A Study on Perchlorate Exposure and Absorption in Beef Cattle

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Perchlorate exposure and potential effects were evaluated in large mammals by monitoring heifer calves placed on a site with access to streamwater fed by a perchlorate-contaminated groundwater spring (~25 ng/mL). Blood was collected from the two calves on the site (and two control calves from an uncontaminated site) approximately every 2 weeks for analysis of perchlorate residues and thyroid hormones. During the 14 week study, perchlorate was detected (detection limit = 13.7 ng/ mL) in blood plasma twice (15 ng/mL and 22 ng/mL) in one of the heifer calves drinking perchlorate-contaminated water on consecutive sampling periods 4 and 6 weeks after the beginning of perchlorate exposure. Constant exposure to 25 ppb perchlorate in drinking water had no effect on circulating thyroid hormones (T_3 and T_4) in the heifer calves.

INTRODUCTION

Perchlorate salts are used as oxidizers for solid propellants in rockets, missiles, and fireworks (*1*, *2*). Perchlorate salts are also used in many supporting industries, such as components in air bag inflators, and as additives in lubricating oils, leather tanning, electroplating, aluminum refining, and rubber manufacturing (*1*, *3*). Nonindustrial sources of perchlorate also exist including the well-known source of Chilean nitrates as well as some potashes, evaporites, and agricultural products (e.g., bone meal, and fish meal) (*4*). Large-scale production of perchlorate salts began in the mid-1940s (*3*), and large volumes have been disposed of in various states since the 1950s (*1*, *5*). Perchlorate salts can persist for many decades under typical ground and surface water conditions (*6*−*8*).

The perchlorate anion has been identified, beginning around 1997, as a contaminant in natural waterways and aquifers in the western U.S. (*2*, *9*). Currently, perchlorate is known to be present in water sources in over 20 states. While most occurrences are small and isolated, a number of regions are broadly affected including the Lake Mead-Colorado River system, which supplies water to large portions of Arizona and Southern California, as well as groundwater in the High Plains of Texas (*10*). Both areas are important sources of drinking and irrigation water. Most perchlorate detections in surface water samples from these states have been below 20 ppb (*5*).

There is concern over perchlorate exposure because it can interfere with thyroid function. Perchlorate is similar to iodide in charge and size, which allows perchlorate to compete with iodide for uptake into the thyroid via the sodium-iodide symporter (NIS) (*3*, *11*, *12*). Decreased iodide uptake into the thyroid eventually results in reduced production of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), and increased production of thyroid-stimulating hormone (TSH) via a negative feedback system existing in the hypothalamic-pituitary-thyroid axis (*1*, *8*, *11*, *12*). As an indirect inhibitor of thyroid hormone production, perchlorate exposure may eventually lead to effects on development (*12*). Thus, there is concern for the potential accumulation of perchlorate in aquatic and terrestrial environments, as well as the adverse effects resulting from exposure to perchlorate in drinking water.

Exposure of humans and other animals to perchlorate has largely been focused on ingestion of perchlorate-impacted water and to a lesser degree ingestion of plants irrigated with perchlorate-impacted water. However, organisms can also be exposed through indirect pathways such as trophic transfer. Evidence for trophic transfer includes detection of perchlorate in aquatic organisms inhabiting contaminated surface water bodies and in small mammals from perchlorate-contaminated sites (*13*). However, data on larger mammals (dairy and beef cattle, for example) are scarce, but represent a potential pathway of perchlorate exposure to humans. Cattle raised in impacted areas may be of special importance due to their potential ingestion of perchlorate both through impacted water and their large intake of forage crops, which could also contain significant quantities of perchlorate. This concern is highlighted by the recent study, which identified perchlorate in some commercial milk samples (*14*).

The unique characteristics of perchlorate (soluble anion) make accurate quantitation in biological matrixes difficult. The presence of additional ions, proteins, lipids, and other biomolecules that can clog or foul ion exchange columns further confounds accurate determination of perchlorate concentrations in biological tissues and fluids. We have focused a great deal

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of effort over the last several years in developing methods that could be used to accurately determine perchlorate in these matrixes (*15*, *16*).

Here we report an evaluation of perchlorate residues in blood plasma and tissues, as well as effects on circulating thyroid hormone levels (T$_3$ and T$_4$) in heifer calves raised on a pasture impacted by perchlorate. These animals were inhabiting areas in central Texas near the Naval Weapons Industrial Reserve Plant (NWIRP), a facility that produced many kinds of perchlorate-containing products prior to 1995. Monitoring data indicated that perchlorate was present in some streams and tributaries near NWIRP (*5*), raising concerns that wildlife and domestic animals consumed by humans may contain perchlorate residues and contribute to perchlorate exposure to humans. The results presented may be applicable to other areas in which cattle are raised in perchlorate-impacted areas.

MATERIALS AND METHODS

Study Description and Sample Collection. The study was conducted over 14 weeks during the spring of 2003 near McGregor, McLennan County, Texas. Four Shorthorn heifer calves (∼535 lbs. each) were purchased commercially from an individual seller. All calves were held for 1 week on the "Reference Site". Two calves (E1 and E2) were then separated and sent to a perchlorate-impacted "Treatment Site" while the other two calves (R1 and R2) remained on the Reference Site. The Reference Site was a pasture a sufficient distance from NWIRP such that perchlorate was not present in available on-site surface or groundwater utilized by the cattle, nor was it present in vegetation. The Treatment Site was a pasture near the NWIRP boundary with a spring-fed stream bisecting the pasture. Previous monitoring data for the spring (collected over a 2 year period) indicated that perchlorate was consistently present in the water (range = 20−60 ng/mL) (unpublished data from our laboratory). The spring-fed stream served as the only water source for calves on the Treatment Site. Calves on the Treatment Site also had access to vegetation (mostly grass) near the stream. We had previously monitored vegetation near the stream (trees and aquatic plants) and showed that perchlorate levels in leaves varied with species and distance from the stream (*17*). However, supplemental forage (hay) used during the first 2 weeks of the study on both sites did not contain detectable perchlorate (unpublished data from our laboratory).

Calves on both sites were visually monitored on a daily basis, and blood was collected from each animal every 2 weeks. Blood samples were always collected in the morning (before 9:00 a.m.). Approximately 50 mL of blood was drawn each sampling and placed into four sterile EDTA-coated blood collection vials. The samples were then spun at approximately 3100 rpm for 15 min. Plasma was transferred off and placed in 15-mL falcon tubes. Samples were stored on dry ice during transport back to the laboratory and stored frozen (−80 °C) until analysis. At the time of each blood collection, drinking water that the animals had access to was also collected and placed on ice during transport back to the laboratory, where it was stored at 4 °C until analysis.

At the conclusion of the study, animals were processed in a manner identical to a commercial beef processing operation. The following tissue samples were obtained from each animal for residue analysis: liver, thyroid, and various meat cuts (sirloin steak, round steak, t-bone steak, and roast).

Perchlorate Analyses. A potassium perchlorate (KClO$_4$) standard solution was obtained as a custom standard from AccuStandard, Inc. (New Haven, CT). Sodium hydroxide (NaOH), 50% (w/w) aqueous solution was purchased from Fisher Scientific. All solutions were prepared in 18.2 MΩ Milli-Q water. Ethanol was purchased from Fisher Scientific.

Plasma samples were processed using methods similar to those described previously (*3*, *15*, *18*) prior to analysis. First, 1 mL of plasma was precipitated with 4 mL of ethanol (ice-cold) and then centrifuged (4 °C) at 3750 rpm for 5 min. The supernatant was removed, evaporated to dryness under nitrogen, and reconstituted in 5 mL of Milli-Q water.
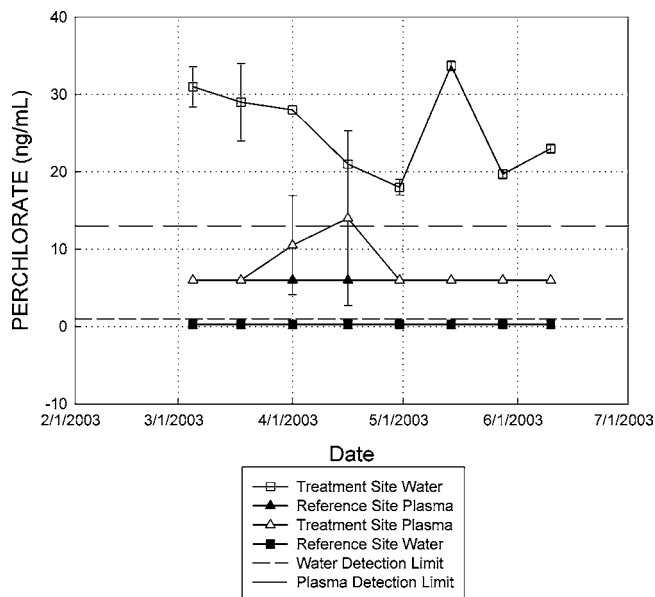

Figure 1. The relationship between perchlorate exposure in drinking water and perchlorate in heifer calf plasma. Each datum point is the mean ± SD of duplicate measurements. For graphing and calculation purposes, nondetects were given a value of $^1/_2$ the respective detection limit for perchlorate in water and plasma.

Samples were then cleaned using Alumina and C18 solid-phase extraction (SPE) cartridges and filtered (0.45 μm) prior to ion chromatography (IC) analysis.

Tissue samples were also processed prior to IC analysis. Samples (10−20 g) were air-dried and then extracted with Milli-Q water using accelerated solvent extraction (ASE; Dionex Corp.) Extraction conditions were as follows: pressure = 1500 psi, temperature = 100 °C, extraction time = 15 min. Sample extract volumes were measured, diluted (5×), and cleaned with Alumina and C18 SPE cartridges. Eluates were filtered (0.45 μm) prior to IC analysis.

Surface water samples collected from the 2 sites during the course of the study were filtered (0.45 μm) prior to IC analysis.

All samples (plasma extracts, tissue extracts, and surface water) were analyzed by ion chromatography similar to EPA Method 314. Analysis was carried out using a Dionex DX-500 Ion Chromatography System equipped with a GP50 gradient pump, a CD20 conductivity detector, and an AS40 automated sampler (Dionex Corp.). PeakNet chromatography software was used to control the system. Ion separation was made with a Dionex IonPac AS16 (250-mm × 4.0-mm) analytical column. Conditions for the system were as follows: flow rate = 1.0 mL/min; eluent = 50 mM sodium hydroxide; injection volume = 1000 μL. Ion detection was by suppressed conductivity in the external water mode. A seven-point standard curve was constructed from constant volume injections of calibration standards of 2.5, 5, 10, 20, 50, 100, 200, and 500 ppb (ng/mL). Computer-generated peak areas were used to measure sample concentrations in an external standard mode. Quality control (QC) samples included blanks, matrix spikes, and check standards. Using the analytical method described above, the detection limits for perchlorate in water, plasma, and tissue were 1.0 ng/mL, 13.7 ng/mL, and 23.2 ng/g, respectively.

Thyroid Hormone Analyses. Thyroid hormones in the heifer calves were assayed as both a biomarker of perchlorate exposure and a biomarker of potential perchlorate effect. Plasma was analyzed for total T$_3$ and total T$_4$, using Coat-A-Count radioimmunoassay (RIA) kits from Diagnostic Products Corporation DPC, Los Angeles, CA. The assay was performed according to manufacturer's instructions. Plasma samples were assayed on a Packard Cobra E5005 gamma counter. Samples were assayed in triplicate, and standards (six) that came with the kit were run at the beginning of each assay and intermittently throughout the assay.
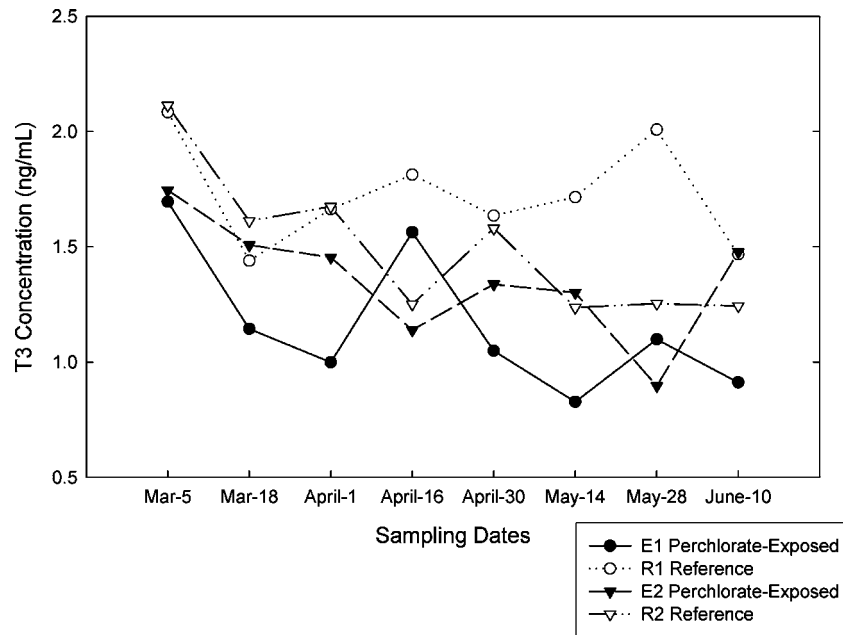
Figure 2. Total T_3 concentrations in plasma from four heifer claves on either perchlorate-contaminated (E1, E2) or reference pastures (R1, R2) near McGregor, TX, 2003.

Figure 3. Total T_4 concentrations in plasma from four heifer calves on either perchlorate-contaminated (E1, E2) or reference pastures (R1, R2) near McGregor, TX, 2003.
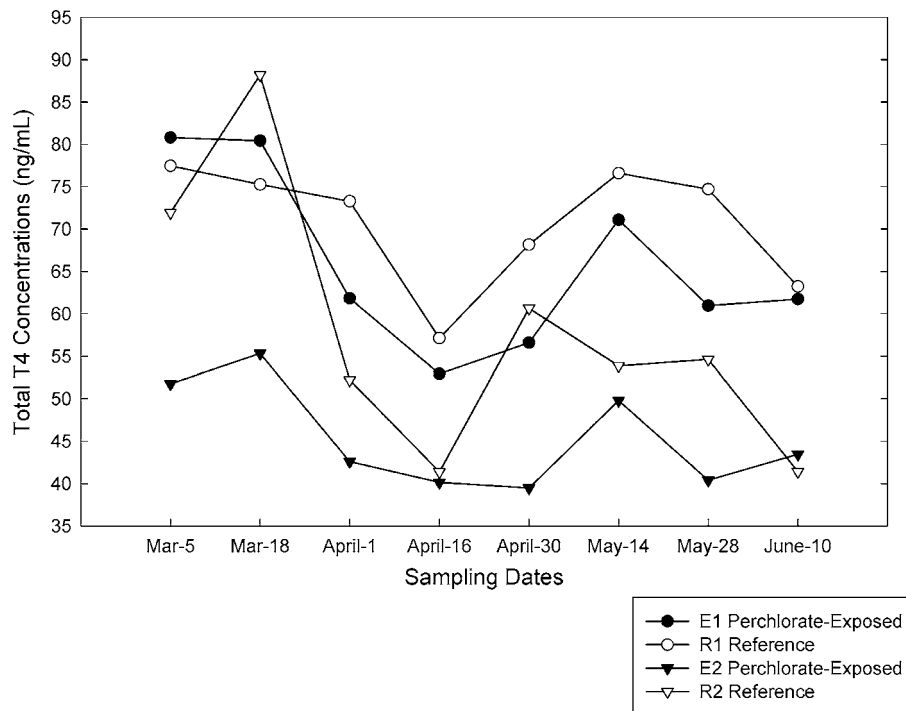
RESULTS AND DISCUSSION

Perchlorate in Drinking Water, Blood Plasma, and Tissues. Perchlorate was not detected in surface water samples collected from the Reference Site (detection limit = 1.0 ng/ mL). In contrast, perchlorate was detected throughout the study (∼14 weeks) in water samples from the Treatment Site (mean ± standard error = 25.4 ± 1.3 ng/mL; n = 8) (**Figure 1**). The concentrations of perchlorate in water from the Treatment Site during our 14-week study are consistent with more than 2 years of monitoring data from that location (unpublished data).

Considerable effort was made to develop a method for perchlorate analysis in blood plasma. The method was able to reduce the background interference significantly, and perchlorate recovery of spiked samples was consistent and reproducible (83.6 ± 0.6%). In addition, the detection limit for perchlorate in blood plasma (S/N = 3) was low, increasing our chances of detecting perchlorate if present in the heifer calves.

Perchlorate was not detected in blood plasma samples from either calf occupying the Reference Site (**Figure 1**). Similar results were obtained for the 2 calves on the Treatment Site with one exception; perchlorate was detected (15 ng/mL and 22 ng/mL) in one of the calves on consecutive sampling periods 4 and 6 weeks after the beginning of the study. Perchlorate was not detected (detection limit = 23.2 ng/g) in any of the tissue
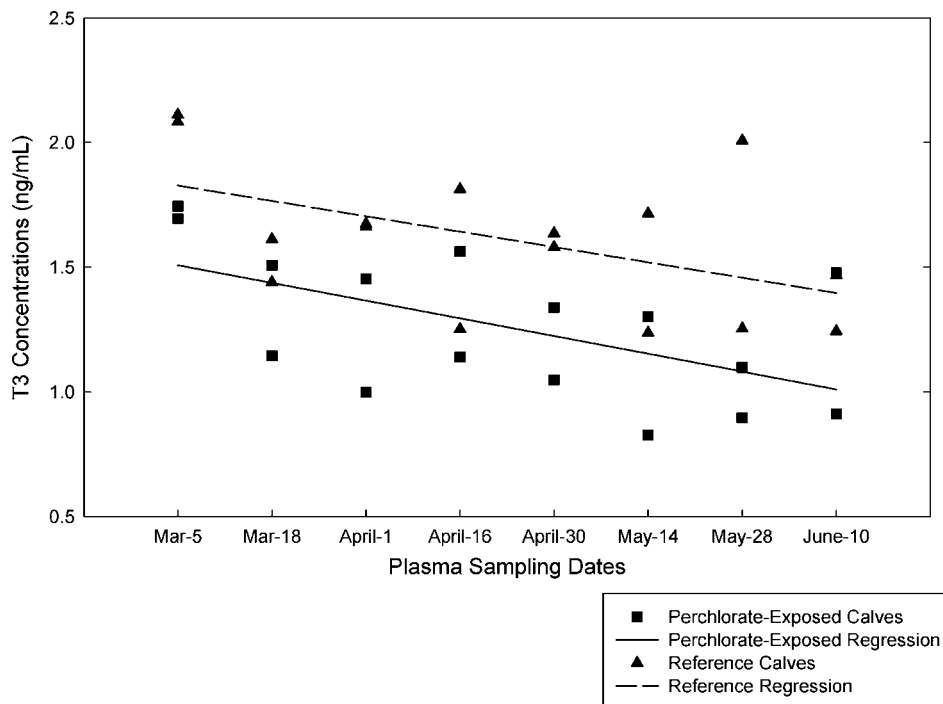
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Figure 4. Linear regression of plasma T_3 concentrations in heifer calves maintained on a perchlorate contaminated or a reference pasture near McGregor, TX, 2003. The slope for the reference calf regression line is −0.06, and the r^2 is 0.25. The slope for the perchlorate-exposed calf regression line is −0.07, and the r^2 is 0.33.
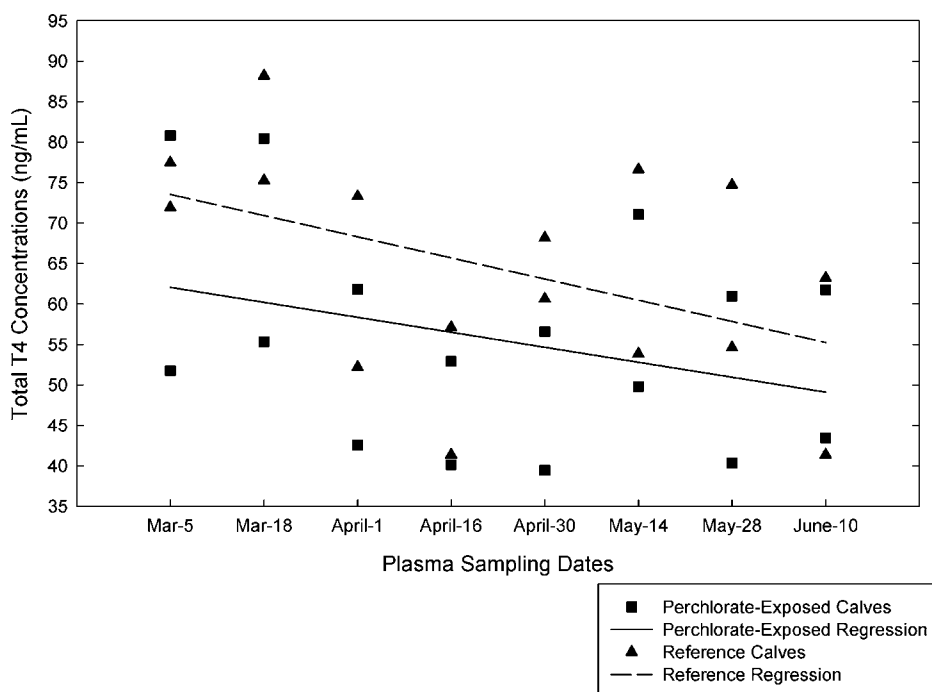

Figure 5. Linear regression of T_4 plasma concentrations in heifer calves maintained on a perchlorate-contaminated or a reference pasture near McGregor, TX, 2003. The slope for the reference calf regression line is −2.61, and the r^2 is 0.21. The slope for the perchlorate-exposed calf regression line is −1.85, and the r^2 is 0.11.

samples collected from calves occupying the Reference Site. In addition, perchlorate was not detected in any of the tissue samples collected from calves occupying the Treatment Site.

Our results indicate that despite the presence of perchlorate in drinking water at the Treatment Site, there was little quantifiable perchlorate exposure measured in blood plasma from heifer calves. Constant exposure to 25 ppb perchlorate in water over 14 weeks does not result in measurable residues in blood plasma or edible tissues. In addition, it is likely that overall

perchlorate exposure in heifers from the Treatment Site was higher than that measured in water as vegetation samples from the Treatment Site also contained perchlorate (*17*).

Water intake in cattle is well understood (*19*). A beef heifer consumes 20−55 liters of water per day and excretes 75% of that intake (2:1 feces/urine). On the basis of that assumption and the measured drinking water concentrations, the heifers on the Treatment Site were ingesting 125−350 μg perchlorate/day. If one makes the assumption that cattle have 60 mL of blood

per kilogram of body weight, the expected perchlorate concentration in plasma should be 9−25 ng/mL. This range is consistent with the 2 detections that we did observe (15 and 22 ng/mL).

Perchlorate can be rapidly excreted in urine, with reported urinary excretion half-lives ranging from 8 to 20 h in rats (*3*, *11*). Although Batjoens and co-workers (*20*) reported that prolonged perchlorate administration (4 g/day for 10 days) in cows resulted in a longer excretion period in the urine than that with a single dose, perchlorate accumulation did not occur because of the relatively rapid excretion. The rapid half-life of perchlorate in mammals contributes to the difficulty in assessing perchlorate exposure by monitoring blood. Although we also collected urine from the animals opportunistically, this sample matrix proved to be difficult for IC analysis by Method 314, due to a high signal background.

Perchlorate was detected with greater frequency and at slightly higher concentrations in blood of cattle from two Kansas farms adjacent to facilities that used or handled perchlorate (unpublished data from our laboratory). Cattle on these farms were not restricted to water supplies containing perchlorate (as were those on the pasture near the NWIRP site), although the water that was contaminated had higher perchlorate concentrations (>100 ng/mL). Nonetheless, perchlorate was detected in 4 of 33 and 17 of 26 cattle at the two Kansas farms. The highest plasma perchlorate concentrations observed in the Kansas cattle were 43 and 32 ppb, respectively. Although perchlorate was detected at slightly higher concentrations in these animals, thyroid hormone levels were not different from control (unexposed) cattle.

In humans, some studies have predicted that iodide uptake would not be inhibited by perchlorate doses of approximately 2 mg/day, while other data suggest a no-effect level of 0.5 mg/day (*12* and the literature therein). However, the authors also suggested that the adverse effects produced by perchlorate may begin somewhere near the 1 mg/d dose range. This is equivalent to 500 ng/mL perchlorate in drinking water if we assume a daily intake of 2 L of water per day. The USEPA has provided a drinking water equivalent level (DWEL) of 1 ng/mL for perchlorate based on 2 L daily consumption and a draft reference dose (RfD) of 0.00003 mg/kg/day (*21*).

Thyroid Hormones. Plasma samples were collected approximately every two weeks from March 5, 2003 to June 10, 2003. Individual heifer thyroid hormone levels varied considerably from week to week (**Figures 2** and **3**). Triiodothyronine (T_3) concentrations ranged from 0.83 to 1.75 ng/mL, with a mean (± standard error) concentration of 1.26 ± 0.07 ng/mL in the perchlorate-exposed heifers. Reference heifer T_3 concentrations ranged from 1.24 to 2.11 ng/mL, with a mean (± standard error) concentration of 1.61 ± 0.07 ng/mL. Mean T_3 concentrations for each heifer ranked from highest to lowest were RI = 1.73 ± 0.08 ng/mL, R2 = 1.50 ± 0.11 ng/mL, E2 = 1.36 ± 0.09 ng/mL, and E1 = 1.16 ± 0.11 ng/mL.

Thyroxine (T_4) concentrations ranged from 39.47 to 80.80 ng/mL, with a mean (± standard error) concentration of 55.58 ± 3.36 ng/mL in the perchlorate-exposed heifers. Reference heifer T_4 concentrations ranged from 41.38 to 88.19 ng/mL, with a mean (± standard error) concentration of 64.38 ± 3.38 ng/mL. Mean T_4 concentrations for each heifer ranked from highest to lowest were R1 = 70.73 ± 2.57 ng/mL, E1 = 65.80 ± 3.72 ng/mL, R2 = 58.03 ± 5.55 ng/mL, and E2 = 45.37 ± 2.15 ng/mL. There was a noticeable decrease in T_4 concentrations among all calves on the April 16 sampling period (**Figure 3**), which may have been due to assay-related error. Overall,

there appeared to be a general trend of decreasing thyroid hormones in both the reference (R) and the exposed (E) animals (**Figures 4** and **5**).

There was considerable variability in thyroid hormone concentrations in both perchlorate-exposed and reference heifers, but there did not appear to be any perchlorate-related reductions in thyroid hormone concentrations. Perchlorate is known to cause hypothyroidism; characterized by a decrease in thyroid hormone levels, an increase in weight, and lethargy (*22*). De Moraes et al. (*23*) reported average blood serum concentrations for T_3 at 1.6 ng/mL and for T_4 at 88.5 ng/mL, collected over a five week period from Brahman cattle. Grigsby and Trenkle (*24*) reported average blood plasma T_3 concentrations of 1.75, 1.22, and 1.52 ng/mL and T_4 concentrations of 72.5, 70.5, and 81.0 ng/mL in Angus, Limousin, and Simmental steers, respectively. These studies illustrate the variability in thyroid hormone concentrations among cattle breeds. Our results are comparable to those described above. The cattle used for this study were a mixed breed (primarily Shorthorn), which may have contributed to the variability observed in thyroid hormone concentrations. The perchlorate-exposed cattle mean thyroid hormone concentrations were lower than those of the reference heifers throughout the study, but the rate of decrease in thyroid hormones throughout the study period was similar between the two groups (**Figures 4** and **5**). In fact, the rate of decline in T_4 concentrations among the reference heifers was slightly greater than that of the perchlorate-exposed heifers. Therefore, it is doubtful that perchlorate inhibited thyroid hormone production in the exposed heifers, which is consistent with our exposure data. The absence of an observed effect of perchlorate (<120 ppb) on thyroid hormones in children or neonates has also been shown in epidemiology studies (*25*, *26*, *27*). Recognizing the limitations of our study with respect to the length of perchlorate exposure and statistical power, low concentrations of perchlorate in water (and forage) do not appear to significantly affect subadult cattle thyroid hormone concentrations. Additional studies are needed to assess potential developmental effects in perchlorate-exposed calves and the potential effects of longer-term exposures.

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