Health Effects Assessment for Environmental Perchlorate Contamination: The Dose Response for Inhibition of Thyroidal Radioiodine Uptake in Humans

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Application of a sensitive new detection method has revealed widespread perchlorate contamination of groundwater in the southwestern United States, typically at 0.005–0.020 mg/L (5–20 ppb). Perchlorate is a competitive inhibitor of the process by which iodide is actively transported from the bloodstream into the thyroid. This inhibitory action of perchlorate is the basis of its pharmaceutical use (in the treatment of hyperthyroidism) as well as its potential toxicity. To establish the dose response in humans for perchlorate inhibition of thyroidal iodide uptake and any short-term effects on thyroid hormones, we gave perchlorate in drinking water at 0.007, 0.02, 0.1, or 0.5 mg/kg-day to 37 male and female volunteers for 14 days. In 24 subjects we performed 8- and 24-hr measurements of thyroidal ^{123}I uptake (RAIU) before exposure, on exposure days 2 (E2) and 14 (E14), and 15 days postexposure (P15). In another 13 subjects we omitted both E2 studies and the 8-hr P15 study. We observed a strong correlation between the 8- and 24-hr RAIU over all dose groups and measurement days. We found no difference between E2 and E14 in the inhibition of RAIU produced by a given perchlorate dose. We also found no sex difference. On both E2 and E14, the dose response was a negative linear function of the logarithm of dose. Based on the dose response for inhibition of the 8- and 24-hr RAIU on E14 in all subjects, we derived estimates of the true no-effect level: 5.2 and 6.4 µg/kg-day, respectively. Given default body weight and exposure assumptions, these doses would be ingested by an adult if the drinking-water supply contained perchlorate at concentrations of approximately 180 and 220 µg/L (ppb), respectively. On P15, RAIU was not significantly different from baseline. In 24 subjects we measured serum levels of thyroxine (total and free), triiodothyronine, and thyrotropin in blood sampled 16 times throughout the study. Only the 0.5 mg/kg-day dose group showed any effect on serum hormones: a slight downward trend in thyrotropin levels in morning blood draws during perchlorate exposure, with recovery by P15. *Key words:* clinical, human, iodine, perchlorate, risk assessment, sodium-iodide symporter, thyroid. *Environ Health Perspect* 110:927–937 (2002). [Online 14 August 2002]

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The ammonium salt of the perchlorate ion is manufactured primarily for use by the Department of Defense, the National Aeronautics and Space Administration, and the aerospace industry as a source of oxygen in solid propellant systems for rockets and missiles. Ammonium perchlorate is also manufactured for use as an oxidizer in fireworks and matches and for pharmaceutical use. In addition, perchlorate can occur naturally in nitrate-rich mineral deposits used as fertilizers. Analysis of nine commercial fertilizers revealed perchlorate in all samples tested at levels ranging from 0.15% to 0.84% by weight (*1*).

Application of a sensitive new detection method has revealed widespread perchlorate contamination of groundwater, particularly in Utah, California, Nevada, and Arizona. Perchlorate contamination is also found in surface waters, including Nevada's Lake Mead. Recent testing by the Los Angeles Metropolitan Water District revealed 8 µg/L at its Lake Mead intake, and the Southern Nevada Water authority found 11 µg/L in tap water (*2*). Sampling by the California Department of Health Services revealed 5–9 µg/L in the Colorado River. Of 2,459 drinking-water sources tested in California, 48 (2.0%) contained concentrations of perchlorate higher than the state's action level of 18 ppb (*3*). Assuming a default consumption rate of 2 L/day, perchlorate at 18 ppb (18 µg/L) in drinking water results in ingestion of 36 µg/day (0.51 µg/kg-day for a 70-kg person).

Perchlorate is a competitive inhibitor of the process by which iodide, circulating in the blood, is actively transported into thyroid follicular cells (*4,5*). The site of this inhibition is the sodium-iodide symporter, a membrane protein located on the basolateral side of the follicular cell, adjacent to the capillaries supplying blood to the thyroid (*6*). The thyroid follicle is the functional unit of the thyroid; a single layer of follicular cells at the surface surrounds a colloidal protein matrix. At the colloid interface, organification of iodide occurs. Organification is a complex, enzyme-dependent process whereby iodide is oxidized and bound to tyrosyl residues within thyroglobulin, ultimately forming the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). If sufficient inhibition of iodide uptake occurs, formation of thyroid hormones is depressed. Depression of thyroid hormone formation secondary to inhibition of thyroidal iodide uptake is the precursor of any potentially adverse effect of perchlorate and is also the basis for its major current and former pharmaceutical usages.

Treatment of thyrotoxicosis (including Graves' disease) with 600–2,000 mg potassium perchlorate (430–1,400 mg perchlorate) daily for periods of several months or longer was once common practice, particularly in Europe (*7,8*). According to Wolff (*9*), although 400 mg of potassium perchlorate divided into four or five daily doses was used initially and found effective, higher doses were introduced when 400 mg/day was discovered not to control thyrotoxicosis in all subjects. Also according to Wolff, seven case reports of fatal aplastic anemia between 1961 and 1966 curtailed the therapeutic use of perchlorate at that time (*9*). However, two decades later, physicians reported treating thyrotoxicosis successfully with lower maintenance doses of potassium perchlorate (40–200 mg/day) for 2 years or longer, in the absence of adverse effects (*10,11*). More recently, perchlorate has been used (alone or in combination with other antithyroid drugs) to treat amiodarone-induced thyrotoxicosis or hypothyroidism, conditions in which underlying thyroid abnormalities are unmasked when the iodine-containing drug amiodarone is given to control cardiac arrhythmia (*9*). Treatment regimens include 500 mg potassium perchlorate twice per day for 18–40 days (*12*) and, for mild cases, 250 mg/day for 4–6 weeks (*13*). When we began the present study, we hoped our results would allow us to estimate, for the temporal dosing pattern

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tested, a maximum effect level for perchlorate inhibition of iodide uptake. With this information in hand, physicians might be prompted to evaluate the efficacy of perchlorate doses lower than those frequently employed.

Thyroid hormones are essential to the regulation of oxygen consumption and metabolism throughout the body. Thyroidal iodine metabolism and thyroid hormone levels in serum and tissues are regulated by a number of homeostatic mechanisms (*14*). Thyrotropin (TSH), a hormone synthesized and secreted by the anterior pituitary gland (hypophysis), is the primary regulator of thyroidal iodide uptake and other aspects of thyroid function (*15*).

When hypothyroidism occurs in a woman early in pregnancy, the fetus is at risk for impaired physical and mental development, the severity of the impairment depending upon the degree of hypothyroidism. In the human fetus, the thyroid and the hypophysial TSH system begin to function at around 11 weeks of gestation, and T_4 secretion begins at around 18–20 weeks of gestation (*14*). Hypothyroidism during infancy is also a major risk factor for mental retardation and other manifestations of impaired neurodevelopment (*14*). To ensure that perchlorate in drinking water is well below levels that would produce even mild hypothyroidism, particularly in pregnant women and infant children, it is important to determine the dose response for perchlorate inhibition of iodide uptake and the relationship between iodide uptake inhibition and depression of thyroid hormone levels.

Before we began our study, the potential health effects in humans of long-term exposure to perchlorate at doses below the therapeutic range were examined in two cross-sectional occupational studies of perchlorate plant workers. In both studies, serum tests of thyroid function revealed no evidence for any adverse effect of perchlorate. In one study, the worker population consisted of 35 males and 2 females, 40% of whom had been employed for more than 5 years; the mean perchlorate doses absorbed during a single work shift, based on urinary excretion, were estimated as 4.0, 11, and 34 mg (0.057, 0.16, and 0.48 mg/kg), with values ranging from 0.4 to 69 mg (0.006–0.99 mg/kg) overall (*16*). In the other study, the worker population consisted of 39 males and 9 females with employment durations of 1–27 years (mean, 8.3 years); the mean work-shift perchlorate exposure was estimated as 0.036 mg/kg, with values ranging from 0.0002 to 0.44 mg/kg overall (*17*).

In a recent clinical study by Lawrence et al. (*18*), inhibition of thyroidal radioiodine uptake (RAIU) was measured in nine male volunteers given perchlorate at 10 mg/day in drinking water for 14 days. In a second study by the same group, eight male volunteers were given a dose of 3 mg/day (*19*). Body-weight adjusted doses were in the range of 0.089–0.140 mg/kg-day (mean, 0.12 mg/kg-day) in the 10-mg/day study and 0.033–0.041 mg/kg-day (mean, 0.038 mg/kg-day) in the 3-mg/day study (*20*).

The present investigation, conducted before publication of Lawrence et al.'s 3-mg/day study (*19*), was designed to provide expanded data on the dose response for perchlorate inhibition of RAIU in humans and to obtain pharmacokinetic data. Whereas Lawrence et al. used a fixed milligram dose in each person (*18,19*), we used body-weight-adjusted doses to provide greater precision. One important purpose of the present study was to establish a no-observed-effect level (NOEL) for perchlorate inhibition of the thyroidal uptake of iodide during 2-week exposure. The U.S. Environmental Protection Agency (U.S. EPA) defines the NOEL as "an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control" (*21*). Here we use the term NOEL more narrowly to indicate the highest exposure level tested at which inhibition of thyroidal iodide uptake is not statistically or biologically significant.

At the time the improved detection method for perchlorate in aqueous solution was developed, the toxicologic database on perchlorate was extremely limited. For example, few or no experimental data existed on the subchronic, reproductive, or developmental toxicity of perchlorate. To fill gaps in the toxicologic database, eight animal studies were designed by a consortium that included the U.S. EPA and the Department of Defense. The results of those animal studies were considered by the U.S. EPA in the development of its 1998 draft risk assessment for perchlorate (*22*). The dose response and time course of the effects of perchlorate in rats are being elucidated further by additional studies and data reevaluations, several designed in response to the external peer-reviewers' 1999 report (*23*) on the U.S. EPA's 1998 draft risk assessment. The additional studies include investigations of perchlorate pharmacokinetics, inhibition of thyroidal iodide uptake, and effects on serum levels of thyroid hormones and TSH in adult males, pregnant and nursing females, and, to a limited extent, fetuses and pups, at doses in the 0.01–10 mg/kg-day range (*24–26*). These data, along with data gathered in the clinical study reported here, are being used to develop and validate an interspecies (rat/human), physiologically based, pharmacokinetic model (*27–31*).

Although the physiology of the pituitary-thyroid axis is very similar in the rat and the human, the rat thyroid is much more rapidly responsive to any perturbation of iodine metabolism leading to decreased thyroid hormone formation (*32,33*). Thyroid hormone storage capacity is a major determinant of the difference in responsiveness. If thyroid hormone synthesis is prevented, the rat thyroid contains only enough hormone to last a few days (*32,33*), whereas the human thyroid has enough thyroid hormone to last several months (*34,35*).

An additional and potentially important interspecies difference is evidenced by the rapid up-regulation of thyroidal iodide uptake in rats in response to multiday treatment with perchlorate, a phenomenon not observed in humans. In rats, the extent of inhibition of thyroidal iodide uptake in the hours following single-dose exposure to perchlorate was found to be attenuated over the course of multiday exposure (*24*).

In a single-dose experiment, male rats were injected intravenously with a perchlorate dose of 0, 0.01, 0.1, 1, or 3 mg/kg-day followed 2 hr later by intravenous injection of radioiodine (^{125}I). Group mean values for thyroidal ^{125}I binding relative to the controls were reported for the six rats per dose sacrificed at 2, 6, or 9 hr following injection of ^{125}I. The dose response for inhibition of ^{125}I binding was similar at all three time points. At the 9-hr sacrifice, the extent of inhibition was statistically significant in the 0.1, 1, and 3 mg/kg-day groups (*24*).

In a multiday experiment, male rats were exposed to drinking water containing perchlorate at doses of 0, 1, 3, or 10 mg/kg-day. On exposure day 1 (E1), E5, and E14, a total of six rats per dose were administered ^{125}I by intravenous injection and sacrificed 2 hr later. The inhibition of thyroidal ^{125}I uptake (presumably measured as the bound fraction) observed on E1 was dose related and much more pronounced at each dose than on E5, when inhibition was observed only in the 3- and 10-mg/kg-day groups. By E14, inhibition was observed only in the 10-mg/kg-day group (*24*).

Additional evidence in rats for rapid homeostatic adjustment (i.e., up-regulation of uptake) in response to diminished thyroidal availability of iodine is provided by a study of the effects of switching from a high-iodine to low-iodine diet, the physiologic equivalent of introducing an inhibitor of iodide uptake. Following the diet shift, rats exhibited a 50% increase in radioiodine uptake in 1 day (*33*). Evidence that humans lack rapid up-regulation of thyroidal iodide uptake in response to perchlorate comes from the two clinical studies of Lawrence et al. (*18,19*), described above. At the end of 14-day exposure via drinking water, a dose-related inhibition of uptake was observed, with statistical significance achieved at the higher dose (mean, 0.12 mg/kg-day).

Given this disparity between rats and humans in the homeostatic up-regulation response, a more precise determination of the time course and dose response for perchlorate inhibition of thyroidal iodide uptake in humans should be extremely helpful in evaluating the health risks of environmental exposures to perchlorate.

In this article we report the results of radioiodine studies and serum tests of thyroid function. The results of perchlorate and iodine analyses in serum and 24-hr urine collected before and throughout exposure, along with an evaluation of the extent to which population variability in the magnitude of the response to perchlorate is related to iodine nutrition, will be presented in a separate report.

Materials and Methods

Sample collection and testing. Biological samples (i.e., whole blood, serum, and urine) were collected in the Oregon Health & Science University (OHSU) General Clinical Research Center (GCRC) by the nursing staff. All samples other than those to be analyzed for perchlorate or iodine were processed by the OHSU Hospital's Lab Central, which arranged for testing at the OHSU Hospital's Core Lab or a contract laboratory. In the following, analysis at the OHSU Hospital's Core Lab is understood unless otherwise specified.

Dose selection. When designing our study, we relied upon the observation by Lawrence et al. (*18*) that a mean perchlorate dose of 0.12 mg/kg-day inhibits RAIU to about 60% of baseline. We chose to test doses of 0.02, 0.1, and 0.5 mg/kg-day to broadly span the effective inhibitory range, thinking it possible that a dose of 0.02 mg/kg-day would be a NOEL. By the time the first 14 subjects had been tested at these three doses, it was clear that 0.02 mg/kg-day was not a NOEL. Regression analysis of the E14 uptake relative to baseline for the first 14 subjects predicted that a perchlorate dose of 0.007 mg/kg-day would produce no inhibition of RAIU; this became the fourth dose tested.

Subject screening and informed consent. During an initial interview, each prospective subject was given verbal information about the study's purpose, exclusionary criteria, and requirements. At that time we provided the appropriate consent form and asked the subject to take it home to review. The two consent forms, one for each protocol, were approved by the institutional review board at OHSU, where the study was conducted. Both forms listed the study's sponsor and explained the purpose, procedures, risks and discomforts, benefits, alternatives to participation, confidentiality, compensation provided ($1,200 for the main-study protocol, $650 for the uptake-study protocol), and the voluntary nature of participation. The consent form was signed at the screening visit by the subject, the principal investigator, and a witness.

The screening visit consisted of a history, a physical examination, blood sampling (for complete blood count, routine serum chemistry, and serum thyroid function tests), and urine sampling (for routine urinalysis, screening for drug abuse, and pregnancy testing for women who had not had a hysterectomy or tubal ligation). We excluded candidates if they had a history of thyroid disease, recent ingestion of any iodine-containing pharmaceutical (including thyroid hormone), or significant thyroid enlargement. Following one woman's enrollment and assignment to the 0.007-mg/kg-day dose group (uptake study), laboratory analysis of her screening-visit blood draw revealed elevated TSH (18 mIU/L). Because her thyroid hormone levels and physical examination were normal, we decided to keep her in the study to evaluate, in a subject with subclinical hypothyroidism, the effect of perchlorate at a dose predicted to be the NOEL. All 37 subjects who presented themselves for the screening visit met the screening criteria and were entered into the study.

Subject assignment. The first 24 subjects were tested according to our initial (main-study) protocol and the next 13 subjects according to our modified (uptake-study) protocol. We developed the uptake-study protocol as a means of including additional subjects with less expenditure of time and resources than required by the main-study protocol. The 37 subjects tested according to one or the other protocol were between 18 and 57 years old (mean ± SD, 38 ± 12 years; median, 40 years). All enrolled subjects remained in the study until its termination.

In the main study, four subjects of each sex received a perchlorate dose of 0.02, 0.1, or 0.5 mg/kg-day. In the uptake study, six women and one man received a dose of 0.007 mg/kg-day and one additional subject of each sex received a dose of 0.02, 0.1, or 0.5 mg/kg-day. Even though we found no statistically significant difference between men and women at the three doses tested in the main study, when testing the new dose in the uptake study we opted to concentrate on women because protecting the developing fetus is of primary concern.

The logistics of the study were such that only three subjects could be started each week. To preclude skewing the dose response through potential temporal variations in measured parameters, in the main study we gave each of the three subjects started each week a different perchlorate dose (0.02, 0.1, or 0.5 mg/kg-day), deviating from this schedule only to balance the sexes in each dose group as necessary. We assigned the first three subjects in the uptake study the 0.007 mg/kg-day dose and thereafter rotated subject enrollment through the four doses tested (0.007, 0.02, 0.1, and 0.5 mg/kg-day).

Study size. Considering that Lawrence et al. (*18*) found highly significant inhibition of uptake in nine subjects given perchlorate at 10 mg/day, a dose approximately the same as our 0.1 mg/kg-day dose and 4-fold lower than our 0.5 mg/kg-day dose, we considered it highly probable that a comparable group size (8–10 per dose) would yield a significant degree of inhibition at these two doses.

Perchlorate solutions: preparation and ingestion. Pharmaceutical-grade Perchloracap capsules, each containing 200 mg potassium perchlorate (144 mg perchlorate), were obtained from Mallinckrodt Medical, Inc. (St. Louis, MO). We emptied the contents of one or more capsules into a beaker, mixed the powder with 0.5–1.0 mL lemon juice to make a slurry, and added sufficient commercial spring water (Crystal Geyser, Mount Shasta, CA) to make a stock solution containing 50 mg $KClO_4$/100 mL. We prepared dosing solutions by appropriate dilution of the stock solution with spring water to a volume of 400 mL in a half-liter plastic bottle. The concentration of perchlorate in some of the stock solutions and in each subject's individual dosing solution was analyzed and confirmed at the Air Force Research Laboratory (AFRL; Wright-Patterson Air Force Base, OH). The stock solution samples were found by AFRL to contain perchlorate at 98% of the nominal concentration, indicating essentially complete solubility. Samples of the unadulterated spring water were also analyzed by AFRL to ensure the absence of perchlorate contamination. Some of the filler in the capsule was insoluble and remained visible as a fine white sediment in the stock solution.

We prepared and bottled dosing solutions several times for each subject during the 14 days of perchlorate administration so that storage in the subject's refrigerator would not be a problem. Subjects were given two 250-mL clear plastic cups that had been marked with a horizontal line to indicate the 100-mL level, one to keep at home and one to leave at work. They were instructed to drink 100 mL at 0800, 1200, 1600, and 2000 hr on each scheduled perchlorate ingestion day and to record the time and volume of each ingestion on a preprinted log sheet for additional verification. They were also instructed to empty the bottle completely into their 2000 hr aliquot to ensure that the entire prescribed perchlorate dose was ingested each day.

Design of the main and uptake studies. In the clinical evaluation of thyroid function, measurement of the accumulated thyroidal radioiodine (i.e., RAIU) at a given time point integrates the radioiodine uptake at earlier times. If inhibition of the rate of thyroidal iodide uptake was significantly greater at an earlier interval compared with later

intervals, this would be reflected in reciprocally increased urinary radioiodide excretion early on, such that less radioiodide would be available for thyroidal uptake at later intervals. After the rate of uptake becomes apparent (~2 hr following radioiodine administration), a mathematical model can be used to predict the thyroidal radioiodine uptake at any time during the accumulation period from a single uptake value measured at any other time (*36,37*). This relationship holds until approximately 24 hr, by which time all radioiodide has either been taken up by the thyroid or excreted in the urine. Measurement at 4–24 hr is more accurate (i.e., the signal-to-noise ratio is higher) than at times < 4 hr for two reasons: First, a variable time is required for radioiodide absorption from the gut into the bloodstream. Second, at earlier times, more radioiodide will be in the blood (i.e., not yet taken up by the thyroid or excreted in the urine), thereby contributing a higher proportion of the radioactivity measured over the thyroid and making background correction for thyroidal uptake less accurate (*38*). In subjects not undergoing treatment with a thyroactive drug, the 3-, 6-, and 8-hr RAIU values have been found to be highly correlated with the 24-hr RAIU values (*36,37*). Likewise, in subjects treated for 2 weeks with perchlorate at 10 mg/day, pairwise comparison revealed no significant differences among the 4-, 8-, and 24-hr radioiodine uptakes relative to baseline (*20,39*). In the present study, although we expected to find no difference between the 8- and 24-hr relative uptakes once a quasi-steady-state equilibrium between perchlorate absorption and elimination was established (i.e., in which perchlorate concentrations in blood fluctuated with the dosing schedule in a regular daily pattern, with no further net perchlorate accumulation over time), we chose to perform both 8- and 24-hr uptake measurements primarily because we were uncertain whether such an equilibrium would be achieved by E2.

The main study design was as follows:

- Ingestion of ^{123}I at 0900 hr on 4 days: the baseline visit (1 day before the start of perchlorate exposure), E2, E14, and postexposure day 15 (P15); measurement of RAIU at 1700 hr on the day of ^{123}I ingestion and 0900 hr the following morning.
- In addition to the blood draw at the screening visit, a total of 22 blood draws on 11 days throughout the study period of 35 days.
- Collection of 24-hour urine in five pooled collections on 5 days (the day before the baseline visit, E1, E2, E8, and P1); collection of 24-hr pooled urine on 3 days (E14, P2, and P14); recording the time of urine collection and the amount of any discarded urine on a preprinted log sheet.

The uptake study design was as follows:

- Ingestion of ^{123}I at 0900 hr on 3 days: the baseline visit (1 day before the start of perchlorate exposure), E14, and P15. Baseline visit and E14: measurement of RAIU at 1700 hr on the day of ^{123}I ingestion and 0900 hr the following morning. P15: measurement of RAIU only at 0900 hr on the day following ^{123}I ingestion.
- In addition to the blood draw at the screening visit, a blood draw on E8 and on E14.
- Collection of 24-hr pooled urine on the day before the baseline visit and on E14.

Radioiodine studies. We conducted all radioiodine studies in the Nuclear Medicine facility of the OHSU Hospital. ^{123}I capsules (nominal specific activity, 100 μC_i) were obtained from Mallinckrodt Inc. (Portland, OR). An Atomlab 950 Thyroid Uptake System (Biodex Medical Systems, Inc., Shirley, NY) was programmed to measure and record the ^{123}I counts per minute (cpm) in the capsule just before ingestion (time zero). The instrument was also programmed to measure and record the cpm over the thyroid, the cpm over the thyroid adjusted for radioactive decay of the ingested ^{123}I, and the percentage uptake of the ingested ^{123}I by the thyroid since time zero. We calibrated the instrument each day before use.

Thyroid function tests. Analysis of serum levels of total thyroxine (TT_4), free thyroxine (FT_4), total triiodothyronine (TT_3), and TSH was performed by Kaiser Permanente Regional Laboratory (Portland, OR) and the results were transmitted electronically to the OHSU Hospital's Lab Central on a shared laboratory software system. Analysis of serum antibodies to thyroglobulin (anti-Tg) and thyroid peroxidase (anti-TPO) was performed by Esoterics Inc. (Calabasas, CA) and the results were output by the company to a printer located in OHSU Hospital's Lab Central. In the main study, serum TT_4, FT_4, TT_3, and TSH were analyzed in blood drawn on 16 occasions: the screening (unspecified time) and baseline (0800 hr) visits, E1 (1200 and 1600 hr), E2 (0800, 1200, and 1700 hr), E3 (0900 hr), E4 (0800 and 1200 hr), E8 (0900 hr), E14 (0800, 1200, and 1700 hr), P1 (0900 hr), and P15 (0900 hr). In the uptake study, these hormones were analyzed only in blood drawn at the screening visit (unspecified time) and on E14 (0800 hr). Serum Anti-TPO levels were measured in blood drawn at the screening visit and on P15 for all subjects in the main study. We likewise ordered analysis of serum anti-Tg in the same two samples, but the lab reported anti-Tg results in both samples for only eight main-study subjects and in the P15 sample alone for the remaining 16 main-study subjects and one uptake-study subject (the woman with abnormally elevated TSH).

Serum chemistry and hematology. Measurement of serum thyroxine-binding globulin (TBG) was performed by Esoterics Inc. and the results were transmitted to OHSU in the same manner as the antibody data. In the main study, a serum chemistry panel (analysis of sodium, potassium, calcium, chloride, total CO_2, glucose, urea nitrogen, total bilirubin, albumin, TBG, total protein, creatinine, aspartate transaminase, and alkaline phosphatase) and a complete blood count (including differential) were performed on blood samples drawn at the screening visit and on E2, E14, and P15. We also requested analysis of alanine aminotransferase (ALT) in the same samples, but the test was inadvertently omitted from the serum chemistry panel until midway through the study; only 16 main-study subjects were tested for ALT at one or more scheduled time points. In the uptake study, the above tests (excluding analysis of TBG) were performed only in blood samples drawn at the screening visit.

Statistical analysis. We analyzed data using standard statistical techniques, including linear regression and correlation analysis to reveal regression slopes and correlation coefficients (r values). To the extent possible, RAIU data from the main and uptake studies were combined. When analysis of variance (ANOVA) was used to investigate the dose dependence of outcome variables, dose was entered as a categorical variable. For pairwise comparisons with baseline values, we used the two-tailed t-test for dependent samples and the nonparametric Wilcoxon matched pairs test. All statistical analyses were run on Statistica (StatSoft, Tulsa, OK). Our selected criterion for statistical significance was $p < 0.05$; however, we also report all p values < 0.1. Results are given as mean ± SE (or regression fit ± SE) unless otherwise indicated.

Quality assurance/quality control. At the request of the U.S. EPA, the study data were subjected to an intensive quality assurance/quality control (QA/QC) audit by outside auditors. The RAIU data were audited by M. G. Schneider under the management of the AFRL. All study data other than the RAIU data were audited by Toxicology/Regulatory Services, Inc. (TRS; Charlottesville, VA) under the management of Toxicology Excellence for Risk Assessment (Cincinnati, OH). The TRS audit led to the discovery of five FT_4 data points that had been entered into the database in error. All results reported in this article reflect the database corrected to exclude these five data points, as described by the study co-investigator (G.G.) in her written response to the TRS QA/QC audit report of 11 April 2001. The audit reports prepared by Schneider and by TRS (and the co-investigator's response to the TRS report) were submitted to the U.S. EPA by the audit managers.

Results

Thyroidal radioiodine uptake. As expected, given its 13-hr half-life, no ^{123}I was detectable in the thyroid just before administration of any subsequent ^{123}I dose.

Baseline uptake. Thyroidal radioiodine uptake at baseline varied widely among subjects: 5.6–25.4% for the 8-hr uptake and 9.8–33.7% for the 24-hr uptake. Our findings concerning the relationship between the baseline uptake and the 24-hr urinary iodine (UI) excretion will be described elsewhere.

Effect of perchlorate ingestion. Table 1 gives descriptive statistics for the 8- and 24-hr raw uptakes (expressed as a percentage of ingested ^{123}I) and the 8- and 24-hr relative uptakes (expressed as a percentage of the baseline uptake). The suppression of radioiodine uptake was linearly related to the logarithm of perchlorate dose. Pairwise comparison revealed no statistically significant difference between the suppression of radioiodine uptake on E2 and E14 at either 8 or 24 hr after radioiodine administration ($p > 0.7$), indicating the achievement of quasi-steady-state inhibition by E2 and the absence of a cumulative effect. Figure 1 shows the relationship between the 24-hr relative uptakes on E2 and E14 for all subjects (and doses) tested at both time points ($r = 0.824$, $n = 24$); we observed a similar relationship for the 8-hr relative uptakes on E2 and E14 ($r = 0.782$, $n = 24$). Regression analysis of the 8- and 24-hr relative uptakes (RU_8 and RU_{24}) against the logarithm of the perchlorate dose (D) in mg/kg-day yielded Equations 1A and 1B for E2 and Equations 1C and 1D for E14:

$$(RU_8)_{E2} = (-0.374 \pm 0.041)\log_{10}D + (0.209 \pm 0.047) \quad [1A]$$

$$(RU_{24})_{E2} = (-0.373 \pm 0.041)\log_{10}D + (0.202 \pm 0.047) \quad [1B]$$

$$(RU_8)_{E14} = (-0.337 \pm 0.037)\log_{10}D + (0.229 \pm 0.052) \quad [1C]$$

$$(RU_{24})_{E14} = (-0.359 \pm 0.034)\log_{10}D + (0.213 \pm 0.048) \quad [1D]$$

In Equations 1A–1D, the intercept is the regression-model prediction of the relative uptake when the perchlorate dose is exactly 1 mg/kg-day ($\log_{10}D = 0$). Analysis of the correlation of $(RU_8)_{E2}$, $(RU_{24})_{E2}$, $(RU_8)_{E14}$, and $(RU_{24})_{E14}$ with $\log_{10}D$ yielded correlation coefficients of –0.888 ($n = 24$), –0.889 ($n = 24$), –0.844 ($n = 36$), and –0.870 ($n = 37$), respectively.

The lowest dose producing no statistically significant inhibition of uptake was 0.007 mg/kg-day (Table 1). Thus, in this study, 0.007 mg/kg-day (7 µg/kg-day) was a NOEL for inhibition of RAIU. To achieve a more refined estimate of the true no-effect level (NEL), we extrapolated from the E14 regression relationships for RU_8 and RU_{24} (Equations 1C and 1D). We used the E14 data and not the E2 data in the extrapolation because the 7-µg/kg-day dose group (along with the other uptake-study subjects) had no uptake measurement on E2. With $(RU_8)_{E14}$ and $(RU_{24})_{E14}$ set equal to 1.00 (equivalent to 0% inhibition of uptake) in Equations 1C and 1D, the predicted dose values corresponding to the true NEL are 5.19 and 6.40 µg/kg-day (0.36 and 0.45 mg/day in a 70-kg person), respectively. With $(RU_8)_{E14}$ and $(RU_{24})_{E14}$ set equal to 0.95, 0.90, 0.85, or 0.80 (equivalent to 5%, 10%, 15%, or 20% inhibition of uptake), Equation 1C predicts dose values of 7.29, 10.3, 14.4, and 20.3 µg/kg-day (0.51, 0.72, 1.0, and 1.4 mg/day in a 70-kg person), respectively, and Equation 1D predicts dose values of 8.82, 12.2, 16.8, and 23.1 µg/kg-day (0.62, 0.85, 1.2, and 1.6 mg/day in a 70-kg person), respectively. Plugging the predicted dose values corresponding to uptake inhibition of 0–20% back into the regression model, we derived 95% upper confidence limits on uptake inhibition (Table 2).

We also used Equations 1C and 1D to provide estimates of the 50% inhibitory dose (ID_{50}). With $(RU_8)_{E14}$ and $(RU_{24})_{E14}$ set equal to 0.5 (equivalent to 50% inhibition of uptake), Equations 1C and 1D predict dose values of 0.157 and 0.159 mg/kg-day (11.0 and 11. 1 mg/day in a 70-kg person), respectively. Although an asymptotic flattening of the log-linear dose response is expected with increasing dose, a lower limit on the 100% inhibitory dose (ID_{100}) can be estimated by assuming linearity at doses beyond the measurement range. With $(RU_8)_{E14}$ and $(RU_{24})_{E14}$ set equal to 0 (equivalent to 100% inhibition of uptake), Equations 1C and 1D predict dose values of 4.76 and 3.92 mg/kg-day (333 and 274 mg/day in a 70-kg person), respectively.

Recovery from perchlorate ingestion. On P15, the 8- and 24-hr uptakes were statistically indistinguishable from their respective baseline uptakes ($p > 0.4$ by pairwise comparison), indicating complete recovery from the inhibitory effect of perchlorate. The mean (±SE) 8-hr uptakes relative to baseline were 111.7 ± 8.1%, 103.5 ± 10.4%, and 107.7 ± 11.3% in the 0.02-, 0.1-, and 0.5-mg/kg-day dose groups, respectively. The mean (±SE) 24-hr uptakes relative to baseline were 100.3 ± 8.4%, 105.3 ± 5.5%, 106.6 ± 9.1%, and 104.6 ± 9.4% in the 0.007-, 0.02-, 0.1-, and 0.5-mg/kg-day dose groups, respectively. Figure 2 shows the 24-hr uptake at baseline, on E14, and on P15 for each individual subject in each dose group. The woman with

Table 1. Descriptive statistics for the 8- and 24-hr thyroidal RAIU by dose.

	8-hr uptake (mean ± SE)			24-hr uptake (mean ± SE)		
Dose	No.	Raw (% ^{123}I)	Percent of baseline	No.	Raw (% ^{123}I)	Percent of baseline
0.5 mg/kg-day						
Baseline visit	10	14.1 ± 1.4	—	10	21.6 ± 2.0	—
E2	8	4.4 ± 0.4	31.6 ± 2.9**	8	6.5 ± 0.6	30.6 ± 2.6**
E14	10	4.5 ± 0.5	32.6 ± 3.3**	10	6.9 ± 0.9	32.9 ± 3.8**
P15	8	14.7 ± 1.4	107.7 ± 11.3	10	21.7 ± 2.0	104.6 ± 9.4
0.1 mg/kg-day						
Baseline visit	10	12.8 ± 1.5	—	10	19.9 ± 2.1	—
E2	8	7.7 ± 1.1	59.4 ± 2.0**	8	11.8 ± 1.7	59.2 ± 3.5**
E14	9	7.4 ± 1.3	56.7 ± 5.2**	10	11.0 ± 1.6	55.3 ± 3.9**
P15	8	12.9 ± 1.8	103.5 ± 10.4	10	20.8 ± 2.2	106.6 ± 9.1
0.02 mg/kg-day						
Baseline visit	10	11.8 ± 1.0	—	10	18.4 ± 1.2	—
E2	8	10.2 ± 1.0	83.8 ± 6.3*	8	15.7 ± 1.4	82.8 ± 5.6*
E14	10	9.4 ± 0.7	81.8 ± 4.2**	10	15.2 ± 1.1	83.6 ± 4.1**
P15	8	13.5 ± 1.2	111.7 ± 8.1	10	19.1 ± 1.3	105.3 ± 5.5
0.007 mg/kg-day						
Baseline visit	7	12.6 ± 2.5	—	7	18.1 ± 3.1	—
E14	7	10.6 ± 1.1	93.8 ± 9.0	7	16.5 ± 1.6	98.2 ± 8.3
P15	—	—	—	7	17.3 ± 2.5	100.3 ± 8.4

*$p < 0.05$; **$p < 0.005$ (pairwise comparison to baseline).

elevated TSH at the screening visit had a 24-hr uptake of 10% at the baseline visit, the lowest value observed in her dose group (0.007 mg/kg-day) or any group. Her 24-hr uptake was 13.9% on E14, or 139% of baseline (Figure 2D). Her case illustrates a general phenomenon in the reporting of uptake as a percentage of baseline: For subjects with low baseline uptake, small changes in absolute uptake, up or down, appear large in terms of relative uptake. Figure 3 shows the mean 24-hr uptake as a percentage of baseline for each dose group on each measurement day.

Effect of sex. In a two-way model testing the effect of sex and dose on the uptake relative to baseline for each of the six postbaseline measurements (8- and 24-hr uptakes on E2, E14, and P15), ANOVA revealed no effect of sex for any measurement ($p > 0.4$). Similarly, two-way ANOVA revealed no effect of sex on the 8- and 24-hr raw uptakes at the baseline visit ($p > 0.7$) or on E2, E14, or P15 ($p > 0.3$). Table 3 gives summary statistics for the raw and relative 24-hr uptakes by sex and dose; we found similar results for the 8-hr uptakes. We calculated the power of the study to detect a 20% difference between males and females in the uptake relative to baseline. The highest power values were found for E2: 83% and 95% for the respective 8-hr and 24-hr measurements in the 0.5-mg/kg-day dose group and 99% for the 8-hr measurement in the 0.1-mg/kg-day dose group.

Correlation of 8- and 24-hr uptakes and relative uptakes. Over all measurement days and dose groups, the 8- and 24-hr uptakes were linearly related ($r = 0.975$, $n = 121$). Linear regression analysis yielded the equation:

$$U_{24} = (1.40 \pm 0.03)\,U_8 + (0.013 \pm 0.003) \quad [2]$$

Pairwise comparison of the U_{24}/U_8 ratio at baseline with the U_{24}/U_8 ratios for measurements made during perchlorate administration (E2 and E14 combined) or on P15 revealed no significant difference, indicating that the ratio is independent of perchlorate treatment. The 8- and 24-hr relative uptakes for E2, E14, and P15 combined were likewise linearly related ($r = 0.967$, $n = 84$). Figure 4 shows the relationship between the 8- and 24-hr relative uptakes on E14 for each dose group.

In a 1951 investigation by Greer (*37*) of euthyroid subjects under basal conditions and after treatment with various doses of thyroid hormone to suppress TSH secretion, the U_{24} versus U_8 regression slope was 1.43 ± 0.02 ($n = 75$). The similarity of the regression slopes observed in the 1951 study (*37*) and the present study is remarkable given the differences in basal radioiodine uptake and treatment. The mean basal 24-hr uptake in the present study was approximately half that observed in the 1951 study (*37*), presumably reflecting a higher dietary iodine intake in the current U.S. population. The nature of the suppression of thyroid function in the two studies was also different; perchlorate, at the doses given, suppresses only iodide transport, whereas thyroid hormone, at doses sufficient to decrease TSH secretion, suppresses almost all aspects of thyroid function.

Comparison with uptake results of Lawrence et al. Whereas we tested body-weight adjusted doses in the present study, the above-described studies by Lawrence et al. (*18,19*) tested doses unadjusted for body weight. To facilitate comparison with the perchlorate inhibition of thyroidal RAIU observed in our study, we obtained body weights (*20*) for all subjects in the two studies performed by Lawrence et al. (*18,19*) and calculated a body weight-adjusted dose for each subject. For each E14 uptake measurement in the three studies, we calculated an effect parameter (analogous to the slope of the dose–response relationship) by dividing the uptake as a percentage of baseline by the logarithm of the body-weight-adjusted dose. Using a nonparametric test sensitive to the shapes of distributions (Kolmogorov-Smirnov), we found significant differences ($p < 0.01$) in the effect parameter for both the 8- and 24-hr uptakes when we compared the data from our study with the combined data from the other two studies. Figure 5A shows the 24-hr uptake on E14 as a function of perchlorate dose for each subject in the present study and the two studies by Lawrence et al. (*18,19*).

Table 2. Doses predicted to produce 0–20% inhibition of iodide uptake by the thyroid and the calculated 95% upper confidence limits on uptake inhibition at these doses.[a]

	Predicted dose (μg/kg-day)		95% UCL on uptake inhibition	
Uptake inhibition (%)	8-hr	24-hr	8-hr (%)	24-hr (%)
0	5.2	6.4	9.5	8.3
5	7.3	8.8	13.6	12.5
10	10.3	12.2	17.8	16.8
15	14.4	16.8	21.9	21.1
20	20.3	23.1	26.3	25.6

[a]Data are based on regression models describing the 8-hr (Equation 1C) and 24-hr (Equation 1D) uptake data for E14. First we used each model to predict the perchlorate dose corresponding to a given level of inhibition of iodide uptake. Using the same models but with dose as the independent variable, we then derived confidence limits on the inhibition of iodide uptake at those doses.

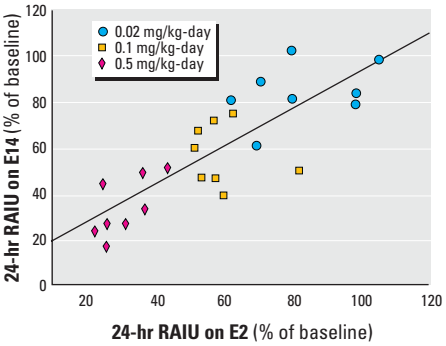

Figure 1. The 24-hr RAIU relative to baseline on E14 compared with E2. The line is the linear regression fit to the data.

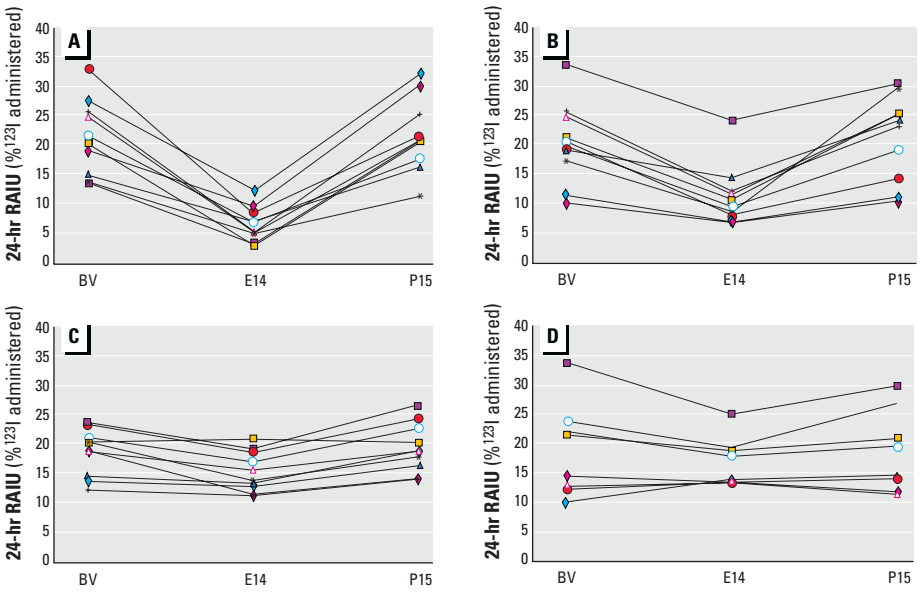

Figure 2. The 24-hr RAIU at the baseline visit and on E14 and P15 for each subject in the (*A*) 0.5-, (*B*) 0.1-, (*C*) 0.02-, and (*D*) 0.007-mg/kg-day dose groups.

Lawrence et al. (*18,19*) performed the final RAIU 2 weeks after perchlorate withdrawal (on P14) and found a statistically significant overshoot relative to baseline at both doses tested. We performed the final RAIU 2 weeks after perchlorate withdrawal (on P15) and found no statistically significant overshoot in any dose group. Figure 5B shows the 24-hr uptake at 2 weeks postexposure as a function of perchlorate dose for each subject in all three studies.

Perchlorate elimination rate. Analysis of urinary excretion data from a 1929 study in an adult man given a single oral perchlorate dose of 1.4 g (*40*) revealed that > 90% of the ingested perchlorate was removed with a half-life ($t_{1/2}$) of 7.7 hr (*39*). In the present study, we calculated the rate of perchlorate elimination from levels in serum following perchlorate withdrawal. For the eight main-study subjects in the 0.5-mg/kg-day dose group, $t_{1/2}$ values ranged from 6.0 to 9.3 hr (average, 8.1 hr). Fewer data points were available for the 0.1-mg/kg-day subjects because levels fell more rapidly below the analytical detection limit of 5 ppb after perchlorate withdrawal. Serum levels in the 0.02-mg/kg-day dose group were almost entirely below detection throughout the exposure period. Additional data and analysis pertaining to serum and urine perchlorate measurements (as well as serum and urine iodine measurements) will be reported elsewhere. The perchlorate measurements on our subjects' serum and urine samples were performed by AFRL scientists, who have presented some of the data previously (*29–31*).

Serum hormones (FT_4, TT_4, TT_3, and TSH). **Effect of perchlorate ingestion.** TSH, FT_4, TT_4, and TT_3 levels throughout the study were in the normal range for all subjects except one woman in the 0.007-mg/kg-day dose group (uptake study) who had abnormally high TSH (18 and 15 mIU/L) on both occasions at which the hormone was measured (screening visit and E14, respectively). Table 4 presents summary statistics for serum hormones measured at all blood draw events in the main study.

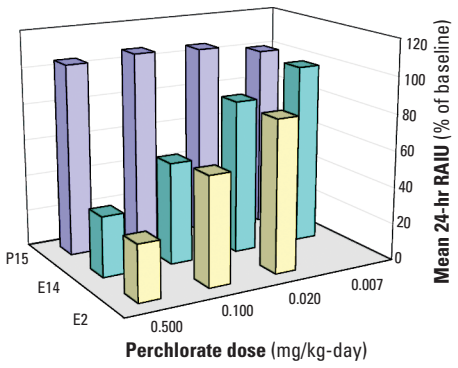

Figure 3. The mean 24-hr RAIU relative to baseline for each dose group on E2, E14, and P15.

Because only limited data were available for uptake-study subjects, we confined evaluation of the effect of perchlorate exposure on serum hormones to the 24 subjects in the main study. To facilitate our initial statistical analysis, we defined a categorical exposure variable (before, during, and after perchlorate) and a categorical dose variable (high, middle, and low). Two-way ANOVA applied to the exposure and dose variables revealed no significant dependence of any serum hormone on the exposure variable. By chance, the 0.5-mg/kg-day group had significantly higher baseline-visit TSH levels than did the 0.1-mg/kg-day group ($p < 0.014$, t-test for independent samples). Similarly, the 0.5-mg/kg-day group had significantly higher screening-visit ($p < 0.046$) and marginally higher baseline-visit ($p < 0.058$) TT_3 levels than did the 0.02-mg/kg-day group. The disparity in pre-exposure values skewed the comparison of TSH and TT_3 across dose groups. To eliminate the problem of intergroup differences in pre-exposure serum hormone values and elucidate any possible effects of perchlorate, we performed one-way ANOVA for each dose group separately, once against the exposure variable and once against blood-draw event (16 draws between the screening visit and P15). The results revealed no association of FT_4, TT_4, or TT_3 with blood-draw event in any dose group. However, we found a marginally significant association of TSH with blood-draw event in the 0.5-mg/kg-day dose group ($p = 0.09$).

Influence of circadian variation. To eliminate any potential dampening of the above association by circadian variation in TSH levels, we defined a time-of-day variable with three categories: morning (before 1030 hr), mid-day (1030–1359 hr), and late afternoon (1400 hr or later); these categories refer to the recorded time of each blood draw, not the target (nominal) time. ANOVA performed separately within each dose group for each time-of-day category (a total of 3 × 3 = 9 analyses) yielded a significant relationship between TSH and blood-draw event only in the morning draws of the 0.5-mg/kg-day dose group ($p = 0.03$). Figure 6 shows the distribution of TSH for each blood-draw event in the 0.5-mg/kg-day dose group, categorized according to the time-of-day variable. The data suggest an overall downward trend during exposure with recovery by P15.

Analysis of the dependence of FT_4, TT_4, and TT_3 on blood-draw event (ANOVA performed separately within each dose group for each time-of-day category) indicated that TT_3 was significantly related to blood-draw event in the late-afternoon draws of subjects in the 0.1-mg/kg-day dose group ($p = 0.03$). However, the effect appears to be attributable to the fact that two of the screening-visit draws for the 0.1-mg/kg-day group fell into the late-afternoon category and both happened to be low (80 and 84 ng/dL). All other late-afternoon draws in the 0.1-mg/kg-day group occurred at the scheduled late-afternoon events on E1, E2, and E14; mean TT_3 values (± SE) for these draws in the eight subjects tested were 102.9 ±

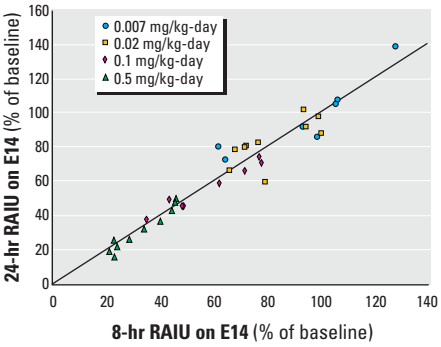

Figure 4. Comparison of the 24-hr and 8-hr RAIU values relative to baseline on E14. The line is the linear regression fit to the data.

Table 3. Descriptive statistics for the 24-hr thyroidal RAIU by sex and dose.

Dose	24-hr uptake in men (mean ± SE)			24-hr uptake in women (mean ± SE)		
	No.	Raw (% ^{123}I)	Percent of baseline	No.	Raw (% ^{123}I)	Percent of baseline
0.5 mg/kg-day						
Baseline visit	5	21.7 ± 3.8	—	5	21.4 ± 1.9	—
E2	4	6.2 ± 0.9	26.6 ± 1.4	4	6.9 ± 0.8	34.7 ± 4.4
E14	5	6.7 ± 1.7	30.5 ± 4.8	5	7.1 ± 0.8	35.3 ± 6.3
P15	5	21.4 ± 3.3	103.4 ± 14.7	5	22.1 ± 2.5	105.7 ± 13.4
0.1 mg/kg-day						
Baseline visit	5	17.5 ± 1.7	—	5	22.3 ± 3.8	—
E2	4	11.6 ± 2.3	63.6 ± 6.5	4	12.0 ± 2.9	54.7 ± 1.3
E14	5	9.5 ± 1.3	54.8 ± 6.0	5	12.5 ± 2.9	55.9 ± 5.6
P15	5	20.4 ± 3.4	116.6 ± 17.0	5	21.2 ± 3.3	96.5 ± 3.0
0.02 mg/kg-day						
Baseline visit	5	17.4 ± 1.5	—	5	19.4 ± 2.0	—
E2	4	13.8 ± 1.7	83.9 ± 10.6	4	17.5 ± 2.0	81.7 ± 6.0
E14	5	14.4 ± 0.8	83.8 ± 5.1	5	16.1 ± 2.0	83.4 ± 7.0
P15	5	18.7 ± 1.0	109.3 ± 8.5	5	19.6 ± 2.6	101.2 ± 7.3
0.007 mg/kg-day						
Baseline visit	1	21.5	—	6	17.5 ± 3.6	—
E14	1	18.7	87.0	6	16.1 ± 1.9	100.0 ± 9.6
P15	1	20.7	96.3	6	16.8 ± 2.8	101.0 ± 9.9

3.9 ng/dL, 104.9 ± 2.9 ng/dL, and 106.3 ± 3.4 ng/dL, respectively. For the two subjects with respective TT_3 values of 80 and 84 ng/dL at the screening visit, TT_3 values on E1, E2, and E14 were 92, 91, and 95 ng/dL in the first subject and 100, 99, and 112 ng/dL in the second. TT_3 levels in the 0.5-mg/kg-day dose group were independent of blood-draw event in every time-of-day category ($p > 0.7$). Consideration of all the available data does not suggest an effect of perchlorate on TT_3 in this study.

Serum anti-Tg and anti-TPO. Serum anti-Tg levels were below detection (< 40 IU/mL) in all samples tested. Levels of anti-TPO were above normal (> 20 IU/mL) in two subjects. One was a 56-year-old male in the 0.02-mg/kg-day group whose anti-TPO levels at the screening visit and on P15 were 63 and 59 IU/mL, respectively. The other was the previously mentioned 49-year-old female in the 0.007-mg/kg-day group with elevated TSH. Her screening-visit anti-TPO level was 75 IU/mL; she was not tested a second time. Both subjects were clinically euthyroid, and their other thyroid function test results remained within normal limits throughout the study.

Serum chemistry and hematology. Serum chemistry and hematology results were within normal limits throughout the study in all subjects. We found no significant change in any of these parameters during or after perchlorate administration.

Discussion

Interpretation of the estimated true NEL for inhibition of uptake. In this study, 0.007 mg/kg-day (7 µg/kg-day) was the NOEL for inhibition of thyroidal RAIU. We estimated true NEL values of 5.2 and 6.4 µg/kg-day based on the dose–response for inhibition of the respective 8-hr ($n = 36$) and 24-hr ($n = 37$) RAIU on E14 in all subjects tested, one with subclinical autoimmune hypothyroidism, the rest euthyroid. Based on the variability observed in these subjects, there is a 95% probability that thyroidal iodide uptake will be inhibited by no more than 8.3–9.5% at a dose of 5.2–6.4 µg/kg-day. We expect that inhibition of thyroidal iodide uptake by 9.5% would be physiologically insignificant in persons with sufficient iodine intake. Indeed, observations in iodine-sufficient perchlorate plant workers (discussed below) lead us to conclude that perchlorate exposure at levels associated with a relatively high percentage inhibition of thyroidal iodide uptake produces no adverse health consequences in such individuals.

Assuming a body weight of 70 kg and a drinking-water intake of 2 L/day, a perchlorate dose of 5.2 or 6.4 µg/kg-day would be consumed if drinking-water supplies contained perchlorate at a concentration of approximately 180 or 220 µg/L (ppb), respectively. Thus, assuming that the drinking-water supply is the only significant source of exposure to perchlorate or other inhibitors of the thyroidal uptake of iodide, a perchlorate concentration of 180–220 ppb (and possibly much higher) should be of no health concern in iodine-sufficient populations.

Interpretation of serum hormone results. Despite significant inhibition of thyroidal radioiodine uptake in the 0.02, 0.1, and 0.5 mg/kg-day dose groups by respective average values of 17%, 44%, and 67%, we found no change in serum levels of thyroid hormones (i.e., FT_4, TT_4, or TT_3). We observed a significant effect of perchlorate on serum levels of TSH only in the 0.5-mg/kg-day dose group: a downward trend in morning (before 1030 hr) blood draws during perchlorate exposure, with recovery by P15. Our findings of no effect on thyroid hormones or TSH during 2-week perchlorate exposure at a dose of 0.1 or 0.02 mg/kg-day agree with those of Lawrence et al. (*18,19*), who reported no change in thyroid hormones or TSH during 2-week exposure at a mean dose of 0.12 or 0.038 mg/kg-day. Our finding of marginally decreased TSH levels during 2-week perchlorate exposure at a dose of 0.5 mg/kg-day is reminiscent of the significant TSH depression observed in five subjects who ingested perchlorate at a dose of 900 mg/day (~13 mg/kg-day) for 4 weeks (*35*).

A downward trend in TSH is the reverse of what is expected when iodide uptake is blocked or iodine nutrition is insufficient (*41*). If iodine deficiency is severe enough and sufficiently prolonged to compromise adequate production and secretion of thyroid hormones, TSH secretion is increased because of decreased negative feedback of thyroid hormones on the hypothalamic–pituitary control centers. We have no explanation for the slight decrease in TSH observed during exposure to perchlorate at 0.5 mg/kg-day (the present study) or a higher dose (*35*).

Subjects in the 0.02-, 0.1-, and 0.5-mg/kg-day dose groups experienced partial inhibition of thyroidal iodide uptake for 14 days; the absence of an effect on serum thyroid hormones is expected given any extent of inhibition lasting only a few weeks. For a simplified view of the relevant dynamics, consider the following: The human thyroid gland contains a huge reserve store of hormone within the intrafollicular thyroglobulin. Under iodine-replete conditions, the iodine content of the human thyroid gland averages 0.5% of wet weight, or 100 mg for a 20-g thyroid (*32*). Approximately 46% (46 mg) of the iodine in thyroglobulin is in the form of iodothyronines. T_4 is 65% iodine; if all stored iodothyronine were T_4, there would be 71 mg of stored T_4. The typical oral replacement dose of T_4 in a hypothyroid individual is 0.1 mg/day. Assuming a constant secretion rate of 0.1 mg T_4 daily, no conversion to T_3, and complete inhibition of thyroidal iodide uptake, the quantity of stored hormone should be sufficient to last for over a year. In fact, 20% of the iodothyronine in thyroglobulin is T_3 (*34*). Given that T_3 is only 58% iodine but has four times the potency of T_4, the quantity of stored hormone should be sufficient to last an even longer period. Because the rate of secretion is proportional to the amount stored in the thyroid gland (*42,43*), blood levels of thyroid hormone would drop continuously during the entire time that thyroidal iodide uptake is completely blocked, although it should take several weeks or longer for this decrease to become detectable.

Consistency with the uptake results of Lawrence et al. We noted small but significant differences in the response to perchlorate observed in our study compared with that observed by Lawrence et al. (*18,19*) in their two studies. In our study there was more inhibition of radioiodide uptake for a given dose of perchlorate. Although there were small interstudy differences in baseline uptake, these differences cannot account for the observed discrepancy in the response to perchlorate. The most likely explanation is that the different exposure regimens (four times daily in our study; *ad libitum* in the studies by Lawrence et al.) led to different temporal patterns in blood levels of perchlorate. We hypothesize that in the *ad libitum* studies, the subjects tended to drink disproportionately large fractions of their dosing

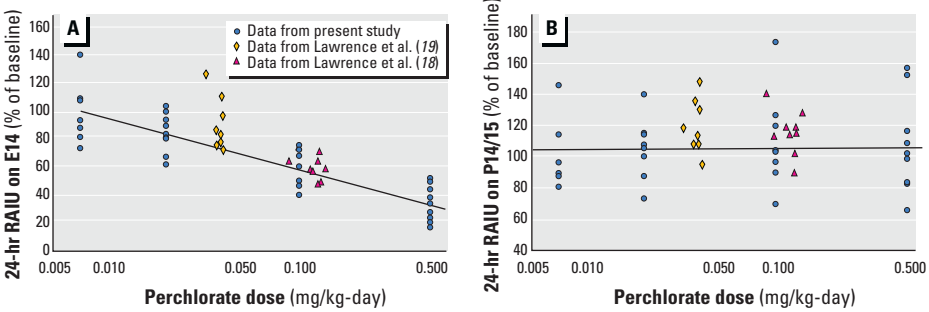

Figure 5. The 24-hr RAIU relative to baseline on E14 (*A*) and on P14 [Lawrence et al. (*18,19*)] or P15 (*B*) as a function of the perchlorate dose. The line in each plot is the linear regression fit to the data.

solutions just before their scheduled clinic visits, leading to higher perchlorate concentrations in sampled blood, higher peak concentrations, faster excretion, and lower time-averaged concentrations compared to subjects drinking perchlorate at four prescribed times throughout the day. Whereas blood sampled in the *ad libitum* studies might reflect recent ingestion of a disproportionate amount of the daily perchlorate dose, the 24-hr RAIU measurements in both studies must reflect perchlorate levels averaged over the entire 24-hr accumulation period. Our temporal hypothesis is supported by the observation that in the 10-mg/day *ad libitum* study, significantly greater inhibition was revealed by the 4-hr RAIU than by the 24-hr measurement (*20,39*). It is also supported by the observation that serum perchlorate levels measured in the 10-mg/day (mean, 0.12 mg/kg-day) *ad libitum* study were much higher than the values measured in our study at a comparable dose. In the 10-mg/day *ad libitum* study, mean serum perchlorate concentrations in samples taken at unspecified times on E7 and E14 were 0.61 and 0.59 µg/mL, respectively (*18*). In our 0.1-mg/kg-day dose group, mean serum perchlorate concentrations in the single (morning) sampling event on E8 and the three (morning, noon, and late afternoon) sampling events on E14 were 0.10–0.17 µg/mL; our 0.5-mg/kg-day dose group had mean serum perchlorate concentrations of 0.45–0.85 µg/mL at the same four time points (data not shown). The serum perchlorate levels measured in our

Table 4. Descriptive statistics for serum hormone concentrations.[a]

	TSH (mIU/L)		FT_4 (ng/dL)		TT_4 (µg/dL)		TT_3 (ng/dL)	
Dose	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ±SE	No.	Mean ± SE
0.5 mg/kg-day								
SV	7	2.146 ± 0.442	7	1.129 ± 0.071	8	7.46 ± 0.76	8	109.4 ± 4.6
BV	8	2.713 ± 0.375	8	1.163 ± 0.080	8	7.31 ± 0.64	8	115.1 ± 7.3
E1A	8	2.304 ± 0.400	8	1.138 ± 0.050	8	7.69 ± 0.75	8	110.8 ± 4.9
E1B	8	2.663 ± 0.616	8	1.150 ± 0.050	8	7.26 ± 0.74	8	108.1 ± 5.7
E2A	8	2.391 ± 0.306	8	1.225 ± 0.070	8	7.24 ± 0.54	8	111.3 ± 6.9
E2B	7	1.641 ± 0.244	8	1.200 ± 0.085	8	7.29 ± 0.55	8	109.0 ± 5.8
E2C	7	2.029 ± 0.305	8	1.150 ± 0.057	8	7.41 ± 0.59	8	108.5 ± 5.7
E3	8	1.818 ± 0.198	8	1.213 ± 0.044	8	7.69 ± 0.65	8	111.0 ± 5.5
E4A	7	2.337 ± 0.330	7	1.186 ± 0.074	7	7.39 ± 0.84	7	122.9 ± 7.0
E4B	8	1.855 ± 0.346	8	1.138 ± 0.078	8	6.95 ± 0.42	8	108.6 ± 6.8
E8	8	1.684 ± 0.237	8	1.300 ± 0.053	8	8.48 ± 0.50	8	110.8 ± 6.1
E14A	8	1.896 ± 0.362	7	1.200 ± 0.049	7	7.46 ± 0.58	7	112.7 ± 8.7
E14B	8	1.644 ± 0.362	8	1.163 ± 0.032	8	7.89 ± 0.63	8	117.1 ± 6.6
E14C	7	1.464 ± 0.341	7	1.257 ± 0.117	8	7.41 ± 0.54	8	114.9 ± 4.7
P1	8	1.599 ± 0.255	8	1.175 ± 0.067	8	7.91 ± 0.65	8	122.3 ± 8.2
P15	8	3.025 ± 0.471	8	1.213 ± 0.058	8	7.84 ± 0.68	8	120.5 ± 10.2
0.1 mg/kg-day								
SV	7	1.481 ± 0.262	8	1.200 ± 0.053	8	7.06 ± 0.53	8	101.1 ± 5.4
BV	8	1.600 ± 0.118	8	1.188 ± 0.055	8	7.13 ± 0.38	8	102.5 ± 3.4
E1A	8	1.700 ± 0.158	8	1.188 ± 0.064	8	7.11 ± 0.49	8	107.4 ± 4.5
E1B	7	1.584 ± 0.181	8	1.238 ± 0.053	8	7.09 ± 0.43	8	102.9 ± 3.9
E2A	8	1.613 ± 0.129	8	1.225 ± 0.075	8	6.56 ± 0.38	8	104.4 ± 3.5
E2B	7	1.443 ± 0.194	7	1.200 ± 0.105	7	7.24 ± 0.32	7	103.1 ± 3.2
E2C	8	1.550 ± 0.165	8	1.150 ± 0.042	8	6.86 ± 0.41	8	104.9 ± 2.9
E3	8	1.233 ± 0.107	8	1.250 ± 0.068	8	7.05 ± 0.61	8	103.3 ± 4.9
E4A	8	1.409 ± 0.146	8	1.188 ± 0.040	8	6.36 ± 0.36	8	99.1 ± 3.7
E4B	8	1.206 ± 0.077	8	1.088 ± 0.044	8	7.33 ± 0.69	8	108.3 ± 6.5
E8	8	1.523 ± 0.151	8	1.275 ± 0.025	8	7.90 ± 0.88	8	110.9 ± 6.2
E14A	7	1.729 ± 0.310	7	1.186 ± 0.077	8	6.46 ± 0.57	8	104.6 ± 5.4
E14B	8	1.598 ± 0.230	7	1.129 ± 0.064	8	6.74 ± 0.59	8	106.1 ± 4.4
E14C	7	1.314 ± 0.152	7	1.129 ± 0.075	8	6.60 ± 0.57	8	106.3 ± 3.4
P1	8	1.483 ± 0.197	8	1.200 ± 0.080	7	6.83 ± 0.55	8	106.9 ± 4.8
P15	8	1.763 ± 0.226	8	1.138 ± 0.068	7	7.09 ± 0.52	7	105.6 ± 4.1
0.02 mg/kg-day								
SV	8	1.811 ± 0.327	8	1.125 ± 0.041	8	6.90 ± 0.78	8	95.5 ± 4.3
BV	8	2.155 ± 0.415	8	1.125 ± 0.041	8	7.58 ± 0.83	8	98.1 ± 3.8
E1A	7	2.263 ± 0.638	7	1.114 ± 0.040	7	7.31 ± 0.80	7	99.3 ± 3.7
E1B	7	1.724 ± 0.256	8	1.150 ± 0.089	8	7.18 ± 0.76	8	101.3 ± 6.2
E2A	7	2.403 ± 0.463	7	1.186 ± 0.080	7	7.30 ± 0.87	7	101.4 ± 6.5
E2B	8	1.668 ± 0.225	8	1.225 ± 0.070	8	7.56 ± 0.76	8	95.8 ± 6.4
E2C	8	2.094 ± 0.437	8	1.125 ± 0.073	8	7.10 ± 0.75	8	95.0 ± 5.3
E3	8	1.875 ± 0.280	8	1.238 ± 0.065	8	7.59 ± 0.70	8	100.3 ± 5.6
E4A	8	2.128 ± 0.331	8	1.213 ± 0.090	8	7.10 ± 0.78	8	102.5 ± 7.1
E4B	8	1.859 ± 0.328	8	1.113 ± 0.106	8	7.35 ± 0.76	8	94.4 ± 6.8
E8	8	1.901 ± 0.343	8	1.200 ± 0.027	8	7.68 ± 0.74	8	103.0 ± 5.3
E14A	7	2.067 ± 0.335	6	1.250 ± 0.072	7	7.71 ± 0.80	7	103.6 ± 4.0
E14B	8	1.516 ± 0.224	7	1.214 ± 0.063	8	7.51 ± 0.65	8	97.3 ± 3.2
E14C	8	2.158 ± 0.407	7	1.157 ± 0.048	8	7.28 ± 0.68	8	100.1 ± 3.9
P1	8	1.769 ± 0.334	8	1.163 ± 0.065	8	7.89 ± 0.89	8	101.4 ± 4.7
P15	8	2.019 ± 0.359	8	1.175 ± 0.056	8	7.60 ± 0.82	8	99.1 ± 4.3

[a]E1A and E1B: nominal 1200 and 1600 hr draws on E1; E2A, E2B, and E2C: nominal 0800, 1200, and 1700 hr draws on E2; E4A and E4B: nominal 0800 and 1200 hr draws on E4; E14A, E14B, and E14C: nominal 0800, 1200, and 1700 hr draws on E14. SV, screening visit; BV, baseline visit. The SV draw was not at a specified time or on a specified day. In some cases, weeks or months elapsed between the SV and the BV. The mean times of the SV blood draw in the 0.02-, 0.1-, and 0.5-mg/kg-day dose groups were 1327, 1330, and 1243 hr; the overall mean (± SE) was 1313 hr ± 24 min. The BV blood draw was nominally at 0800 hr; the E3, E8, P1, and P15 blood draws were nominally at 0900 hr.

study's 0.5-mg/kg-day dose group span those observed in subjects given a 4-fold lower dose in the 10-mg/day *ad libitum* study. Note that interlaboratory variability can be excluded as an explanation for the discrepancy because in both cases the perchlorate analyses were performed by the AFRL. In a separate article we will address at greater length the issue of temporal effects related to the observed interstudy differences.

Lawrence et al. (*18,19*) found that the thyroidal radioiodine uptake measured 2 weeks postexposure was significantly elevated relative to the baseline uptake. We did not observe a significant postexposure overshoot in the present study, nor was one seen in six subjects given perchlorate at a dose of 144 mg/day for 7 days (*44*). In the latter study, measurement of baseline thyroidal radioiodine uptake immediately preceded the start of perchlorate administration and repeat uptake measurements were performed at 2, 4, 7, and 14 days postexposure; the results showed that the mean uptake returned to the baseline value within 2–4 days. Therefore, we consider it likely that the postexposure overshoot in radioiodine uptake observed by Lawrence et al. was a consequence of chance variability or some other factor unrelated to perchlorate exposure.

Inhibition of uptake in iodine insufficiency. It is difficult to say what level of thyroidal iodide uptake inhibition (if any) would be nonadverse in a person with any particular degree of iodine insufficiency.

The third National Health and Nutrition Examination Survey (NHANES III) (*45*), conducted in 1988–1994, found that the percentages of males and females with urinary iodine (UI) concentrations below 5 µg/dL were substantially higher in every age category than in the 1971–1974 survey. Although the NHANES III data reveal a major decline in iodine intake over a 25-year period, we do not believe that iodine insufficiency should be inferred. The NHANES data were obtained from single spot urines. In comparison with the population distribution of UI based on a 1-year average of monthly spot urines, the distribution based on single spot urines is much broader, leading to overestimation of the number of individuals at both the low and high ends (*46*). Although single spot urines are a convenient means for obtaining crude information about iodine nutrition in a population, gauging the adequacy of iodine intake in an individual requires collection of one or more 24-hr urines (*47,48*). The NHANES III investigators fully appreciated that it was not possible for them to determine, based on their spot-urine results alone, whether thyroid function was adversely affected in any members of the population as a result of insufficient iodine nutrition (*45*): "Whether the reduced UI seen in 1988–1994 can be directly correlated to measurable changes in thyroid function in the population is not known at this time because the results of thyroid function tests and thyroid antibodies in the 1988–1994 study are not available as yet." They went on to say that "We know of no population-based study in the United States, such as transient neonatal hypothyroidism or recent goiter surveys, that has shown changes that resulted from decreased iodine intake." The decline in iodine nutrition in the 25 years before 1988–1994 also brought a substantial reduction in the percentage of the population with excessive iodine intake. The investigators noted (*45*): "In 1971–1974, 27.8% of the population had excessive UI concentrations (> 50 µg/dL), and the decline of those with excessive UI to 5.3% in 1988–1994 may be seen as beneficial in possibly reducing diseases due to iodine excess such as Hashimoto's thyroiditis and perhaps Graves' disease." In the United States as elsewhere, if iodine insufficiency occurs in any person or population group, then it should be corrected by iodine supplementation. Such supplementation should occur irrespective of whether there is significant known exposure to environmental perchlorate, other inhibitors of the sodium-iodide symporter such as nitrate and thiocyanate (*49*), or foods that naturally contain antithyroid compounds or their precursors (*41*).

Protection against depressed thyroid function in perchlorate plant workers. The average perchlorate absorption in workers subjected to the highest exposure has been estimated as 34 mg/shift, or approximately 0.5 mg/kg-day (*16*). In our study, a perchlorate dose of 0.5 mg/kg-day inhibited uptake by 67% on average. The fact that studies of chronically exposed perchlorate plant workers have thus far failed to detect any abnormalities of thyroid function (*16,17*) may well be attributable to high daily intake of iodine among the workers. If iodine intake is sufficiently high, even an average reduction in thyroidal iodide concentration to one-third its former level may still leave these individuals sufficient iodide to produce thyroid hormone at a normal rate. Further, given that perchlorate has a half-life of about 8 hr, the perchlorate plant workers, many of whom worked 12-hr shifts, might recover from the inhibitory effects of exposure during off-shift periods. Thus, the discontinuous nature of occupational exposure, which resembles the discontinuous nature of drinking-water exposure, might be a major factor in the observed tolerance to long-term perchlorate exposure at levels capable of producing pronounced inhibition of uptake. Prolonged exposure to perchlorate may also lead to an adaptive increase in the efficiency of thyroidal iodide uptake.

Although it has been argued, on the basis of data from a very small group of subjects, that serum TSH is lowest in persons whose iodine nutrition falls within certain narrow limits (*50*), normal TSH levels are typically observed over a wide range of iodine intake. A study of 2,300 euthyroid subjects found that serum TSH levels were statistically indistinguishable among groups with single-sample UI concentrations of 51–100, 101–200, and 201–300 µg/g creatinine; only the group

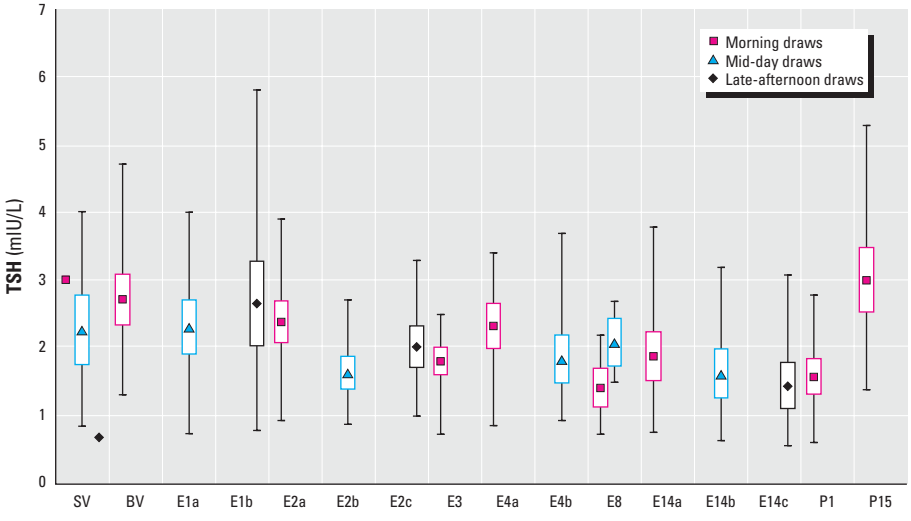

Figure 6. Values of serum TSH in all blood samples from main study subjects in the 0.5-mg/kg-day dose group, categorized according to the 16 scheduled blood-draw events. BV, baseline visit; SV, screening visit. Where more than one blood draw was scheduled on a given day, a, b, and c denote the first, second, and third draw, respectively. In the SV category, the square and diamond correspond to the lone morning and late-afternoon blood draws, respectively, for that event. Boxes indicate SE; whiskers indicate maximum and minimum. The *x*-axis is not to scale regarding the timing of events. For all events except the following, $n = 8$. Morning draws: SV ($n = 1$), E4a ($n = 7$), E8 ($n = 5$); mid-day draws: SV ($n = 5$), E2b ($n = 7$), E8 ($n = 3$); afternoon draws: SV ($n = 1$), E2c ($n = 7$), E14c ($n = 7$).

with UI < 50 μg/g creatinine had significantly higher serum TSH levels compared to the other groups (*51*). Further, iodine deficiency must be relatively severe to produce any clear clinical or laboratory signs of abnormally low thyroid function (*41*).

Humans are able to maintain normal thyroid function when exposed to relatively high levels of the iodide uptake inhibitor thiocyanate (e.g., from consumption of cassava or cabbage); the prevalence of goiter is not elevated as long as the iodine/thiocyanate ratio in urine is above a critical threshold of approximately 3 μg iodine per 1 mg thiocyanate (*41*). Taking into consideration the relative molecular weights of perchlorate and thiocyanate and the fact that perchlorate is about 20-fold more potent an inhibitor of thyroidal iodide uptake (*52*), extrapolation from this guideline for the iodine/thiocyanate ratio suggests that ingestion of 36 μg iodine per 1 mg perchlorate should be sufficient to avoid goiter. Based on this crude calculation, 150 μg iodine daily would protect against goiter in a person whose perchlorate ingestion is 4 mg/day (0.057 mg/kg-day in a 70-kg adult), a dose that would inhibit iodide uptake by approximately 35%, according to the dose–response we observed in the present study. It is not clear what ratio of iodine to perchlorate is necessary to protect against effects on thyroid function more subtle than goiter, but from the observations on thiocyanate, it can be inferred that adequate iodine intake is key to the resiliency of the thyroid–pituitary axis when challenged with perchlorate or any other inhibitor of thyroidal iodide uptake.

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