestational exposure status to the T_4 levels measured in each newborn. It was not necessary to use GIS in the assessment of exposure in the control group from Hertzlia. This population was considered to be homogeneous with respect to exposure to perchlorate in drinking water.

Details on drinking water sources in pregnancy

Telephone interviews with mothers were done to assess actual exposure to perchlorate from drinking water. The question asked was "Which water did you usually drink during your pregnancy (including water for tea, coffee and concentrate)?" The options were "water from the tap," "filtered tap water," and "bottled water." Data were grouped for responses "tap water" and others.

This nonblinded telephone interview was conducted with all mothers in the very high and high exposure groups to determine their habits of drinking water during pregnancy (tap water or bottled water). A comparison of T_4 values was done between newborns in the very high and high exposure groups whose mothers drank tap water regularly during pregnancy.

Serum perchlorate concentrations from blood bank donors

To assess perchlorate exposure of the population residing in the three areas, serum samples of blood bank donors obtained during January 2003–September 2004 (before closing the well in the high exposure area) were identified from the Magen David Adom (MDA), National Blood Services, and the Israeli National Serum Bank. Perchlorate assay of these samples was done at the Centers for Disease Control (CDC), in a double-blind analysis. The usage of serum samples was approved by the Israeli Ministry of Health.

Serum perchlorate, thiocyanate, nitrate, and iodide were analyzed by using modifications to previously published methods (18,19). Briefly, 0.5 mL of serum was spiked with an isotopically labeled internal standard isotropically labeled perchlorate ($Cl^{18}O_4^-$), mixed, and incubated (2 minutes) to allow the internal standard to equilibrate into the sample. Subsequently, serum proteins were precipitated using six volumes of cold (−20°C) ethanol; thus protein-bound iodine was precipitated and only free iodide was measured. Samples were centrifuged (3016×g, −5°C) for 35 minutes. Supernatant was transferred to a clean centrifuge tube and evaporated to dryness under a stream of nitrogen at 60°C. The sample was resuspended in 1.0 mL of deionized (DI) water and added to a preconditioned C18 SPE cartridge. The breakthrough fraction and a subsequent 1 mL wash of DI water were collected and mixed, and 1 mL was transferred to an autosampler vial. This solution was subsequently analyzed using ion chromatography–electrospray ionization–tandem mass spectrometry. Perchlorate was quantified based on the peak area ratio of analyte to stable isotope-labeled internal standard. Two quality control pools were analyzed in each analytical batch with unknown samples. Reported results met the accuracy and precision specifications of the quality control/quality assurance program of the Division of Laboratory Sciences, National Center for Environmental Health, CDC (similar to rules outlined by Westgard) (20). Excellent analytical precision was found when serum quality control pools were repetitively analyzed for perchlorate. Analytical response was linear across the calibration range (0.05–500 μg/L). Contamination was assessed by lot screening all reagents and analyzing blanks with each batch of unknowns; no contamination problems were identified.

Statistical analysis

Statistical analysis was done on SPSS 14. After checking for normal distribution, two-tailed t-tests were used to compare T_4 level means. In addition, analysis of variance (ANOVA) was used for mean comparisons with Bonferroni correction. Chi-square analysis was used for nonparametric data. Pearson correlation was utilized on linear data, and the Spearman Rho correlation was employed in nonparametric analysis. A matched control analysis between the high and low exposure groups was also done. $p < 0.05$ was considered statistically significant.

Results

There were 97 eligible newborns in the very high exposure group, 216 in the high exposure group, and 843 in the low exposure group. The concentrations of perchlorate in drinking water, demographic characteristics, birth weight, gestational age of newborns, maternal age, and corresponding neonatal T_4 levels in these three groups are shown in Table 1.

At the first stage of the study, T_4 levels obtained from the national NBS program for congenital hypothyroidism were

Table 1. Perchlorate Exposure: Demographics and Newborn T_4 Levels

Perchlorate in drinking water (μg/L)[a]	n	*Maternal age*[a,b] *Mean (SD)*	*Birth weight*[a] *(g) Mean (SD)*	*Gestational age*[a]*(week) Mean (SD)*	T_4[a] *(μg/dL) Mean (SD)*	Gender (%)
Very high: Morasha (>340)[a]	97	31.2 (4.8)	3182 (516)	38.8 (2.2)	13.93 (3.8)	M 48.5 F 51.5
High:[a,b] Ramat Hasharon (42–94)	216	32.3 (4.3)[b]	3220 (454)	38.7 (3.5)	13.91 (3.4)	M 50.0 F 50.0
Low:[a] Herzlia (< 3)	843	31.4 (4.6)	3228 (450)	39.0 (1.7)	13.98 (3.5)	M 54.3 F 45.7
Total	1156	31.6 (4.6)	3222 (457)	38.9 (2.2)	13.97 (3.5)	M 48.5 F 51.5
ANOVA	1156	$p = 0.046$[b]	NS $p = 0.648$	NS $p = 0.109$	NS $p = 0.952$	Chi-square NS $p = 0.336$

[a]Two-tailed *t*-tests assuming equal variances with Bonferroni correction revealed no significance in any of the parameters ($p > 0.05$).
[b]ANOVA maternal age significantly older in high exposure area (Bonferroni correction $p = 0.056$).

compared between newborns in the three groups. As shown in Table 1, there was no significant difference in T_4 levels between the groups as indicated by a *p*-value of 0.95. These T_4 values were normally distributed in each of the three groups (Fig. 2).

Residing in an area with a very high concentration of perchlorate in the drinking water does not necessarily denote high perchlorate consumption. Therefore, we further investigated the specific patterns of tap water use (e.g., drinking, cooking) during gestation by telephone interview, as an indicator of potential perchlorate exposure. Data on 31 mothers in the very high exposure group and 62 mothers in the high exposure group, who drank tap water, were compared with the low exposure group (Table 2). There were no statistically significant differences in T_4 levels between these groups.

In addition to perchlorate, we considered the effects of potential confounders on T_4 levels in the study population. Neonatal T_4 levels did not correlate with maternal age, birth weight, gestational age, or gender.

A matched control analysis was also done. Twenty-five newborns from the very high exposure area, whose mothers drank tap water and for whom there were complete data, were matched by gender, gestational age, and maternal age with

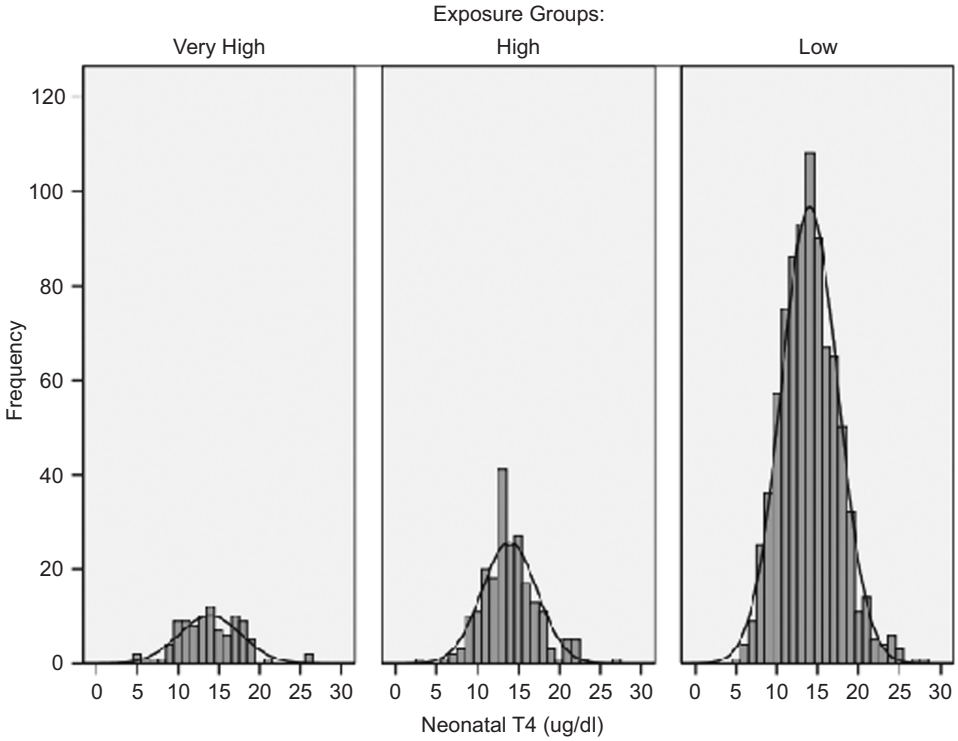

FIG. 2. Normal distribution of T_4 levels in the very high, high, and low exposure groups.

Table 2. Mothers and Newborns from the High and Intermediate Perchlorate Exposure Groups Who Drank Tap Water during Pregnancy Compared with the Low Exposure Group

Perchlorate in drinking water (μg/L)	n	*Maternal age*[a] *Mean (SD)*	*Birth weight (g) Mean (SD)*	*Gestational age (week) Mean (SD)*	*T_4 (μg/dL) Mean (SD)*	Gender (%)
Very high: (≤340) Morasha tap water	31	33.3 (4.9)	3177 (679)	37.8 (3.1)	14.48 (3.9)	M 45.2 F 54.8
High: (42–94) Ramat Hasharon tap water	62	32.3 (4.2)	3164 (519)	38.9 (2.2)	13.76 (3.4)	M 48.4 F 51.6
Low: (<3)[a] Herziliyah	843	31.4 (4.6)[a]	3228 (450)	39.0 (1.7)	13.98 (3.5)	M 54.3 F 45.7
Total	936	31.5 (4.6)	3222 (464)	39.0 (1.8)	13.99 (3.5)	M 53.6 F 46.4
ANOVA		$p = 0.035$[a]	NS $p = 0.699$	$p = 0.058$	NS $p = 0.640$	Chi-square NS $p = 0.418$

[a]Mothers in the low exposure area were significantly younger.

newborns in the low exposure group. The mean T_4 level of newborns in the very high exposure group was 14.7 (SD 4.2) μg/dL and in the low exposure group was 13.5 (SD 3.4) μg/dL; again exposure did not prove to be a significant determinant of neonatal T_4 levels ($p = 0.271$).

Exposure assessment of the populations residing in the very high, high, and low exposure areas was further studied by a double-blind analysis of serum samples from blood bank donors residing in these areas. Figure 3 shows the serum perchlorate levels of blood donors from three proxy populations (residing in the areas corresponding to the three groups). As expected, the serum perchlorate levels of these three proxy populations fall into distinct clusters that correlated well with drinking water perchlorate concentrations from the same areas. Those with the highest serum perchlorate levels (5.99 ± 3.89 μg/L) corresponded with the very high exposure group (≤340 μg/L perchlorate in drinking water); those with high serum perchlorate levels (1.19 ± 1.37 μg/L) corresponded to the high exposure group (42–94 μg/L in drinking water); those with the lowest serum levels (0.44 ± 0.55 μg/L) corresponded to the controls (<3 μg/L perchlorate in drinking water).

Among the blood donors, the difference between serum perchlorate levels of the high and low exposure groups was significant ($p = 0.04$). However, the difference between the very high and low exposure groups was marginally significant ($p = 0.06$), whereas the difference between the very high and high groups was not significant ($p = 0.09$). The lack of statistical significance between the very high and low exposure groups is obviously due to the small sample size in the very high exposure group ($n = 4$).

The potential for inhibition of iodide uptake by the thyroid gland depends on the relative levels of iodide and physiologically relevant competitive inhibitors of iodide uptake (perchlorate, nitrate, and thiocyanate). Therefore iodide, nitrate, and thiocyanate were measured along with perchlorate in the samples from blood donors residing in the combined very high/high (exposed) versus low perchlorate (control) areas. The values (mean ± SD) were as follows: nitrate, 4845 ± 217 and

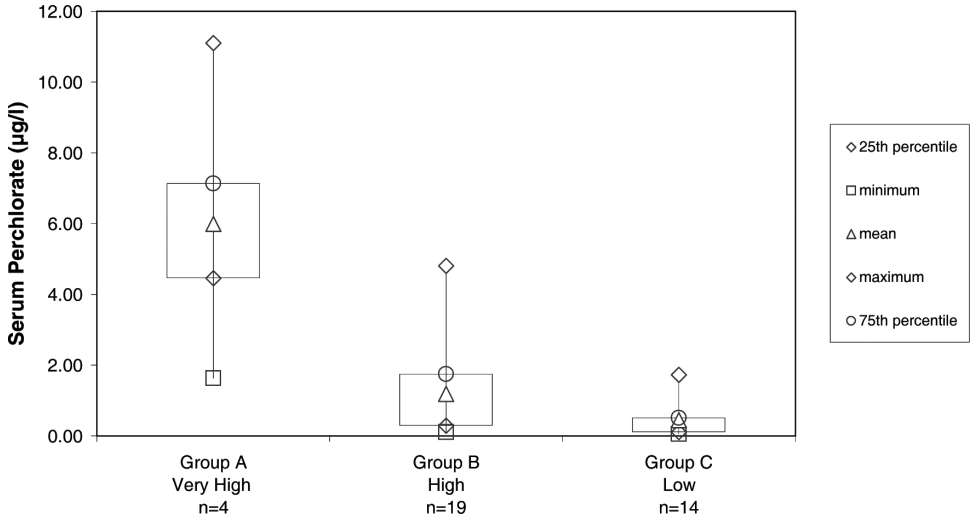

FIG. 3. Serum perchlorate levels of blood donors residing in areas corresponding to groups A (Morasha), B (Ramat Hasharon), and C (Herzlia/control) during 2003–2004.

$4661 \pm 1465\,\mu g/L$ ($p = 0.787$); thiocyanate, 1906 ± 1552 and $1067 \pm 1044\,\mu g/L$ ($p = 0.086$); iodide, 3.10 ± 1.25 and $2.24 \pm 0.85\,\mu g/L$ ($p = 0.031$); and perchlorate, 1.97 ± 2.69 and $0.44 \pm 0.55\,\mu g/L$ ($p = 0.045$), for the exposed and control populations, respectively. p-values are two-tailed.

Discussion

We report on the association between gestational exposure to perchlorate in drinking water and T_4 levels in newborns from Ramat Hasharon, Israel. Serum T_4 of newborns whose mothers drank water with perchlorate concentrations in the range of $42\,\mu g/L$ and above, up to the highest measured level of $340\,\mu g/L$, were similar to those of controls even after exclusion of those whose mothers reported drinking bottled water.

Additionally, serum perchlorate analysis was performed by the CDC on blood donors who resided in the three areas corresponding to various exposure levels. These levels, used as a proxy indicator of exposure, were well correlated with these three exposure groups. Despite likely differences between the proxy blood donors and the pregnant study participants, these proxy samples confirm elevated perchlorate exposure in the residents of Morasha and Ramat Hasharon. It should be noted that perchlorate exposure was measured in blood donors (adults), whereas our study population was pregnant women and their newborns. Our findings indicate that even though pregnant mothers consumed drinking water with perchlorate levels mostly in the range $42–94\,\mu g/L$ and in some at least up to the highest level measured, that is, $340\,\mu g/L$, their newborns presented with normal T_4 levels.

Other potential confounders that may affect T_4 were also measured in blood donors residing in the same areas of the exposed and control population. These donors, used as exposed and control proxy populations, had similar levels of serum thiocyanate and nitrate, while serum perchlorate and iodide levels were significantly elevated in donors residing in the high exposure area.

Iodine intake is important for protecting the thyroid from iodide uptake inhibitors, such as perchlorate. Blount *et al.* (21) recently found that perchlorate exposure is associated with decreased thyroid function in U.S. women with low urinary iodine. Higher serum iodide levels in the high perchlorate exposure area may modulate any perchlorate-induced inhibition of iodide uptake. A previous study of pregnant women in the coastal areas of Israel indicates iodine sufficiency (urinary iodine median $= 143\,\mu g/L$, mean $= 130\,\mu g/L$) (22) based on World Health Organization (WHO) criteria. Another important difference between the current work and the Blount *et al.*'s study is the study population: we examined thyroid function in neonates, while Blount *et al.* (21) studied adults and adolescents. Based on differences in iodine intake and life stage for the two studies, our findings do not contradict those of Blount *et al.* (21).

The critical concern regarding perchlorate contamination of drinking water is the level of exposure that might place sensitive populations at risk. Therefore, it is of the utmost importance to establish a RfD, that is, the dose in mg/(kg body weight · day) that can be tolerated for a lifetime without adverse health effects. The EPA's risk assessment on perchlorate from 2002 (6) was made prior to the appearance of the peer-reviewed publication of the Greer *et al.*'s (1) human exposure study, which showed that $7\,\mu g/(kg \cdot day)$ did not inhibit iodine uptake in healthy adults. A 10-fold safety factor to account for the most sensitive populations applied to this value resulted in the RfD of $0.7\,\mu g/(kg \cdot day)$ proposed by the NRC (2). Ultimately, the EPA also developed independently an RfD of $0.7\,\mu g/(kg \cdot day)$ from which they derived their DWEL of $24.5\,\mu g/L$. NRC recommended an RfD in February 2005. Gibbs (23) noted that $10\,\mu g/L$ perchlorate (converted from Gibbs' original data units of $\mu mol/L$) in serum corresponds to $6\,\mu g/(kg$ body weight · day). This value is very close to the NOEL of $7\,\mu g/(kg \cdot day)$ based on the clinical study of Greer *et al.* (1) and approximately 10-fold greater than the RfD of $0.7\,\mu g/(kg \cdot day)$ proposed by the NRC (2). In our study, serum perchlorate levels of nearly $6\,\mu g/L$ (Fig. 3) were found in subjects residing in the area corresponding to pregnant women in the high exposure group with $\leq 340\,\mu g/L$ perchlorate in their drinking water. If we assume a default drinking water consumption value of 2 L/day (24), subjects in this group drank about $680\,\mu g$/day of perchlorate. When we divide this value by the default body weight of 70 kg (24), it is revealed that subjects in this group consumed approximately $9.7\,\mu g/(kg \cdot day)$ or 38.6% greater than the NOEL [$7\,\mu g/(kg \cdot day)$]. Similarly the high exposure group (drinking water perchlorate levels of $42–94\,\mu g/L$) extrapolates to doses of $1.2–2.7\,\mu g/(kg \cdot day)$, values less than the NOEL, but about 1.7–3.8 times higher than the RfD.

Keeping in mind the concern for fetal and neonatal development, there have been several human studies that testify to the lack of adverse affects in neonatal thyroid function from exposure to perchlorate in drinking water (8–12,25). Most of these considered perchlorate concentrations in the range $6–16\,\mu g/L$, that is, lower than the DWEL of $24.5\,\mu g/L$.

Of the epidemiological studies examining thyroid function in neonates with perchlorate exposure, one ecological study by Brechner *et al.* (3) reported significantly higher median thyroid-stimulating hormone (TSH) levels in age-adjusted newborns attributed to perchlorate. However, this study was criticized by Goodman (13) and Lamm (14) on several grounds. Other cross-sectional studies of newborns in Chile and Nevada did not show the differences reported by Brechner *et al.* (3). Lamm (14) reevaluated the same sampling data used by Brechner *et al.* (3) and found that the differences they reported were regional differences and not due to exposure.

Women from Ramat Hasharon putatively consumed drinking water contaminated with typically $42–94\,\mu g/L$, and those from Morasha, $\leq 340\,\mu g/L$ (the highest measured level of perchlorate in drinking water) during pregnancy. These levels exceed any threshold value recommended to date and, to the best of our knowledge, rival the highest drinking water exposure levels reported during gestation. Despite these exposures, neonatal T_4 levels were in the range of normal and not different from those of infants born to mothers from areas where perchlorate levels were $<3\,\mu g/L$.

Crump *et al.* (8) studied the effect of very high perchlorate exposure in drinking water ($100–120\,\mu g/L$) on thyroid function in schoolchildren. No difference was found in TSH levels or goiter prevalence among lifelong residents from this area compared with those living in a low exposure area (after adjusting for age, sex, and urinary iodine). No presumptive cases of congenital hypothyroidism were detected in those areas with very high to intermediate levels ($5–7\,\mu g/L$) of perchlorate in drinking water, whereas seven cases were detected in control area (perchlorate undetectable). Neonatal TSH levels were significantly lower in the highly exposed group compared with controls; this is opposite to the known pharmacological effect of perchlorate, and of small magnitude. Both our study and the Crump study (8) indicate that perchlorate in drinking water at concentrations as high as $100–120\,\mu g/L$ does not suppress thyroid function in newborns or school-age children, respectively. We note that perchlorate exposure levels in Ramat Hasharon ($48–92\,\mu g/L$) approach those in Taltal, Chile, and the serum

perchlorate levels in Ramat Hasharon (nd–6 μg/L) approach the serum perchlorate levels (2.2–8.9 μg/L) found in children in Taltal (26). In a more recent report of the same areas of Chile studied by Crump *et al*. (8), Tellez *et al*. (12) found comparable thyroid function during pregnancy and postpartum pregnant women from subjects exposed to various perchlorate concentrations.

The relative resistance of the thyroid gland to perchlorate exposure is most likely explained by normal homeostatic mechanisms of thyroid hormone metabolism. Inhibition of iodine uptake via the sodium/iodide symporter (NIS) is only the first step in a complex sequence of physiological events that regulate thyroid gland function (27), and it is reversible (28). Below the threshold for inhibition of iodine uptake, there would not be any effect, and some studies have indicated that TSH is not affected by long-term exposure to higher perchlorate levels (8,29). Lawrence *et al*. (30) showed that whereas daily consumption of 10 mg perchlorate for 2 weeks significantly decreased iodide uptake by the thyroid gland, neither circulating thyroid hormone nor TSH concentrations were affected. An occupational study of healthy male workers (29) showed that the thyroid can compensate for long-term exposure to perchlorate by increasing the efficiency of iodine uptake. In their physiologically based pharmacokinetic analysis of perchlorate inhibition of iodine uptake in the rat, Clewell *et al*. (31) emphasized that overall risk to the fetus may be less than to the neonate because during gestation the fetus can obtain thyroid hormones from the mother. According to Clewell *et al*. (31), "the maternal hormones may compensate for the increased inhibition seen in the fetal thyroid resulting in less chance of adverse developmental effects." However, the maternal–fetal T_4 transfer is not sufficient to prevent the effects of *in utero* hypothyroidism (18). This was demonstrated by very high TSH and the lack of epiphyseal nuclei in athyreotic newborns of euthyroid mothers (18).

A public health goal for perchlorate is prevention of its potential to cause neurodevelopmental deficits in newborns that might arise from maternal or neonatal hypothyroidism. More definitive evidence of the safety of gestational perchlorate exposure in Ramat Hasharon will require further study of neurobehavioral development in infants whose mothers were exposed to high perchlorate levels during pregnancy. In their ecological study of Nevada children, Chang *et al*. (32) found no evidence of increased risk of attention-deficit hyperactivity disorder or autism caused by perchlorate contamination of drinking water up to 24 μg/L (the DWEL proposed by the EPA). These authors also reported no differences in fourth-grade school performance. However, as recognized by Chang *et al*., there were limitations to their study pertaining to diagnostic criteria and the ability to ascertain geographic and demographic differences and, perhaps more importantly, to data on individual residence and water consumption during pregnancy. Such studies are important for Ramat Hasharon, where perchlorate contamination far exceeded that in Nevada.

In conclusion, our data and those of others (6–10,32) found no association between neonatal thyroid function and maternal consumption of drinking water that contains perchlorate at levels in excess of the DWEL (24.5 μg/L). Because this DWEL derives from the RfD, the perchlorate RfD of 0.7 μg/(kg · day) is likely to be protective of thyroid function in neonates of mothers with adequate iodine intake.

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Disclaimer

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Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States

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Background: Perchlorate is commonly found in the environment and known to inhibit thyroid function at high doses. Assessing the potential effect of low-level exposure to perchlorate on thyroid function is an area of ongoing research.

Objectives: We evaluated the potential relationship between urinary levels of perchlorate and serum levels of thyroid stimulating hormone (TSH) and total thyroxine (T_4) in 2,299 men and women, ≥ 12 years of age, participating in the National Health and Nutrition Examination Survey (NHANES) during 2001–2002.

Methods: We used multiple regression models of T_4 and TSH that included perchlorate and covariates known to be or likely to be associated with T_4 or TSH levels: age, race/ethnicity, body mass index, estrogen use, menopausal status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate, and selected medication groups.

Results: Perchlorate was not a significant predictor of T_4 or TSH levels in men. For women overall, perchlorate was a significant predictor of both T_4 and TSH. For women with urinary iodine < 100 µg/L, perchlorate was a significant negative predictor of T_4 ($p < 0.0001$) and a positive predictor of TSH ($p = 0.001$). For women with urinary iodine ≥ 100 µg/L, perchlorate was a significant positive predictor of TSH ($p = 0.025$) but not T_4 ($p = 0.550$).

Conclusions: These associations of perchlorate with T_4 and TSH are coherent in direction and independent of other variables known to affect thyroid function, but are present at perchlorate exposure levels that were unanticipated based on previous studies.

Key words: exposure, iodine, NHANES, perchlorate, thyroid, thyroxine, TSH. *Environ Health Perspect* 114:1865–1871 (2006). doi:10.1289/ehp.9466 available via *http://dx.doi.org/* [Online 5 October 2006]

Perchlorate is an inorganic anion used for a variety of products such as road flares, explosives, pyrotechnics, and solid rocket propellant (Mendiratta et al. 1996). Perchlorate can also form naturally in the atmosphere, leading to trace levels of perchlorate in precipitation (Dasgupta et al. 2005). Natural processes are considered to concentrate perchlorate in some locations such as regions of west Texas (Dasgupta et al. 2005) and northern Chile (Urbansky et al. 2001). A combination of human activities and natural sources has led to the widespread presence of perchlorate in the environment. As of November 2005, perchlorate was detected in drinking water samples from 4.1% of community water supplies in 26 different states, with levels ranging from the method detection limit of 4 µg/L to a maximum at 420 µg/L [U.S. Environmental Protection Agency (EPA) 2005]. Most of this drinking-water contamination is likely due to contaminated source waters, although in rare instances perchlorate formation has been reported to occur in water distribution systems (Jackson et al. 2004). Additionally, perchlorate exposure from the diet is probable because of the contamination of milk (Kirk et al. 2005), vegetables (Sanchez et al. 2005), fruit (Sanchez et al. 2006a), grain (Sanchez et al. 2006b), and forage crops (Jackson et al. 2005). Perchlorate contamination has also been reported in dietary supplements and flavor enhancers (Snyder et al. 2006).

Trace levels of perchlorate in the environment leads to human exposure. Direct measurement of perchlorate in biological samples collected from people [National Research Council (NRC) 2005] is considered an excellent assessment of their exposure. We recently assessed perchlorate exposure in a nationally representative sample of 2,820 U.S. residents, ≥ 6 years of age, who participated in the National Health and Nutrition Examination Survey (NHANES) during 2001 and 2002 (Blount et al. 2006).

Environmental perchlorate exposure is of potential health concern because much larger doses of perchlorate have been shown to competitively inhibit iodide uptake (Greer et al. 2002; Wyngaarden et al. 1953). Populations with low intake of iodine or increased demand for iodine may be more vulnerable to inhibition of iodide uptake. Sustained inhibition of iodide uptake can lead to hypothyroidism, although perchlorate-induced changes to thyroid function have not been previously demonstrated in any human population exposed to perchlorate, even at doses as high as 0.5 mg/kg body weight per day (NRC 2005). The thyroid plays a crucial role in energy homeostasis and neurologic development. Hypothyroidism can lead to metabolic problems in adults and abnormal development during gestation and infancy (Braverman and Utiger 2000). Severe hypothyroidism due to iodine deficiency during pregnancy is a preventable cause of cretinism, a permanent cognitive impairment of the developing fetus (Glinoer 2000). Mild hypothyroidism during pregnancy has been associated with subtle cognitive deficits in children (Haddow et al. 1999; Klein et al. 2001), leading the NRC to recommend that consideration be given to adding iodide to all prenatal vitamins (NRC 2005). Therefore, we examined relationships between urinary perchlorate and serum thyroid hormones in men and women, ≥ 12 years of age, who participated in NHANES 2001–2002.

Subjects and Methods

Study design. NHANES is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). This survey is designed to assess the health and nutrition status of the civilian, noninstitutionalized U.S. population. NHANES uses a complex multistage probability sampling designed to be representative of the U.S. population based on age, sex, race/ethnicity, and income. Data reported in the present study were collected using an extensive household interview addressing health conditions and health-related behaviors and a standardized physical examination including medical blood and urine tests, which were conducted in mobile examination centers. NHANES 2001–2002 was conducted in 30 locations throughout the United States. Overall, the

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survey interview response rate was 83.9% and the exam response rate was 79.6%. A full description of the NHANES survey is available on the NHANES website (CDC 2004). The study protocol was reviewed and approved by the CDC institutional review board; additionally, informed written consent was obtained from all subjects before they took part in the study.

Urinary perchlorate levels were measured by the Division of Laboratory Sciences, National Center for Environmental Health, on a representative random one-third subsample consisting of 2,820 study participants (males and females), ≥ 6 years of age (Blount et al. 2006). For ages ≥ 12 years, 2,517 persons were in the random subsample. Serum levels of thyroid stimulating hormone (TSH) and total thyroxine (T_4) were only available for 2,299 participants ≥ 12 years of age.

Demographic variables. Sociodemographic data were self-reported by study participants. Race/ethnicity was derived from self-reported questionnaire data and categorized as non-Hispanic white, non-Hispanic black, Mexican American, and "other." Each of these race/ethnicity categories was used in the regression modeling. Non-Hispanic whites were used as the referent group in regression analysis.

Laboratory methods. During the physical examinations, whole blood and spot urine specimens were collected from participants, aliquoted, and stored cold (2–4°C) or frozen until shipment. Whole blood was collected into a red-top 15-mL Vacutainer tube, mixed, allowed to clot 30–45 min, and centrifuged; approximately 1 mL serum was stored frozen in a cryovial for future analysis for TSH and T_4. Serum samples collected in 2001 were assayed for TSH and T_4 by the Coulston Foundation (Alamogordo, NM) using a microparticle enzyme immunoassay for the quantitative determination of TSH, and a Hitachi 704 chemistry analyzer (Hitachi Chemical Diagnostics, Mountain View, CA) for the quantitative determination of T_4 (CDC 2003). Serum samples collected in 2002 were assayed for TSH and T_4 by Collaborative Laboratory Services (Ottumwa, IA) using a chemiluminescent immunoassay (Access Immunoassay System; Beckman Instruments, Fullerton, CA) (CDC 2003). The National Center for Health Statistics evaluated the TSH and T_4 data sets from the two laboratories and determined that the values are comparable across the 2 years.

Surplus urine samples from NHANES 2001–2002 were shipped on dry ice to the Division of Laboratory Sciences and analyzed for perchlorate, thiocyanate, and nitrate using ion chromatography tandem mass spectrometry (Blount et al., 2006; Valentin-Blasini et al. 2005). These samples were stored frozen (–70°C) for up to 4 years before perchlorate analysis. Experiments evaluating storage at –70°C for > 2 years indicated no changes in urinary levels of this analyte (Blount et al. 2006). Reported results for all assays met the division's quality control and quality assurance performance criteria for accuracy and precision [similar to specifications outlined by Westgard et al. (1981)]. Urine samples from the same study participants had previously been analyzed for iodine using inductively coupled plasma mass spectrometry (Caldwell et al. 2005).

Statistical analysis. Initial multiple regression analysis found perchlorate to be a significant predictor of both T_4 and log TSH in women, but perchlorate did not predict either T_4 or log TSH in men (data not shown). Therefore, we present subsequent analysis focused on women.

Of the 1,318 women ≥ 12 years of age, 92 had missing TSH and T_4 values, leaving 1,226. Of these 1,226 women, 91 were excluded from analysis because they reported a history of thyroid disease or current use of thyroid medications, leaving 1,135 women. Of these 1,135 women, 3 had extreme values of T_4 and/or TSH and were excluded. One of these women had a total T_4 of 27 µg/dL and a TSH of 0.04 IU/L. This woman was clearly hyperthyroid and thus was excluded from the analysis. Two other women had very high TSH levels (43 and 68 IU/L) and were excluded. Of the remaining 1,132 women, 21 had missing perchlorate measurements, leaving a sample size of 1,111 women.

The major design variables for NHANES are age, sex, race/ethnicity, and income related to the poverty level. The values of these variables for the initial 1,318 women and the final 1,111 women, respectively, are as follows: mean age, 41.6 and 39.8 years; percent non-Hispanic whites, 70.8% and 69.4%; percent non-Hispanic blacks, 11.8% and 12.5%; percent Mexican Americans, 7.0% and 7.0%; and percent below the poverty level, 13.9% and 14.9%.

We chose covariates for the multiple regression analyses that are known to be or likely to be associated with T_4 or TSH. We selected a broad number of covariates to evaluate the independence of the perchlorate relationship. These covariates were age, race/ethnicity, body mass index (BMI), serum albumin, serum cotinine (a marker of tobacco smoke exposure), estimated total caloric intake, pregnancy status, postmenopausal status, premenarche status, serum C-reactive protein, hours fasting before sample collection, urinary thiocyanate, urinary nitrate, and use of selected medications.

For these covariates, Table 1 provides means (or geometric means if lognormally distributed) for continuous variables, percent in category for categorical variables, and number of missing results for each covariate. Thyroid

Table 1. Means and percent in category for covariates used in the multiple regression, women ≥ 12 years of age, NHANES 2001–2002.[a]

Variable	No.	No. missing	Arithmetic mean (95% CI)	Geometric mean (95% CI)	Percent in category (95% CI)
Age (years)	1,111	0	39.8 (38.1–41.6)		
Fasting (hr)	1,111	0	10.4 (9.85–10.9)		
Serum albumin (g/dL)	1,111	0	4.20 (4.17–4.23)		
Serum T_4 (µg/dL)	1,111	0	8.27 (7.97–8.58)		
Total kilocalories (kcal/1,000)	1,072	39	1.93 (1.87–1.99)		
BMI	1,075	36		25.8 (25.2–26.5)	
Serum cotinine (µg/L)	1,104	7		0.33 (0.23–0.48)	
Serum C-reactive protein (mg/dL)	1,111	0		0.16 (0.14–0.18)	
Serum TSH (IU/L)	1,111	0		1.36 (1.31–1.42)	
Urine creatinine (mg/dL)	1,109	2		81.4 (76.7–86.5)	
Urine iodine (µg/L)	1,111	0		126 (115–138)	
Urine nitrate (µg/L × 1,000)	1,106	5		38.0 (35.9–40.3)	
Urine perchlorate (µg/L)	1,111	0		2.84 (2.54–3.18)	
Urine thiocyanate (µg/L × 1,000)	1,104	7		1.20 (1.08–1.33)	
Race/ethnicity					
Non-Hispanic white	1,111	0			69.4 (62.9–75.4)
Non-Hispanic black	1,111	0			12.5 (7.49–19.1)
Mexican American	1,111	0			7.02 (5.14–9.34)
Other race	1,111	0			11.1 (7.04–16.3)
Medication usage					
Furosemide	1,111	0			1.99 (1.25–3.01)
Glucocorticoids and androgens	1,111	0			2.23 (1.24–3.67)
Beta-blocker	1,111	0			4.48 (3.34–5.87)
Estrogen	1,111	0			17.1 (13.2–21.7)
Other drug	1,111	0			1.04 (0.52–1.88)
Menopausal or postmenopausal	1,028	83			35.9 (30.1–41.9)
Pregnant	1,111	0			3.84 (2.74–5.21)
Premenarchal	1,019	92			1.06 (0.48–2.02)

CI, confidence interval.
[a]Excludes women with missing TSH, T_4, or perchlorate, women with history of thyroid disease or taking thyroid drugs, and three women with outlier values of T_4 or TSH (see text).

function has been previously reported to vary with the constitutional variables of age, race, sex, pregnancy, and menopause (Braverman and Utiger 2000). Serum cotinine is a marker of tobacco smoke exposure, and smoking is associated with altered thyroid function (Bertelsen and Hegedus 1994). We included serum C-reactive protein as a marker for inflammatory conditions that have been associated with alterations in thyroid function. Both total caloric intake [based on a 24-hr dietary recall survey and a U.S. Department of Agriculture (USDA) database (Food and Nutrition Database for Dietary Studies; USDA 2004)] and BMI are related to thyroid function, but the interrelationship as to cause or effect is unclear.

Serum albumin was included in our analysis as a possible surrogate for T_4 serum protein binding. NHANES 2001–2002 included total T_4 measurements but not free T_4 measurements; total T_4 varies with the concentrations of specific binding proteins. Concentrations of these proteins can change with physiologic state and health conditions. Free T_4 varies less with such protein concentration changes than does total T_4. Serum albumin accounts for 15–20% of T_4 binding, with thyroid binding protein and prealbumin (not measured in NHANES) accounting for the remaining percentage (Robbins 2000). Thyroid autoantibody measurements were not available for 2001–2002. For autoantibodies to affect the relationship between perchlorate and T_4 or TSH, presence of autoantibodies would have to correlate with perchlorate levels. We have found no such correlation in the literature and we are unaware of a rationale for such an association.

Medications known to affect thyroid function were also considered. As noted above, women taking medication containing thyroid hormone (e.g., levothyroxine) or antithyroid drugs (e.g., methimazole or propylthiouracil) were excluded. Use of beta-blockers, estrogen formulations, steroids, and furosemide were each modeled using an indicator variable in the regressions. An "other drug" category was also modeled by an indicator variable. This "other drug" category consisted of a heterogeneous group of other medications that have possible effects on thyroid function, protein binding, or measurements, including salicylates, dopaminergics, anticonvulsants and barbiturates, narcotic analgesics, androgenic agents, lithium, and several others (a total of 28 drug codes).

We included the log of urinary creatinine in the models to adjust for variable water excretion. A nonlinear relationship was evaluated by adding the square of the log of perchlorate to final models, but it was not significant. Models were also checked for significance of interaction terms involving main effects. We examined partial regression plots to identify any unduly influential data points; no unduly influential points were found. Indicator variable coefficients in the models (e.g., for non-Hispanic blacks) were interpreted as follows: 1 = group member, and 0 = not a group member. Urine samples were collected in three sessions of the day from 0800 hours through 2200 hours. Mean perchlorate levels were not statistically different across sessions ($p = 0.49$).

We examined univariate statistics and distribution plots for each dependent and independent variable to look for outliers and to assess the distribution shape. TSH, perchlorate, cotinine, BMI, urinary thiocyanate, urinary nitrate, and C-reactive protein were $\log_{10}$-transformed to normalize their distributions.

Regression models, including log of perchlorate as one of the predictor variables, were constructed separately for thyroxine and log of TSH. For the initial phase of analysis, we used ordinary least-squares regression (OLS) (SAS Proc Reg, version 9.0; SAS Institute, Cary, NC) and purposefully did not adjust for the NHANES complex survey design in order to obtain a broad group of potentially significant predictor variables. Forward stepwise and backward elimination procedures were used on both population-weighted and unweighted data. The entry p-value for forward elimination models was 0.10 and the retaining p-value for backward elimination was 0.10 in order to identify significant and borderline significant predictors. The forward stepwise and backward elimination approaches produced models that were generally in good agreement.

This OLS analysis produced a generous list of significant and borderline-significant variables for regression analysis using SUDAAN (version 9.0.1; Research Triangle Institute, Research Triangle Park, NC), which provides an analysis that adjusts for the complex survey design. SUDAAN regression models were tested using a manual backward elimination approach starting with the variables obtained from the OLS regression modeling. Selected variables that were excluded in the SUDAAN backward elimination process were added to the final model to ensure they were not significant. The stability of the perchlorate coefficient was monitored during the SUDAAN backward elimination process.

In the main SUDAAN regression analysis, we used population weights to represent women ≥ 12 years of age in the U.S. population for the years 2001 and 2002. In addition, we performed separate regression analyses with SUDAAN using unweighted data and verified that regression coefficients were in good agreement with those obtained using population weights. Reported regression model results in the tables use the population-weighted analysis.

Women were categorized based on a urinary iodine cut point of 100 µg/L and analyzed separately. The 100 µg/L cut point was used based on the World Health Organization (WHO) definition of sufficient iodine intake in populations (WHO 1994). The WHO noted that the prevalence of goiter begins to increase in populations with median urinary iodine < 100 µg/L. A urine iodine level of 100 µg/L represents about the 36th percentile of urinary iodine concentrations in women living in the United States (Caldwell et al. 2005). Women with lower iodine intake could be more vulnerable to perchlorate's effects to impair iodine uptake. From this analysis, the significance of urinary perchlorate as a predictor of thyroid function in women was found to be largely determined by women with urinary iodine < 100 µg/L. Consequently, we report here results for women divided into groups based on urinary iodine levels.

Compared to the use of average multiple spot urine measurements or 24-hr urine specimens, the use of a single spot urine for perchlorate and iodine measurement has more imprecision in estimating true urine levels (Andersen et al. 2001). This imprecision is a source of random error (not bias) and therefore decreases statistical power to detect an association between perchlorate and either TSH or T_4 compared to these other urine collection approaches.

Results

For all women ≥ 12 years of age, multiple regression analysis found urinary perchlorate to be a significant predictor of serum TSH and a significant predictor of serum T_4 (data not shown). Because low iodine levels had potential to affect the relationship of perchlorate with T_4 and TSH, women with urinary iodine < 100 µg/L were analyzed separately from women with urinary iodine ≥ 100 µg/L. Results of this analysis are presented in Tables 2 and 3 for T_4 and in Tables 4 and 5 for TSH.

For women with urinary iodine < 100 µg/L, multiple regression analysis found perchlorate to be a significant predictor ($p < 0.0001$) of T_4 with a coefficient for log perchlorate of –0.8917. The result of regression of T_4 on perchlorate and urinary creatinine without other covariates yielded a coefficient of –0.8604 ($p < 0.0001$). Perchlorate was also a significant predictor ($p = 0.0010$) of log TSH with a coefficient of 0.1230. The result of regression of log TSH on perchlorate and urinary creatinine without other covariates found a coefficient of 0.1117 ($p = 0.0031$). The signs of these coefficients are coherent, with increased perchlorate associated with less production of T_4 and an increase in TSH to stimulate additional T_4 production. For women with urinary iodine ≥ 100 µg/L, perchlorate was not a significant predictor of T_4 ($p = 0.5503$) but remained a significant predictor of log TSH ($p = 0.0249$). The regression analysis results

in Tables 2–5 include variables that were borderline significant ($0.05 \le p < 0.10$) to give ample opportunity for other variables to explain variance and better evaluate the independence of the perchlorate effect.

Regression results for men (not shown) indicated that perchlorate was not a significant predictor of either T_4 or log TSH. This finding also held when examining men with urinary iodine levels < 100 µg/L.

From the regression coefficients for women with urinary iodine < 100 µg/L, we calculated the predicted effect size (i.e., the change in T_4 and TSH) for different levels of perchlorate exposure. We chose perchlorate levels corresponding to the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles of urinary perchlorate in women ≥ 12 years of age. The minimum and maximum perchlorate values are observed results for this population sample; they are not estimates of the 0th and 100th percentiles for the U.S. population. As such, they would be expected to change in another population sample. The effect size was calculated from the difference between the minimum level of perchlorate measured in women and the level of perchlorate corresponding to the specific percentile. For example, the 50th percentile of urinary perchlorate for women was 2.9 µg/L and the minimum level was 0.19 µg/L. Increasing exposure from 0.19 µg/L to 2.9 µg/L would result in a predicted decrease in T_4 of 1.06 µg/dL.

For TSH, one more step is needed in the calculation. Because TSH was modeled as log TSH, the change in TSH from a given change in perchlorate depends on the starting level of TSH. In our calculations we used the approximate 50th and 90th percentiles of TSH as starting points to estimate the predicted perchlorate effect size for TSH. Results of these calculations for T_4 and TSH are presented in Table 6. For comparison, the normal range is 5–12 µg/dL for T_4 and 0.3–4.5 IU/L for TSH.

To search for a threshold for the perchlorate relationship with T_4 and TSH, piecewise regression models (Neter et al. 1985) were fit to the data. No inflection point was found for the perchlorate relationship with T_4 or TSH. However, statistical power is limited to detect such a threshold, if present.

Table 2. Regression of serum T_4 on perchlorate and covariates for women ≥ 12 years of age with urine iodine < 100 µg/L, NHANES 2001–2002.

Independent variable	Coefficient	SE	*p*-Value
Intercept	8.6508	0.5428	< 0.0001
Log (urinary perchlorate)	–0.8917	0.1811	< 0.0001
Log (urinary creatinine)	0.6897	0.3338	0.0391
Estrogen use	1.5117	0.4421	0.0007
Log (C-reactive protein)	0.8249	0.1774	< 0.0001
Mexican American[a]	0.6296	0.3684	0.0878
Menopause	–0.5908	0.2578	0.0221
Pregnant (by test)	0.7389	0.3662	0.0439
Total kilocalorie intake (÷ 1,000)	–0.3334	0.1173	0.0046
Premenarche	0.6401	0.2722	0.0189

Dependent variable: serum T_4 ($n = 348$; $R^2 = 0.240$).
[a]Referent group for race is non-Hispanic white.

Table 3. Regression of serum T_4 on perchlorate and covariates for women ≥ 12 years of age with urine iodine ≥ 100 µg/L, NHANES 2001–2002.

Independent variables	Coefficient	SE	*p*-Value
Intercept	10.6652	1.2345	< 0.0001
Log (urinary perchlorate)	0.2203	0.3687	0.5503
Log (urinary creatinine)	1.3138	0.7183	0.0677
Estrogen use	0.8278	0.2722	0.0024
Log (C-reactive protein)	0.5783	0.1247	< 0.0001
Mexican-American[a]	0.5763	0.2522	0.0225
Pregnant (by test)	1.6175	0.3334	< 0.0001
Log (urinary nitrate)	–1.1215	0.4994	0.0249
Hours of fasting	0.0290	0.0156	0.0630

Dependent variable: serum T_4 ($n = 724$; $R^2 = 0.149$).
[a]Referent group for race is non-Hispanic white.

Table 4. Regression of serum TSH on perchlorate and covariates for women ≥ 12 years of age with urine iodine < 100 µg/L, NHANES 2001–2002.

Independent variables	Coefficient	SE	*p*-Value
Intercept	0.2654	0.1183	0.0403
Log (urinary perchlorate)	0.1230	0.0373	0.0010
Log (urinary creatinine)	–0.0954	0.0761	0.2103
Beta-blocker use	0.1881	0.0595	0.0016
Estrogen use	–0.0918	0.0404	0.0233
Premenarche	0.1288	0.0262	< 0.0001

Dependent variable: log of serum TSH ($n = 356$; $R^2 = 0.061$).

Table 5. Regression of serum TSH on perchlorate and covariates for women ≥ 12 years of age with urine iodine ≥ 100 µg/L, NHANES 2001–2002.

Independent variables	Coefficient	SE	*p*-Value
Intercept	–0.6948	0.3415	0.0600
Log (urinary perchlorate)	0.1137	0.0506	0.0249
Log (urinary creatinine)	–0.1198	0.0910	0.1884
Age in years	0.0025	0.0006	< 0.0001
Log (BMI)	0.4812	0.1346	0.0004
Non-Hispanic black[a]	–0.1125	0.0335	0.0008
Log (urinary nitrate)	0.1087	0.0591	0.0660
Log (urinary thiocyanate)	–0.0816	0.0352	0.0206

Dependent variable: log of serum TSH ($n = 697$; $R^2 = 0.145$).
[a]Referent group for race is non-Hispanic white.

Discussion

Increased urinary perchlorate was associated with increased TSH and decreased T_4 for women with urinary iodine levels < 100 µg/L, a group possibly more susceptible to competitive inhibition of thyroid iodine uptake by perchlorate. The statistically significant associations of urinary perchlorate with decreased serum T_4 and increased serum TSH were consistent with competitive inhibition of iodide uptake.

For women with urine iodine ≥ 100 µg/L, perchlorate was also a statistically significant predictor for TSH but not for T_4. Greater iodine intake may have diminished the effect of perchlorate on T_4 in these women. The significant association with TSH, but not with T_4, in this group may be due to the greater sensitivity of TSH to impairment of thyroid function; that is, normal T_4 levels are maintained by increasing TSH to compensate for impaired thyroid function.

Predicted changes in serum TSH and T_4 with increasing perchlorate exposure (Table 6) can span a notable portion of the normal medical range of TSH and T_4 values. Compared with a urine level of 0.19 µg/L, urinary perchlorate of 13 µg/L (95th percentile) yields a predicted decrease in T_4 of 1.64 µg/dL. The normal range for T_4 is 5–12 µg/dL. A similar exposure would increase TSH by 2.12 IU/L for a woman starting with a TSH level of 3.11 IU/L (90th percentile for TSH in

women ≥ 12 years of age). The normal range for TSH is 0.3–4.5 IU/L. Effect size estimates that start with the 90th percentile of TSH have more uncertainty than estimates starting with the 50th percentile because the predicted TSH levels fall further from the central portions of the original data.

The mechanism of perchlorate's effect is competitive inhibition of iodide uptake by the thyroid (Clewell et al. 2004; Wolff 1998). Based on this mechanism, individuals with less iodide available to compete with perchlorate may be more vulnerable to impaired iodide uptake. Chronically impaired iodide uptake could lead to changes in serum thyroid hormones, consistent with the increased TSH and decreased T_4 we find associated with increased perchlorate exposure in women with urinary iodine < 100 µg/L. The WHO (2004) has identified median urinary iodine levels ≥ 100 µg/L as indicating sufficient iodine intake for a population. Based on concerns about adequate iodine intake, the NRC (2005) recently recommended that consideration be given to adding iodine to all prenatal vitamins.

In the present study, perchlorate was not found to be a significant predictor of T_4 or TSH in men. Previous studies report that women have a much higher risk of goiter than do men, especially in populations with marginal iodine intake (Laurberg et al. 2000). The increased vulnerability of women may partially be caused by increased susceptibility to autoimmune thyroid disease in women, the increased demands on the thyroid during pregnancy, or the effect of estrogens on thyroid function. Estradiol has been shown to block TSH-induced sodium/iodide symporter (NIS) expression in the FRTL5 rat follicular cell line (Furlanetto et al. 1999). Impaired NIS expression could lead to reduced ability of the thyroid follicular cells to import iodide, and thus an increased vulnerability to NIS-inhibitors such as perchlorate. Also, estrogens increase T_4-binding globulin and thus increase the demand for T_4 so that free T_4 levels can remain constant.

Covariates in the regression models predicted T_4 and TSH levels in a manner generally consistent with previous studies. We found that estrogen use was a significant, independent, and positive predictor of T_4 in both low and sufficient iodine models of women ≥ 12 years of age, but was not a significant predictor in either of the TSH models. Similar to estrogen use, pregnancy was a significant or borderline significant predictor of T_4 but not TSH. Both estrogen use and pregnancy raise estrogen levels, increase thyroid binding proteins, and increase serum T_4 concentrations (Glinoer 1997). Menopause lowers estrogen levels and was a significant predictor of T_4 in the regression for women with urinary iodine levels < 100 µg/L.

In NHANES III (1988–1994), non-Hispanic blacks were reported to have lower TSH than other groups, and Mexican Americans had higher T_4 levels than non-Hispanic blacks and whites (Hollowell et al. 2002). The models for TSH and T_4 in the present study were consistent with these previous findings concerning race/ethnicity. Non-Hispanic blacks have also been shown to have lower urinary perchlorate levels than non-Hispanic whites, although the reason for this difference is not known (Blount et al. 2006). Age was positively associated with TSH in women with urinary iodine levels ≥ 100 µg/L, but not significant for women with urinary iodine levels < 100 µg/L. A positive association of age and TSH was seen in NHANES III and other studies (Canaris et al. 2000; Hollowell et al. 2002).

BMI was significant in the TSH model for women with urinary iodine levels ≥ 100 µg/L, and total caloric intake was significant in the T_4 model for women with urinary iodine levels < 100 µg/L. Thyroid function clearly has an effect on BMI, as seen clinically and documented in populations (Nyrnes et al. 2006). The reverse is also true, because BMI and total caloric intake can influence the hypothalamic–pituitary–thyroidal axis, although usually at the extremes of body weight and caloric intake (Acheson et al. 1984; Burger et al. 1987; Danforth et al. 1979; Loucks et al. 1992; Loucks and Heath 1994). Total caloric intake in NHANES is a 24-hr recall of food intake. Depending on how well recent intake reflects long-term intake, total caloric intake may parallel the effect of BMI, which was not seen in the present study. Increased caloric intake is known to increase thyroid hormone disposition through deiodination pathways (Burger et al. 1987; Danforth et al. 1979), increasing the conversion of T_4 to the active form, triiodothyronine (T_3), and increasing conversion of T_3 to inactive forms. The effect of changes in calories and carbohydrate composition of the diet on thyroid disposition may have different short- and long-term effects on T_3 and T_4 levels. In the present study, hours of fasting before sample collection was a borderline significant predictor in one regression model: T_4 in women with sufficient iodine. Fasting for 60 hr can reduce TSH in humans, but fasting for shorter periods has unknown effects on thyroid function.

Beta-blocker drugs are commonly used to treat hypertension and other cardiovascular conditions. Beta-blockers inhibit the conversion of T_4 to the more active form, T3, and increase serum TSH (Kayser et al. 1991). Use of these drugs was positively associated with TSH in the regression for women with urinary iodine < 100 µg/L. Serum C-reactive protein was positively associated with T_4 in women in each of the iodine groups. C-reactive protein is an acute phase reactant protein increased in many inflammatory conditions in response to production of tissue-generated cytokines, particularly interleukin-6, and has been used as a marker for both specific and systemic low-level inflammation conditions. It is unclear if C-reactive protein is associated with thyroid function other than thyroiditis (Jublanc et al. 2004; Pearce et al. 2003; Tuzcu et al. 2005). However, the stimulus for C-reactive protein, interleukin-6, has a firm inverse relationship with serum T_3 in nonthyroidal illnesses. Also, C-reactive protein and serum T_4 binding proteins are synthesized by the liver; C-reactive protein may vary with an unrecognized health or physiologic condition that affects the synthesis of both proteins. The association of C-reactive protein and T_4 in our study is unclear.

Other variables that are known to possibly affect thyroid function or measurements were not significant predictors in the regression models, including the categories of medications (other than estrogen use and beta-blockers), serum albumin, and serum cotinine. Generally, other medication categories were small and unlikely to have significant effects. Serum albumin did not appear in the final models. Factors such as estrogen use that increase protein binding of thyroid hormones may have accounted for variance in T_4 due to protein binding that serum albumin may have otherwise explained. Serum cotinine is a marker of tobacco smoke exposure, and

Table 6. Predicted change in serum T_4[a] and serum TSH[b] levels based on changes in urinary perchlorate levels in women ≥ 12 years of age, with urine iodine < 100 µg/L, NHANES 2001–2002.

		Change in TSH (IU/L)[c]	
Change in urine perchlorate[d]	Change in T_4 (µg/dL)	Initial TSH of 1.40 IU/L (50th TSH percentile)	Initial TSH of 3.11 IU/L (90th TSH percentile)
0.19 to 0.65 µg/L (5th percentile)	0.48	0.23	0.51
0.19 to 0.92 µg/L (10th percentile)	0.61	0.30	0.67
0.19 to 1.6 µg/L (25th percentile)	0.83	0.42	0.93
0.19 to 2.9 µg/L (50th percentile)	1.06	0.56	1.24
0.19 to 5.2 µg/L (75th percentile)	1.28	0.70	1.56
0.19 to 9.0 µg/L (90th percentile)	1.49	0.85	1.89
0.19 to 13 µg/L (95th percentile)	1.64	0.95	2.12
0.19 to 100 µg/L (maximum)	2.43	1.63	3.61

[a]Normal range for T_4: 5–12 µg/dL. [b]Normal range for TSH: 0.3–4.5 IU/L. [c]Depends on initial TSH level. [d]Minimum level measured, 0.19 µg/L.

smoking is associated with altered thyroid function (Belin et al. 2004; Bertelsen and Hegedus 1994). However, tobacco smoke also contains other factors that can inhibit TSH secretion (Bartalena et al. 1995), and perhaps is an explanation for the absence of an association of serum cotinine with either TSH or T_4.

Cyanide in tobacco smoke is metabolized to thiocyanate, a competitive inhibitor of iodide uptake (Tonacchera et al. 2004). Also, nitrate from dietary sources and from formation by intestinal bacteria can compete with iodide. *In vitro* studies indicate that perchlorate is a more potent inhibitor of human NIS, with potencies 15, 30, and 240 times greater than thiocyanate, iodide, and nitrate, respectively (Tonacchera et al. 2004). Thus, the ability of NIS to transport adequate amounts of iodide depends on the relative concentrations of these competing anions. Based on the relative concentrations of perchlorate, nitrate, and thiocyanate likely to be found in human serum, several researchers have predicted that nitrate and thiocyanate are more likely than perchlorate to impair thyroid function (DeGroef et al. 2006; Gibbs 2006). Thiocyanate-induced NIS inhibition is a plausible explanation of the association of smoking with goiter in populations with low iodine intake (Knudsen et al. 2002) and is analogous to the association of perchlorate exposure with thyroid hormone levels observed in our study. However, in women with urinary iodine levels ≥ 100 µg/L, urinary thiocyanate was negatively associated with serum TSH, a direction unexpected based on a mechanism of NIS inhibition. The explanation for this is unclear. Urinary nitrate was negatively associated with serum T_4 in women with urinary iodine levels ≥ 100 µg/L, a direction consistent with inhibition of NIS. Goitrogenic effects of nitrate intake in animal studies have been observed (Wyngaarden et al. 1953), but there are few studies in humans.

Recently the NRC (2005) evaluated the potential health effects of perchlorate ingestion. Based on studies of long-term treatment of hyperthyroidism and clinical studies of healthy adults, the NRC panel estimated that a perchlorate dose of > 0.40 mg/kg/day would be required to cause hypothyroidism in adults, although lower doses may lead to hypothyroidism in sensitive subpopulations (NRC 2005).

Comparison of our results to previous studies requires consideration of *a*) target population group studied, *b*) estimated dose of perchlorate, *c*) duration of exposure to perchlorate dose, and *d*) sample size (statistical power). First, for men, we found no relationship with perchlorate and T_4 or TSH. This finding is in general agreement with predicted effects of this level of perchlorate exposure based on reported studies of exposure in men. Lawrence et al. (2000) administered 10 mg perchlorate daily (~ 0.14 mg/kg) to iodine-sufficient adult males for 14 days and found a 10% decrease in radioactive iodine uptake (RAIU), but with no change in TSH or free T_4.

Greer et al. (2002) administered perchlorate to 16 male and 21 female volunteers for 14 days, and found increasing RAIU inhibition for doses between 0.02 and 0.5 mg/kg/day, with no perchlorate-related change in TSH or free T_4. An unknown number of women in that study may have had urinary iodine < 100 µg/L, but if the women were typical of the U.S. population (Caldwell et al. 2005), the predicted number of women with low urinary iodine would be 7–8. Braverman et al. (2006) administered perchlorate to 13 iodine-sufficient male and female volunteers at daily doses of 0.5 mg and 3 mg for 6 months, and found no change in RAIU, TSH, or free T_4. Two other studies have also found that workers exposed to perchlorate intermittently for long periods did not have significant changes to serum TSH or T_4 levels (Braverman et al. 2005; Lamm et al. 1999). These study populations were either exclusively (Braverman et al 2005) or predominantly (Lamm et al 1999) male.

For women, only two perchlorate studies have focused on women or included a large percentage of women. A recent study of 184 pregnant Chilean women, with mean urinary perchlorate levels near the 99th percentile for women in NHANES 2001–2002, found no perchlorate relationship with thyroid function (Tellez et al. 2005). Of these 184 women, 181 had mean urinary iodine levels ≥ 100 µg/L and only 3 had mean levels < 100 µg/L. Therefore, the results of Tellez et al. (2005) would compare to the present results for women with urinary iodine levels ≥ 100 µg/L. Urinary iodine levels in the Chilean study population (median 269 µg/L) were higher than urinary iodine levels found in the NHANES 2001–2002 population [median 168 µg/L; 95% confidence interval, 159–178 µg/L]. The Chilean women (Tellez et al. 2005) were also pregnant, which increases the variability in T_4 and TSH. This increased variability would make an association between perchlorate and thyroid function harder to find. The second study with a large percentage of women was Greer et al. (2002) discussed above. These two studies are compared with the present study in Table 7.

Table 7 indicates that our study is the first to target and separately analyze results for women with lower levels of urinary iodine, a potentially susceptible population. A second special attribute of the present study is the much larger sample size of women, affording more statistical power to detect a potential effect. By averaging over many women, the current data likely represents a good approximation of a population steady-state exposure to perchlorate that women have had for a long period of time. If a mid- to long-term exposure is needed for perchlorate to affect thyroid function, this data would have a better opportunity to detect that effect than study designs using short-term exposures. The influence of duration of exposure merits further study.

Accurate assessment of exposure is critical to detect biochemical end points potentially related to exposure. Our laboratory recently developed an improved method for measuring urinary perchlorate, which enhances individual perchlorate exposure assessment (Valentin-Blasini et al. 2005). The use of this new urinary perchlorate measurement strengthens the ability of the present study to detect potential associations with T_4 and TSH.

The present study has the general limitations of a cross-sectional analysis. Therefore, the relationship between urinary perchlorate and thyroid function was examined with attention to the potential influences of chance, bias, or confounding. Perchlorate (as with any of the significant predictor variables) could be a surrogate for another unrecognized determinant of thyroid function. We also assumed in this analysis that urinary perchlorate correlates with levels in the thyroid stroma and tissue, a kinetically distinct compartment. This would be the case in a population with stable, chronic exposures, which is likely but not certain in this population. A large sample size helps to average such potential kinetic differences. Finally, a measurement of free T_4 would be an improvement to the study.

Table 7. Comparison of perchlorate studies targeting women or including a high percentage of women.

	Greer et al. (2002)	Tellez et al. (2005)	Present study
No. of females studied	21 (37 total subjects)	184	1,111
No. of females with urine iodine < 100 µg/L	Unknown (estimate 7–8)	3[a]	348, T_4 analysis 356, TSH analysis
Females with urine iodine < 100 µg/L analyzed separately	No	No	Yes
Perchlorate dose and duration of exposure	Up to 0.5 mg/kg/day for 14 days	Long-term environmental exposure	Long-term environmental exposure
Comments		All women pregnant, increasing variability of T_4 and TSH	

[a]Average of one to three spot urine samples.

Conclusions

Urinary perchlorate is associated with an increased TSH and decreased total T_4 in women ≥ 12 years of age with urine iodine levels < 100 µg/L in the U.S. population during 2001–2002. For women with urine iodine levels ≥ 100 µg/L, urine perchlorate is a significant predictor of TSH but not T_4. These effects of perchlorate on T_4 and TSH are coherent in direction and independent of other variables known to affect thyroid function, but are found at perchlorate exposure levels that were unanticipated based on previous studies. Further research is recommended to affirm these findings.

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Long-Term Environmental Exposure to Perchlorate Through Drinking Water and Thyroid Function During Pregnancy and the Neonatal Period

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We have conducted a longitudinal epidemiologic study among pregnant women from three cities in northern Chile: Taltal with 114 μg/L, Chañaral with 6 μg/L, and Antofagasta with 0.5 μg/L perchlorate in the public drinking water. We tested the hypothesis that long-term exposure to perchlorate at these levels may cause a situation analogous to iodine deficiency, thus causing increases in thyrotropin (TSH) and thyroglobulin (Tg) levels and decreased levels of free thyroxine (FT_4), in either the mother during the early stages of gestation or the neonate at birth, or in the fetus cause growth retardation. We found no increases in Tg or TSH and no decreases in FT_4 among either the women during early pregnancy (16.1 ± 4.1 weeks), late pregnancy (32.4 ± 3.0 weeks), or the neonates at birth related to perchlorate in drinking water. Neonatal birth weight, length, and head circumference were not different among the three cities and were consistent with current U.S. norms. Therefore, perchlorate in drinking water at 114 μg/L did not cause changes in neonatal thyroid function or fetal growth retardation. Median urinary iodine among the entire cohort was 269 μg/L, intermediate between that of pregnant women in the United States at National Health and Nutrition Examination Survey (NHANES) I and at NHANES III and consistent with current World Health Organization (WHO) recommendations. Median breast milk iodine was not decreased in the cities with detectable perchlorate. Analysis of maternal urinary perchlorate excretion indicates an additional dietary source of perchlorate.

Introduction

In recent years, perchlorate has been found in drinking water in the United States (1). Concern about the possibility of environmental perchlorate inducing a relative iodine deficiency during pregnancy, especially among women with lower iodine intake, and thereby resulting in adverse neurodevelopmental effects in the fetus has led to proposed drinking water standards as low as 1 μg/L (2).

Recent studies in Europe and the United States have determined that maternal thyroid underfunction during pregnancy, even when mild and considered subclinical, and especially when occurring during early gestation, may be associated with an impairment of normal brain development and intelligence in offspring (3–8). Whether decreased maternal free thyroxine (FT_4) or increased maternal thyrotropin (TSH) is the most important marker for potential adverse neurodevelopmental outcome remains unclear.

Depending on the degree of iodine deficiency during pregnancy, maternal responses include increased thyroid volume and goiter prevalence, increased TSH, thyroglobulin (Tg), and decreased FT_4 (9–22). In addition to the same general pattern of hormonal responses, fetal responses may also include decreased birth weight, length, and head circumference (18,23,24).

We previously conducted a study among first-grade schoolchildren in northern Chile where perchlorate levels in drinking water range from nondetectable by ion chromatography (IC) to 100–120 μg/L (25). No decrease in FT_4, increase in TSH, or goiter attributable to perchlorate levels

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The Centers for Disease Control and Prevention's (CDC) role in this study dealt exclusively with perchlorate exposure assessment. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of CDC.

in drinking water was detected. Analysis of neonatal screening records from the same region did not find elevated TSH levels associated with perchlorate levels in water over a 3-year period. Iodine excretion among the schoolchildren was approximately 3 times higher than among children in the United States in 1988–1994 (National Health and Nutrition Examination Survey [NHANES] III) and 75% higher than that of children age 1–11 in the United States in 1971–1974 (NHANES I).

The International Council for the Control of Iodine Deficiency Disorders has reported on the iodine status in Chile intermittently over the past decade (26–30). Iodized salt was first mandated by law in Chile in 1959. This law was repealed in 1982, but a new law was passed in 1990 mandating that salt contain 100 ppm potassium iodate. In 1991 a survey among schoolchildren indicated mild to moderate iodine deficiency in various regions in Chile. In 1995 a median urinary iodine excretion of 750 μg/L was reported. In 1997 levels for iodized salt were lowered to 40–100 ppm as potassium iodate. Median urinary iodine was reported as 565 μg/L in 1998 and 540 μg/L in 1999, second highest in the world after Japan. Average urinary iodide of 640 μg/g creatinine was reported in pregnant women in Santiago in 1998 (31). Average urinary iodine of 717 μg/L was reported among schoolchildren in northern Chile in 1999 (25). In 2000, the iodide content of salt was reduced to 20–60 ppm. In terms of other common public health parameters (birth rate, life expectancy, and infant death rate), Chile ranks a close third behind Canada and the United States, respectively, in the Western hemisphere.

This longitudinal epidemiologic study was designed to address the possible impact of perchlorate in drinking water at concentrations of 100–120 μg/L on maternal thyroid function during pregnancy, neonatal thyroid function and developmental status at birth, and breast milk iodide and perchlorate levels during lactation. It was conducted in the same three coastal cities in northern Chile in which schoolchildren were previously studied (25). These cities, situated along a 200-mile span of the west coast of Chile, centered at approximately 25° south latitude, have very similar climates, and the population appears racially homogeneous. This region is in the Atacama Desert, which is the driest inhabited area in world. Saltpeter, which is known to contain perchlorate, has been mined in this region for over 200 years. The use of local water for irrigation is not an issue because there is no local agriculture. Municipal drinking water sources, which are different for each city, have been previously described (25).

Materials and Methods

All research activities and consent forms were approved in advance by the review board of the Southeast Metropolitan Health Service in Santiago and by the Coquimbo-Atacama Health Service Institutional Review Board (IRB). Additionally, the research protocol was reviewed and approved by the Centers for Disease Control and Prevention IRB. Informed consent was obtained upon enrollment of the women in the study, both for themselves and for their newborns. The same protocol was applied in each of the three study cities; Antofagasta, Taltal, and Chañaral. The study was conducted between November 2002 and April 2004.

Pregnant women were enrolled in a consecutive manner in Antofagasta, Taltal, and Chañaral when first presenting for prenatal care. Because we were most concerned about maternal thyroid changes during early pregnancy, we excluded women who first presented for prenatal care after 24 weeks' gestation. Additional exclusion criteria included women who had resided in their respective cities less than 6 months, or who were taking thyroid medications or iodine-containing medications during the 3 months before entering the study. The target number of subjects per city was 60. A schematic of the study design is shown as Figure 1.

At the first prenatal study visit, each subject provided socioeconomic and cultural information elicited from a questionnaire, and a sample of home tap water. During the postpartum study visit, a breast milk sample was obtained when possible. During both prenatal study visits and the postpartum study visit, maternal serum and urine samples were obtained, frozen, and subsequently shipped by air to Santiago as was the newborns' cord blood serum. All serum and urine samples were analyzed at the Catholic University Clinical Laboratories in Santiago. TSH, FT_4, and triiodothyronine (T_3) were analyzed using chemiluminescence (ADVIA Centaur, Bayer HealthCare, Berkshire, United Kingdom). Tg was analyzed using radioimmunoassay and antibodies to Tg were analyzed using radioimmune separation (32). Antibodies to thyroid peroxidase (TPO) were analyzed using hemagluttination (Thymune-M, Murex, Dartford, England). Urine iodine determinations were made using oxidation with ammonium persulfate (33).

Serum and urine samples were analyzed as they were received by the laboratory. FT_4 was observed to be significantly lower in sequential samples from two sample batches collected in February 2004 than samples from other batches. Retained frozen serum samples corresponding to those tested in February were retested at the Catholic University Laboratory in June 2004 and the June results substituted into the final data set. No batch effect was evident with TSH analyses.

As an external laboratory quality control, 194 remaining frozen samples of cord serum, and maternal serum from the first and second prenatal visits (with sufficient quantity), were re-analyzed at the Boston Medical Center Laboratory for TSH, T_3, T_4, FT_4, and T_3 uptake as a single batch in July 2004. The Boston laboratory also uses the Bayer ADVIA Centaur Automated Chemiluminescence System for these analyses.

During both prenatal study visits and the postpartum visit, a physical examination of the maternal thyroid was conducted by an endocrinologist (Dr. Téllez in Taltal, Drs. Michaud and Téllez in Antofagasta, and Drs. Reyes and Téllez in Chañaral). The criteria recommended by WHO/UNICEF/CIL TCCY (34) were used to classify goiters.

Home tap water samples, breast milk samples, serum samples, and urine samples were shipped via air from Santiago to the United States. Thirty-one tap water samples from Antofagasta, 58 tap water samples from Taltal, and 51 from Chañaral were analyzed for perchlorate at the U.S. Air Force Research Laboratory (AFRL) using ion chromatography (U.S. EPA Method 314.0). Thirty-four tap water samples from Antofagasta and 4 from Taltal were analyzed by the Kerr-McGee Chemical LLC laboratory in Oklahoma City using the

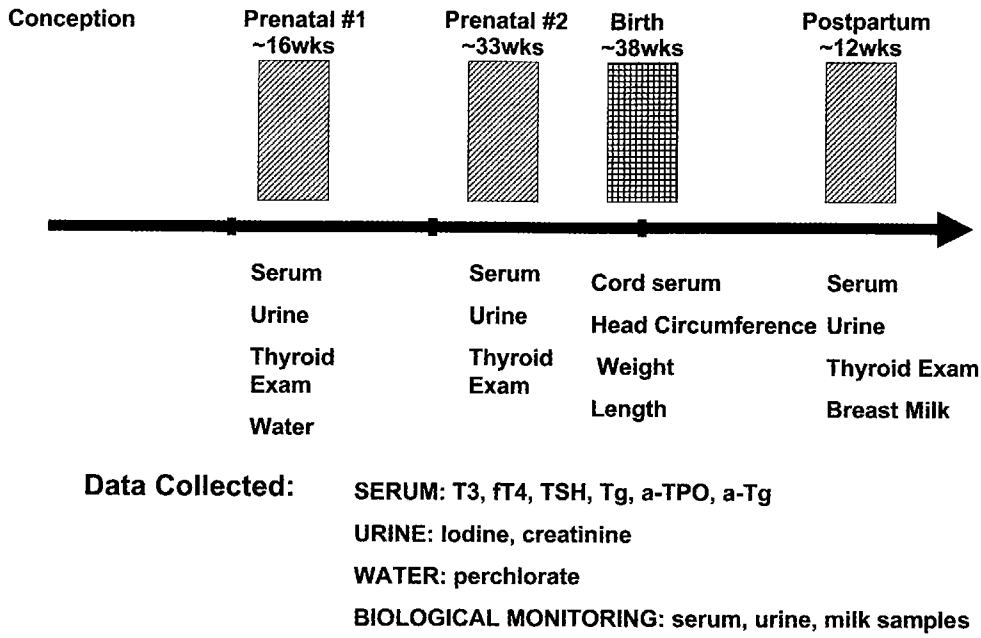

FIG. 1. Schematic of study design.

same methodology. Six water samples from Antofagasta were analyzed by the Centers for Disease Control and Prevention (CDC) for perchlorate using ion chromatography coupled with electrospray ionization tandem mass spectrometry (IC-MS/MS) (35). A total of 63 serum samples were analyzed for perchlorate at the AFRL using the method of Narayanan et al. (36) to further the development of a physiologically based pharmacokinetic (PBPK) model for perchlorate. All breast milk samples were analyzed at Dr. Lewis Braverman's laboratory in Boston for perchlorate using ion chromatography, and for iodine using the method of Benotti et al. (37). A total of 297 urine samples were analyzed by the CDC for perchlorate using IC-MS/MS.

Nitrate, selenium, iodine, lithium, and arsenic are known to be present in mineral deposits in the Atacama desert and to some extent all have been linked to thyroid dysfunction. In order to determine if these substances may be present in the study region at levels that might confound the results, five of the home tap water samples from each of the three study cities were analyzed for iodide, lithium, and arsenic at the MWH Laboratories in Monrovia, California. Iodide was analyzed by EPA method 200.8, while lithium was analyzed using inductively coupled plasma (ICP) and arsenic with ICAP/MS. Six tap water samples from each city were analyzed for nitrate at the AFRL using ion chromatography. Five serum samples from the second prenatal study visit in each city were sent to LabCorp for analysis for selenium using ICP/MS (LabCorp reference interval 79–326 μg/L). Additionally, to ensure that the local diet was not high in cyanogens, a total of 40 nonsmokers' postpartum serum samples were tested for thiocyanate by ion chromatography at Dr. Braverman's laboratory.

Neonatal weight, length, and head circumference were measured routinely for all hospital deliveries and gestational age at birth was estimated as per usual procedure and recorded by the attending physician. The estimated gestational age at birth was considered more accurate than the gestational age based on the mother's recall of her last menstrual period. After gestational age at birth was recorded, gestational age at the first and second prenatal study visits was recalculated.

The nonparametric Kruskal-Wallis (38) test was used to evaluate differences among cities in measures collected from women during the first and second prenatal visits, postpartum visit, and in measures collected in neonates. Additional analysis of continuous variables that controlled for various potential confounders was conducted using regression (analysis of covariance). Transformations of the data were performed in some cases to satisfy the regression requirements of homogeneity of variance and normality of residuals. Differences among cities in binary responses were evaluated using contingency table analysis. Analyses that included values of the dependent variable measured at both the first and second control visit were performed using repeated measure regression that includes estimates of both within-subject and between-subject variance.

Results

Drinking water analyses are reported in Table 1. All water samples were tested for perchlorate using ion chromatography (IC) while approximately 10% of the samples from each city were selected at random for testing for other potential goitrogens known to be present in northern Chile. Mean (range) perchlorate levels in the subjects' tap water as measured by IC were less than 4.0 (all less than 4.0), 5.8 (4.7–7.3), and 114 (72–139) μg/L in Antofagasta, Chañaral, and Taltal, respectively, which is in excellent agreement with previous measurements obtained from public drinking water sources in the same cities (25). Reanalysis of six subjects' tap water samples from Antofagasta for perchlorate using IC coupled with tandem mass spectrometry demonstrated a

TABLE 1. WATER ANALYSES FOR PERCHLORATE AND OTHER POTENTIAL GOITROGENS

	Antofagasta			*Chañaral*			*Taltal*		
	n	*Mean*	*SD*	n	*Mean*	*SD*	n	*Mean*	*SD*
Perchlorate, $\mu g/L^a$	66	N.D.	—	53	5.82	0.63	62	113.9	13.3
Iodide, $\mu g/L^b$	5	156	35	5	87	18	5	114	14
Arsenic, $\mu g/L^c$	5	11	6	5	N.D.	—	5	15	6
Lithium, $\mu g/L^d$	5	848	205	5	370	72	5	75	10
Nitrate nitrogen mg/L[e]	6	0.61	0.47	6	0.64	0.4	6	2.06	0.84
Perchlorate, $\mu g/L^f$	6	0.46	0.29	—	—	—	—	—	—

[a]Perchlorate analyses using IC: not different from measurements previously reported (21).
[b]Iodide: urinary iodide excretion was not different among the three cities.
[c]Arsenic: current U.S. standard is 10 $\mu g/L$.
[d]Lithium: average diet contains 2 mg/d. Therapeutic dose is , 100 mg/d.
[e]Nitrate nitrogen: U.S. standard is 10 mg/L.
[f]Analyses at CDC using IC-MS/MS, range 0.26–1.0 $\mu g/L$ (35).
SD, standard deviation; IC, ion chromatography; MS, mass spectrometry.

mean concentration of 0.46 and range of 0.26–1.0 $\mu g/L$. Although there are some differences among the three water sources in arsenic, lithium, nitrate, and iodine, these levels are not sufficiently high to cause concern about confounding.

Maternal demographics are listed in Table 2. The women enrolled in Chañaral were slightly older, had more previous pregnancies, and had lived in their respective city longer than women enrolled in either Taltal or Antofagasta. Women from Chañaral and Taltal were heavier than those from Antofagasta. There was no difference in cigarette smoking or self-reported tap water consumption among women from the three cities. The subjects from the three cities appeared to be racially homogeneous, so analysis by race is not included. Contingency table analysis of socioeconomic data found no significant difference among women from the three cities in terms of education (categorized as high school graduate or more versus less than high school graduate), economic status (type of house, categorized as owner, renter or other), or marital status (categorized as married or not married). For various reasons (e.g., subject moved away, did not attend subsequent study visits, were not pregnant, early miscarriage, or gave birth in other sites where we could not obtain staff collaboration), a total of 8 women from Antofagasta, 10 women from Taltal and 6 women from Chañaral were lost to follow-up.

Figure 2 compares maternal urinary iodine levels (all three cities, first and second prenatal study visits combined) in the study cohort compared with pregnant women included in two recent National Health and Nutrition Examination Surveys (NHANES). As shown in this figure, iodine excretion among the study cohort is consistent with the current World Health Organization (WHO) recommendations and intermediate between levels in the United States in the early 1970s (NHANES I) and early 1990s (NHANES III).

Table 3 contains maternal measurements from the three study visits. The women from Chañaral had a significantly higher goiter prevalence at both prenatal visits compared with women from the other two cities and this difference could not be explained by age, parity or antithyroid antibodies. The women in Taltal had increased goiter prevalence at the second prenatal visit relative to the first, and at the post partum visit relative to the second prenatal visit. No difference in goiter prevalence among the three cities was ob-

TABLE 2. MATERNAL DEMOGRAPHICS

	Antofagasta			*Chañaral*			*Taltal*			
	n	*Mean*	*SD*	n	*Mean*	*SD*	n	*Mean*	*SD*	P *value*
Mother's Age	65	23.1	6.2	53	28.2	6.3	66	25.0	6.0	<0.0001[a]
Years in City	63	16.1	8.5	50	20.6	11.2	66	16.8	9.5	0.02[a]
Previous Pregnancies	65	0.83	1.18	52	1.92	1.56	66	1.50	1.45	<0.0001[a]
Height (cm)	56	156.4	6.1	46	156.8	5.2	62	157.4	5.8	0.72[a]
Weight (kg)	46	61.4	13.7	47	66.8	13.2	60	66.8	14.6	0.04[a]
Smoking, cigs/week	63	1.62	5.77	53	2.74	8.95	66	0.52	2.72	0.21[a]
Housing—% Owners	65	43.1		52	51.9		66	52.2		0.60[b]
Education—% HS+	65	46.2		52	44.2		66	52.2		0.65[b]
Marital—% Married	65	18.5		52	25.0		66	26.9		0.49[b]
Tap water, L/d	61	1.04	0.63	47	1.03	0.43	66	0.92	0.41	0.38[a]

[a]Kruskal-Wallis *p* value.
[b]Contingency table *p* value.
SD, standard deviation.

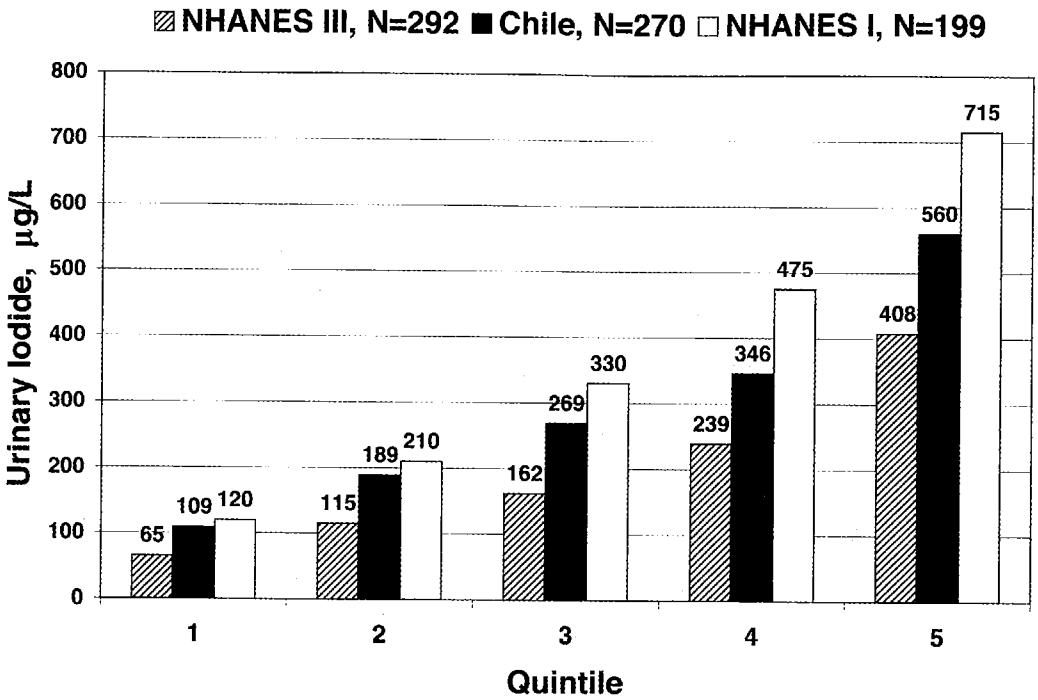

FIG. 2. Comparison of urinary iodine distribution by quintiles in the current study (combined first and second prenatal visits from all three cities) with pregnant women from (NHANES) I and NHANES III surveys.

served at the postpartum visit. With the exception of one subject in Chañaral and one in Antofagasta who had grade 2 (visible) goiters at both prenatal visits, all goiters classified in this study were grade 1.

Urinary iodine excretion was lower in Taltal than the other two cities. The women from Chañaral had a significantly higher T_3 level than women from the other two cities at both prenatal visits, however no differences were observed at the postpartum visit. FT_4 was significantly lower at the second prenatal visit compared to the first visit in all three cities. At the second prenatal visit the Kruskal-Wallis p value is highly significant for a difference in FT_4 levels among the three cities, however, with regression analysis that controlled for differences in maternal age among the three cities (Table 2), the differences among cities were no longer significant. Figure 3 shows maternal FT_4 levels at the first and second prenatal visits compared with weeks of gestation at those visits. TSH remained unchanged throughout gestation and there were no differences among the three cities. Maternal TSH cumulative distributions for the first and second prenatal visits combined were nearly identical for the subjects from the three cities as shown in Figure 4. The results of the 194 frozen serum samples analyzed at the Boston Medical Center for T_3, FT_4, and TSH (not shown) were highly consistent with the results from the Catholic University Laboratory for the same specimens. R^2 values from linear regression of the Catholic University data on the corresponding Boston Medical Center data without intercept were 0.98, 0.96, and 0.83 for FT_4, T_3, and TSH, respectively.

One woman from Chañaral was taking T_4 throughout gestation and was enrolled without recognition of this exclusion factor. Three subjects from Chañaral, four from Taltal, and one from Antofagasta had a serum TSH greater than 4.5 μUI/mL and according to protocol, were maintained on T_4 after the first prenatal visit. One additional subject from each city had a serum TSH greater than 4.5 μUI/mL at the second prenatal visit and was subsequently maintained on T_4 for the duration of pregnancy. Differences among the cities for subjects placed on T_4 were not significant. In all, 12% of the entire cohort (16 from Antofagasta, 13 from Taltal, and 8 from Chañaral) had a TSH greater than the laboratory nonpregnant reference range of 4.2 μUI/mL at either the first or second prenatal visit. The data presented in Table 3 represent the entire data set including the women taking T_4. A similar analysis (not shown) excluding subjects who were taking T_4 at the time of the prenatal and postpartum visits did not change any of the significant findings. One woman from Antofagasta without antithyroid antibodies developed hypothyroidism by the second prenatal visit (TSH = 61.8). Excluding this one subject the mean TSH among 47 women from Antofagasta was 2.46 ± 1.44 μUI/mL. One additional woman from Antofagasta who had a high titer of anti-TPO antibodies became hypothyroid by the time of the postpartum visit. Excluding those two outliers, the mean TSH among the 40 other women from Antofagasta was 2.39 ± 2.8 μUI/mL at the postpartum visit.

Serum selenium levels on a subset of second prenatal visit maternal samples were slightly different among the three cities, but were well within the reference interval, suggesting that selenium deficiency was unlikely among this study population. Serum thiocyanate levels among nonsmoking mothers at the postpartum visit were not significantly different among the three cities and were all in the lower range of what has been generally reported, indicating that dietary thiocyanate was not likely to be a confounder in this study.

TABLE 3. MATERNAL PARAMETERS

	Antofagasta			Chañaral			Taltal			City effects significance	
	n	Mean	SD	n	Mean	SD	n	Mean	SD	Kruskal-Wallis p value	Regression[a] p value
First Prenatal											
Weeks' gestation	46	16.0	4.4	45	17.3	4.4	64	15.3	4.1	0.05	
Weight (kg)	46	62.1	13.5	45	67.6	13.1	60	66.8	14.6	0.04	
% with goiter	46	8.7%		45	24.4%		64	9.4%		0.04[b]	
T_3 (ng/dL)	64	183	35.2	52	207	38.5	65	187	36.1	0.001[c]	0.0004
Free T_4 (ng/dL)	64	0.97	0.15	52	0.95	0.13	65	0.99	0.13	0.19	0.18
TSH (μUI/mL)	64	2.63	1.54	52	2.81	1.78	65	2.61	1.45	0.91	0.75
Thyroglobulin (ng/mL)	58	4.32	3.63	45	3.67	3.49	58	3.64	3.31	0.30	
% Anti-TPO	64	6.3%		52	15.4%		64	9.4%		0.26	
% Anti-Tg	67	9.4%		49	8.2%		64	9.4%		0.97	
Urine Iodine, μg/dL	63	32.1	17.9	40	30.3	18.0	62	32.5	18.4	0.73	
Iodine/g creatinine	62	407	191	39	363	140	62	323	157	0.02	
Urine perchlorate μg/L	61	24.5	23.9	53	66.7	84.9	59	132.9	103.9	<0.0001	
Perchlorate/g creatinine	61	28.4	22	53	80.2	129.6	59	135.5	95	<0.0001	
Serum perchlorate μg/L[d]	3	<4		7	<4		14	10.9	2.1	0.0001	
Second Prenatal											
Weeks' gestation	39	32.2	4.1	33	30.8	4.3	46	33.2	2.7	0.02	
Weight (kg)	38	69.2	14.4	31	74.0	13.0	46	75.3	12.2	0.02	
% with goiter	39	7.7%		33	36.4%		46	15.2%		0.01[b]	
T_3 (ng/dL)	48	196	46.3	40	206	40.7	38	173	39.9	0.003[c]	0.006
Free T_4 (ng/dL)	48	0.86	0.13	40	0.82	0.09	38	0.83	0.12	0.015	0.61
TSH (μUI/mL)	48	3.69	8.69	40	2.55	2.12	38	2.08	0.86	0.63	0.68
Thyroglobulin (ng/mL)	47	2.97	2.05	38	2.99	2.39	35	3.70	2.78	0.23	
% Anti-TPO	49	6.1%		40	7.5%		37	8.1%		0.93	
% Anti-Tg	48	4.2%		38	0.0%		37	5.4%		0.38	
Urine iodine, μg/dL	47	24.2	16.4	27	40.4	19.4	37	21.7	10.9	<0.0001	
Iodine/g creatinine	47	368	192	27	422	123	37	358	120	0.08	
Serum Se, μg/L	5	94.2	12.0	5	133.6	30.7	4	134.0	18.9	0.048	
Urine perchlorate μg/L	35	16	14.3	36	73.2	178.1	27	128.9	127	<0.0001	
Perchlorate/g creatinine	35	23.5	18.2	36	71.9	148.9	27	192.1	138.6	<0.0001	
Serum perchlorate μg/L[d]	5	<4		5	<4		6	13.2	1.7	0.0009	
Postpartum											
Weeks' gestation	45	16.2	10.2	45	13.3	8.7	49	9.2	3.8	0.01	
Weight (kg)	38	60.3	10.2	43	68.0	12.3	48	68.1	13.2	0.01	
% with goiter	45	11.1%		45	24.4%		49	22.5%		0.21	
T_3 (ng/dL)	42	115	40.3	26	114	50.2	46	107	28.1	0.74	0.48
Free T_4 (ng/dL)	42	0.93	0.24	26	1.00	0.41	46	0.95	0.20	0.95	0.97
TSH (μUI/mL)	42	8.25	27.02	26	2.34	1.86	46	1.95	1.88	0.29	0.13
Thyroglobulin (ng/mL)	41	3.80	5.31	20	6.50	8.05	44	5.78	10.03	0.16	
% Anti-TPO	41	7.3%		25	16.0%		36	8.3%		0.49	
% Anti-Tg	42	2.4%		20	0.0%		46	4.3%		0.60	
Urine Iodine, μg/dL	35	32.6	20.3	25	32.3	15.2	40	20.9	11.6	0.004	
Iodine/creatine ratio	34	382	488	25	344	95	39	244	114	0.003	
Urine perchlorate μg/L	6	22.3	23.8	4	17.5	10	16	49.1	35.2	0.016	
Perchlorate/g creatinine	6	49.7	88.8	4	23	18	16	54	36.9	0.19	
Serum perchlorate μg/L[d]	4	4		0			0				
Breast milk perchlorate μg/L	14	81.6	277.1	16	18.3	17.7	25	95.6	54.6	<0.0001	
Breast milk iodine, μg/L	14	45.4	37.4	16	32.5	15.0	25	38.4	15.4	0.41	
Serum SCN, μmol/L	9	20.0	10.9	21	17.4	16.1	10	12.6	6.4	0.072	

[a]Regression with city, parity, antibodies, mother's age and weeks (gestation, postpartum or gestation age), transformations of the dependent variables were used to assure normality of the residuals.

[b]Goiter prevalence was not different between Antofagasta and Taltal at the first (p=0.97) or second (p=0.1) prenatal visits.

[c]T3 was not different between Antofagasta and Taltal at the first (p=0.48) or seconed (p=0.08) prenatal visit.

[d]There was no attempt to measure serum perchlorate in all samples

SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyrotropin; TPO, thyroid peroxidase; Tg, thyroglobulin.

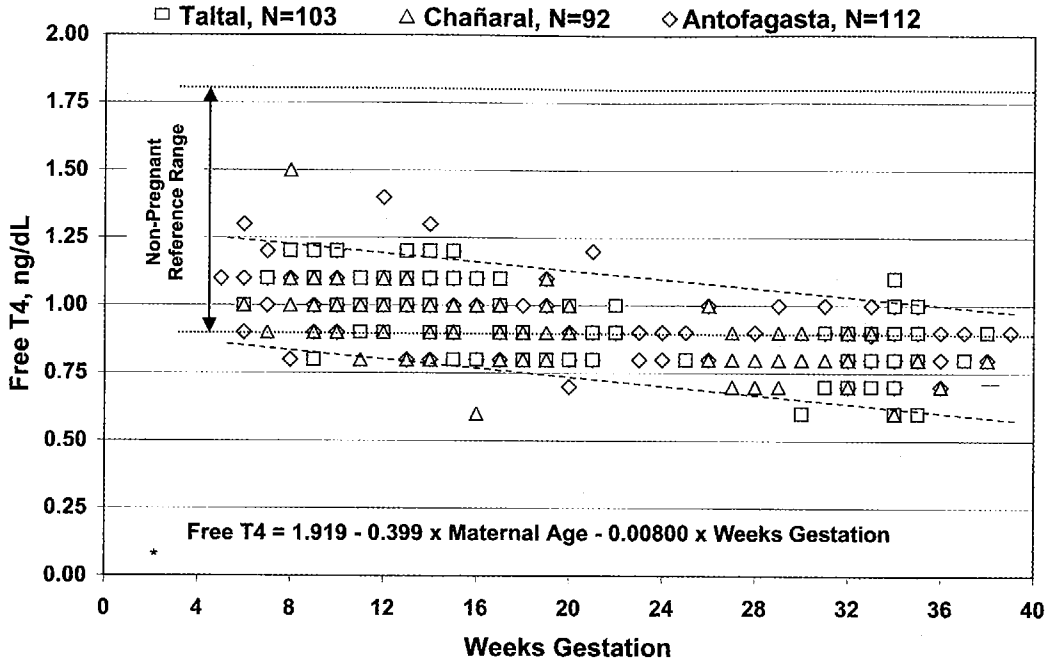

FIG. 3. Maternal free thyroxine (FT_4) levels during gestation (first and second prenatal visits combined) from each city. Initial regression using repeated measures included a city effect ($p = 0.1543$), maternal age ($p = 0.004$), and weeks' gestation ($p < 0.0001$). Above regression was performed excluding a city effect. Mixed model repeated measures regression of FT_4 with maternal age and weeks gestation, $n = 307$, variance = 0.01444. The dashed lines are the 10th and 90th percentiles on the overall regression.

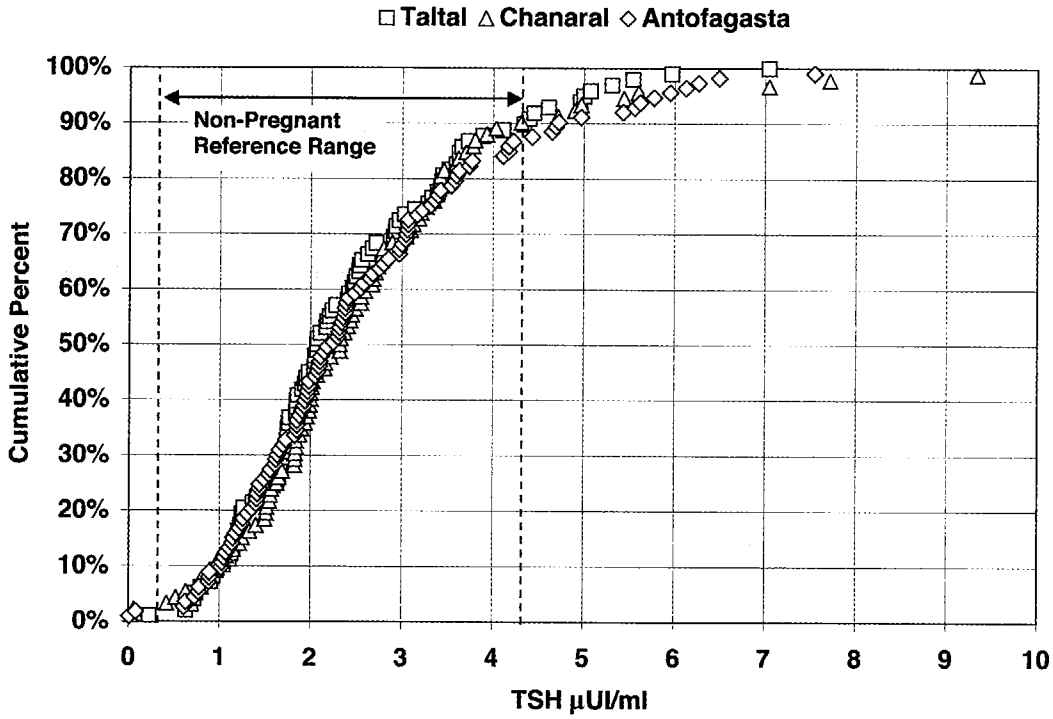

FIG. 4. Cumulative distribution of maternal thyrotropin (TSH) levels during gestation (first and second prenatal visits combined) from each city. Note: Two second prenatal data points with TSH > 10, one from Antofagasta and one from Chañaral not shown on graph.

TABLE 4. NEONATAL PARAMETERS

	Antofagasta			*Chañaral*			*Taltal*			*City effect significance*	
	n	*Mean*	*SD*	n	*Mean*	*SD*	n	*Mean*	*SD*	*Kruskal-Wallis p value*	*Regression*[a]
Gestational age (weeks)	55	38.9	3.9	49	38.2	4.1	55	38.4	2.6	0.01	
% Male	55	49.1%		48	54.2%		55	72.7%		0.03	
% Vaginal birth	56	60.7%		49	69.4%		57	40.4%		0.01	
% Alive	56	96.4%		49	95.9%		57	94.7%		0.90	
Length, cm	55	49.9	2.1	48	49.7	3.1	54	49.6	3.3	0.98	0.46
Weight, g	55	3459	488	48	3464	705	55	3356	593	0.30	0.98
Head circumference, cm	42	35.2	2.8	45	35.1	1.4	50	34.7	1.5	0.32	0.51
T_3 (ng/dL)	35	79	13.4	42	73	17.9	28	82	20.6	0.03	0.003[b]
Free T_4 (ng/dL)	35	1.07	0.16	42	1.04	0.13	28	1.03	0.14	0.73	0.08
TSH (μUl/ml)	33	6.20	2.96	40	6.69	4.13	28	6.31	2.91	0.99	0.69
Thyroglobulin (ng/mL)	30	16.79	9.93	36	14.03	10.98	28	18.11	12.82	0.29	0.01[c]
Serum perchlorate, μg/L[d]	4	<4		1	<4		14	19.9	5.0	0.005	

[a]City significance in regression with city, parity, antibodies, mother's age, gender and gestation age. Transformations of dependent variables were used to assure normality of the residuals.
[b]T_3 was not different between Antofagasta and Taltal (p=0.76).
[c]Thyroglobulin was not different between Antofagasta and Taltal (p=0.95).
[d]There was no attempt to measure serum perchlorate in all samples.
SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyrotropin.

Although the median breast milk perchlorate concentrations at the postpartum visit (less than 4, 19, and 104 μg/L for Antofagasta, Chañaral, and Taltal, respectively) were similar to drinking water concentrations in the respective cities, the breast milk perchlorate levels were highly variable. The levels ranged from less than 4 μg/L in each city to a maximum of 1042, 61, and 204 in Antofagasta, Chañaral, and Taltal, respectively. The woman with the highest (1042 μg/L, confirmed on repeat analysis) breast milk concentration in Antofagasta had less than 4 μg/L perchlorate in her home tap water. Analysis of a urine samples collected on the same day as this milk sample also contained elevated levels of perchlorate (230 μg perchlorate per gram creatinine), confirming recent significant exposure. On a second postpartum visit, her breast milk concentration was 74 μg/L. We were unable to determine the source of the elevated breast milk perchlorate in this individual. No significant correlations could be established between breast milk perchlorate and either urine perchlorate or breast milk iodine concentrations.

Neonatal characteristics for all of the infants are summarized in Table 4. A similar analysis (not shown) excluding infants born to mothers who were taking T_4 at the time of delivery did not change any of the findings. There were no differences in indicators of fetal development (birth weight, length, head circumference) among the three cities. Birth weight distributions among the neonates from the three cities were similar to the distribution reported for U.S. neonates in NHANES III (39) as shown in Table 5. There were no differences in FT_4 or TSH in cord sera attributable to city of origin. T_3 and thyroglobulin were statistically lower among neonates from Chañaral in contrast to the statistically higher maternal T_3 levels. T_3 and Tg were not significantly different between Antofagasta and Taltal. The percentage of male births (72.7%) in Taltal was significantly higher than expected, however, we reported a 50.7% prevalence of males among 484 births occurring between 1996 and 1998 in Taltal (25).

The percentage of cesarean births was significantly higher

TABLE 5. NEONATAL BIRTH WEIGHT DISTRIBUTION (GRAMS)

		Percentile						
	n	*5%*	*10%*	*25%*	*50%*	*75%*	*90%*	*95%*
Antofagasta	53	2710	2924	3160	3390	3700	4068	4306
Chanaral	47	2283	2768	3190	3570	3885	4260	4388
Taltal	56	2645	2870	3080	3350	3740	4055	4090
NHANES III[a]	—	2440	2680	3030	3360	3700	4000	4195

[a]Mean for males and females

in Taltal (57.1%) relative to Antofagasta (39.3%) and Chañaral (30.6%). In contrast to Antofagasta and Chañaral, where the majority of women chose to deliver in their home cities, 45% of the women from Taltal chose to deliver in Antofagasta, which is 125 miles to the north. Of the women from Taltal who delivered in Antofagasta, a significantly higher percentage ($p = 0.013$) delivered via Caesarian section (76.0%) compared to those that delivered via cesarean section in Taltal (41.9%). There was no indication of fetal distress as the reason for higher cesarean-section rates for those women from Taltal who delivered in Antofagasta. Cord TSH levels were slightly but not significantly lower for infants delivered by cesarean relative to those delivered vaginally in each of the three cities, however, the difference nearly reached significance in the three cities combined ($p = 0.06$).

Analyses of 20 maternal prenatal serum samples from Taltal (combined first and second prenatal visits) for perchlorate (Table 3) averaged 12.2 ± 2.4 μg/L, while analyses of 14 cord serum samples from Taltal averaged 19.9 ± 5.0 μg/L (Table 4). Perchlorate was nondetectable in a total of 12 maternal samples each from Chañaral and Antofagasta. Four cord serum samples from Antofagasta and one from Chañaral were also nondetectable for perchlorate. These data have been used by the AFRL to further the development of a PBPK model for perchlorate (40).

Cumulative urine perchlorate excretion (μg/g creatinine) distributions based on the 297 urine perchlorate measurements performed at the CDC are presented in Figure 5. Overall the distributions are significantly different from one another, and are generally consistent with the differences in perchlorate concentrations in the respective municipal water systems. Somewhat surprisingly, there is significant overlap of perchlorate excretion among the women from the three cities. Additionally, the median perchlorate excretions in Antofagasta and Chañaral significantly exceed what would be reasonably expected if the respective municipal water supplies were the only source of perchlorate. Serum thyroid hormones were available corresponding to 281 of the urine perchlorate values. Regression analysis for the entire dataset indicates no significant correlation between perchlorate excretion and T_3, FT_4, TSH, or Tg. Urine perchlorate excretion was not different for those subjects with goiter observed at the first or second prenatal visit (77 μg/g creatinine, $n = 33$) than for those subjects without goiter (84 μg/g creatinine, $n = 238$, $p = 0.76$).

Forty-two of the women who provided breast milk samples during the postpartum visit were nonsmokers while two from Chañaral and one from Antofagasta were smokers. There was no decrease in breast milk iodine associated with the perchlorate in municipal water in Taltal (Table 3). Perchlorate was found in 3 of the 14 breast milk samples from Antofagasta, with the highest being 1042 μg/L. Perchlorate was not detectable in three of the 25 samples from Taltal or in 6 of the 16 samples from Chañaral. Median breast milk iodine to urine iodine ratios are presented in Table 6 for 39 nonsmoking subjects who provided both a urine and a breast milk sample at the postpartum visit. Median breast milk iodine to urine iodine ratios were higher in Taltal relative to the other two cities and this difference reached marginal statistical significance.

Discussion

This is the first epidemiologic study of potential thyroid-related health effects among pregnant women with environmental perchlorate exposure. Perchlorate levels in the

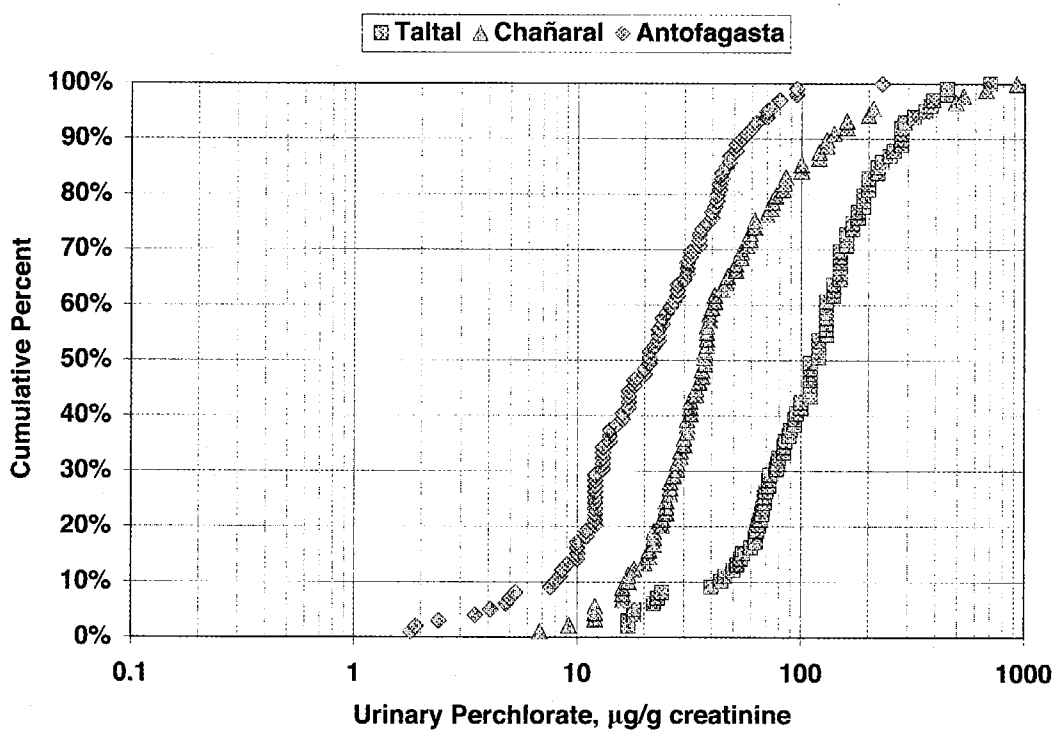

FIG. 5. Cumulative distribution of urine perchlorate excretion by city.

TABLE 6. BREAST MILK/URINE IODINE RATIOS (NON-SMOKERS ONLY)

	Antofagasta			*Chañaral*			*Taltal*			
	n	*Mean*	*Interquartile Range*	n	*Mean*	*Interquartile Range*	n	*Mean*	*Interquartile Range*	*Kruskal-Wallis p value*
Breast milk iodine/ urine iodine	9	1.04	1.05	9	1.10	0.35	21	1.88	2.42	0.03
Breast milk iodine/urine Iodine/grams creatinine	9	1.18	1.28	9	0.81	0.55	20	1.04	1.14	0.06

pregnant women's home tap water were essentially the same as reported in public drinking water samples from the same cities in 2000 (25). The urinary perchlorate excretion distributions indicate a difference in perchlorate dose among the three cities that is generally consistent with the perchlorate levels in the municipal tap water.

In Table 2, the self-reported mean tap water consumption estimates are approximately 1 L/d, approximately half the 2 L/d default assumption used in risk assessments for environmental contaminants. An additional mean fluid consumption from bottled water and sodas, etc., of 670 mL/d was reported by this cohort. The mean creatinine excretion rates among pregnant women has been reported to be 1.08 g/d (41). Table 7 compares estimates of the median daily perchlorate excretion based on this creatinine excretion rate to estimates of the consumption of perchlorate through tap water. In each city, median perchlorate excretion exceeds the intake attributable to tap water by 21.7–33.8 μg/d. While this analysis is for median values, it can be seen in Figure 5 that the actual urine perchlorate concentrations are highly variable. This is consistent with a significant and highly variable dietary source of perchlorate that is similar in all three cities. It is likely that the highly variable breast milk perchlorate concentrations found at the postpartum visit can also be attributed to a variable dietary source of perchlorate. We did not, however, identify any specific dietary source of perchlorate.

In Taltal, perchlorate doses were sufficient to result in detectable perchlorate in all of the cord and maternal serum samples tested using IC, which provides an additional measure of internal dose. Thus perchlorate exposure and dose have both been well defined in this population.

Chile has previously had documented excess iodine excretion levels that likely explain, at least in part, the high goiter prevalence that we previously described as well as the high prevalence of relatives of school children with thyroid conditions (25). Since 2000, iodine excretion levels have dropped considerably in Chile. As shown in Figure 2, iodine excretion is intermediate between levels in the United States in the early 1970s (NHANES I) and early 1990s (NHANES III) and considered acceptable under current WHO criteria, which recommends that the ideal dietary allowance of iodine is 200 μg of iodine per day for pregnant women. We did not find evidence to suggest that nitrate, thiocyanate, selenium, arsenic, or lithium levels were potential confounders in this study. The goiter prevalence observed in this study is higher than current U.S. experience, possibly because of the high iodine levels in the recent past. Goiter was not significant in repeated measures modeling (first and second prenatal visits) of the dependent variables (FT_4, T_3, and TSH) with independent variables including maternal age, parity, antibodies, and weeks' gestation (results not shown).

We do not have an explanation of higher frequency of goiter in Chañaral. To limit the effect of possible differences in their physical examinations of the thyroid, the examiners distributed themselves in the three cities (Dr. Michaud in Antofagasta, Dr. Reyes in Chañaral, and Dr. Téllez in Taltal). The examiners tested their degree of concordance in the physical examination of thyroid gland. Dr. Téllez accompanied both Dr. Reyes and Dr. Michaud to their respective cities several times, and independently, they examined each woman and annotated the results on separate sheets. When there were differences in their examinations, they examined the mother again to get consensus. The agreed upon result was recorded in the study database. Dr. Téllez and Dr. Michaud both examined a total of 37 mothers from Antofagasta and the chance-corrected agreement (κ statistic, 42) between them was 0.84. Dr. Téllez and Dr. Reyes examined a

TABLE 7. ANALYSIS OF DIETARY AND DRINKING WATER SOURCES OF PERCHLORATE

	Antofagasta	*Chañaral*	*Taltal*
Median estimated tap water consumption, L/d	0.84	1.06	0.82
Mean tap water perchlorate, μg/L	0.5	5.8	114
Calculated perchlorate consumed daily from tap water, μg/d	0.42	6.1	93.5
Median measured perchlorate in urine, μg/g creatinine	20.5	37	110
Estimated creatinine excretion, g/day (41)	1.08	1.08	1.08
Calculated median perchlorate excretion, μg/d	22.1	40.0	118.8
Perchlorate gap, μg/d (urine excretion minus water consumption)	21.7	33.8	25.3

Note: individual urine perchlorate levels varied considerably. The difference between median excretion and median water consumption is assumed to represent dietary intake.

total of 80 mothers (some of them were examined both during pregnancy and postpartum) with $\kappa = 0.63$. Most of the thyroid examinations in Chañaral were done by both Dr. Reyes and Dr. Téllez (80/123). Altogether 41.5% of thyroid examinations in Antofagasta were done by both Dr. Michaud and Dr. Téllez, but their concordance was high. Therefore, the higher goiter prevalence in Chañaral cannot be explained by different examiners.

The vast majority of pregnant women present themselves for prenatal care before 24 weeks of pregnancy. In the smaller cities of Taltal and Chañaral, we incorporated almost all pregnant women who presented for prenatal care, except, for a few who did not consent to participate. For this reason, the probability that the study inadvertently excluded more complicated pregnancies, or women with thyroid disease is low. We cannot exclude that some women (very few) self-selected for diversion to other medical care facilities. Overall, we feel that the women who participated in this study are a representative sample.

Numerous studies have reported increases in maternal Tg (15–17,19–21) and/or increases in TSH (4,10,11,13,16,17,19, 20) during gestation or at delivery in regions with iodine deficiency. These observations lead to the conclusion that the combination of pregnancy and relative iodine deficiency causes stress on the maternal thyroid. A decrease in TSH in early gestation in response to the increase in human chorionic gonadotropin (hCG) followed by an increase in later gestation has been reported (22). We did not observe this in the current study, perhaps because the first prenatal visit occurred on average at approximately the 16th week of gestation. We did not observe an increase in either TSH or Tg during gestation in Taltal, indicating that the 114 μg/L perchlorate in the municipal drinking water there does not induce a significant relative iodine deficiency.

We observed an average of 14% decrease in maternal FT_4 throughout gestation in all three cities between the first and second prenatal visits, and the mean FT_4 level at the second prenatal visit was slightly below the nonpregnant reference range. Regression analysis indicated that there is a significant effect of maternal age on FT_4 levels but no difference between cities. Thus, perchlorate at the concentrations seen in Taltal did not diminish maternal FT_4 during gestation. Decreases in FT_4 in the range of 12%–38% between the first and third trimesters have been reported by others (10–13,17, 19–21) with the largest decreases observed in regions with iodine deficiency.

Maternal and cord serum FT_4, TSH, and Tg levels have been reported in both iodine-sufficient and iodine-deficient populations. We can perhaps make some meaningful comparisons between the current study and others by comparing the ratio of cord and maternal serum values, although estimates from the current study are based on the second prenatal visit and not at delivery. The cord mean FT_4 to maternal mean FT_4 ratio in the present overall cohort is 1.25, consistent with a range of 1.03 to 1.49 (12,15,21,43) reported elsewhere. The ratio of median cord TSH to median maternal TSH is 2.87, slightly lower than the available range of 3.09 to 7.05 (11,15,18,21,43,44). The ratio of mean cord serum Tg to mean maternal serum Tg is 5.08, slightly higher than 2.2–3.5 (12,15,21) observed in other recent studies. Thus, combined results from the three cities in this study are reasonably consistent with other published studies. Moreover, the cord serum concentrations of FT_4, TSH, and Tg in Taltal do not suggest that perchlorate in the municipal drinking water stressed the neonatal thyroid.

It has been speculated (45) that perchlorate from environmental sources might cause a decrease in breast milk iodine concentrations. This appears to be the case with thiocyanate because breast milk iodine to urine iodine concentration ratios among women who smoke cigarettes (0.57) (44) and among women in a region that relies heavily on cassava as a food staple (0.57) (46) are lower than those reported in other populations, which range from 0.8 to 2.0 (18,44,47,48). Breast milk iodine concentrations were not decreased, and the breast milk iodine to urinary iodine ratio was not decreased (Table 6), by perchlorate at 114 μg/L in Taltal, which suggests that the effect of perchlorate at these levels is negligible compared with that of thiocyanate from cigarette smoking or cassava consumption in other populations.

Based on urinary iodine excretion, the highest and median perchlorate doses in Taltal were 0.017 and 0.0018 mg/kg per day, respectively. Research in dairy cows (49) has shown a threshold for inhibition of iodine transfer to milk by perchlorate at 0.1 mg/kg per day and maximal effect at 2.0 mg/kg per day. Thus, total perchlorate doses seen in the current study are orders of magnitude lower than doses likely to significantly reduce breast milk iodine.

We found a statistically significant increase in maternal T_3 and goiter prevalence in Chañaral during gestation that was not observed at the post partum visit. It is not likely that these differences are the result of the low perchlorate concentrations in municipal drinking water in Chañaral, considering that the concentration in Taltal was approximately 20-fold higher and no similar changes were observed there.

The mechanism of action of perchlorate is well known. At some serum perchlorate concentration, iodine uptake will be sufficiently inhibited to cause decreases in serum FT_4 and breast milk iodine, and increases in serum Tg and TSH. In this study, we did not see any of these changes in either the mother during gestation or the neonate at birth. This indicates that the perchlorate dose in Taltal was not sufficiently high to stress the maternal or neonatal thyroid.

The mean cord perchlorate concentration in Taltal was 19.9 μg/L (0.199 μmol/L), which can be estimated to cause approximately 13% inhibition of iodine uptake in the neonate (50). A recent report by the National Research Council (51) indicates that iodine uptake inhibition is not an adverse event, and that greater than 75% inhibition would be necessary before adverse effects on thyroid hormones would be likely. It can be estimated that a 75% inhibition of iodine uptake would require a serum perchlorate concentration of approximately 4 μmol/L (50). Thus, the cord serum perchlorate levels in Taltal are 20 times lower than the lowest levels that could be anticipated to cause adverse thyroid hormonal effects.

The potency of perchlorate in iodide uptake inhibition at the human sodium iodide symporter (NIS) has recently been determined to be 15 times that of thiocyanate on a serum molar concentration basis (50). The mean maternal serum perchlorate concentration in Taltal of 12.2 μg/L (0.122 μmol/L), was therefore equivalent to 1.83 μmol/L thiocyanate in iodine uptake inhibition. Typical serum thiocyanate concentrations are 10–70 and 80–120 μmol/L among nonsmokers and smokers respectively (50). Thus, it is ap-

parent that inhibition of iodine uptake at the NIS resulting from the internal perchlorate doses in Taltal is negligible compared with the inhibition of iodine uptake attributable to dietary thiocyanate. This is consistent with the lack of observed effect of perchlorate on breast milk iodine or on serum FT_4, TSH, or Tg in the current study.

In our previous study in this region among schoolchildren, the urinary iodine excretion among the children was found to be excessive (25). It has been postulated that the high dietary iodide intake makes this geographic area unsuitable for studies of the thyroid effects of perchlorate exposure. The contention is that the iodide intake is so high that substantial competition with perchlorate cannot be detected. The National Academy of Sciences recently addressed this concern and concluded (51) "on the basis of the iodide-inhibition analyses, the additional comparisons, and a review of information on urinary iodide excretion, the committee concluded that the data from Chile could be considered in the evaluation of the U.S. experience with perchlorate in drinking water." Because total iodine nutrition among the current cohort of pregnant women is similar to that of U.S. pregnant women, it is clearly not an issue in the current study.

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