Fate of dietary perchlorate in lactating dairy cows: Relevance to animal health and levels in the milk supply

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Perchlorate is a goitrogenic anion that competitively inhibits the sodium iodide transporter and has been detected in forages and in commercial milk throughout the U.S. The fate of perchlorate and its effect on animal health were studied in lactating cows, ruminally infused with perchlorate for 5 weeks. Milk perchlorate levels were highly correlated with perchlorate intake, but milk iodine was unaffected, and there were no demonstrable health effects. We provide evidence that up to 80% of dietary perchlorate was metabolized, most likely in the rumen, which would provide cattle with a degree of refractoriness to perchlorate. Data presented are important for assessing the environmental impact on perchlorate concentrations in milk and potential for relevance to human health.

environment | health risk | human | thyroid hormones

Public concern about perchlorate (ClO_4^-) in the environment, drinking water, and foods has steadily increased in recent years. Within the Colorado watershed, regional water supplies contain perchlorate at concentrations that may reach hundreds of parts per billion (ppb) (or μg/liter), but more typical levels in the drinking water in this region have ranged from 2 to 20 ppb (1, 2). High levels of perchlorate have often been associated with environmental contamination from the production of munitions, primarily in the form of ammonium perchlorate for use as an oxidant in solid rocket fuel. However, perchlorate is also used as an oxidant in matches, fireworks, automobile airbag-inflation systems, and a variety of other industrial applications, including the production of dyes, paints, and rubber (1). Perchlorate also appears to be naturally present in the environment (3–6).

Perchlorate has also been detected in components of the food chain (www.cfsan.fda.gov/~dms/clo4data.html; www.usda.gov/agency/oce/oracba/forum.htm) (7). Alfalfa accumulates perchlorate, making it and other dietary components sources of exposure to livestock (7). Recently, perchlorate has been detected in commercial fluid milk, with concentrations ranging from 0 to 11 ppb in all regions of the country surveyed (www.cfsan.fda.gov/~dms/clo4data.html) (8). We (C.P.R., unpublished data) have detected perchlorate at the same concentration in both manually and mechanically collected milk, indicating that its presence cannot be attributed to contamination during automated milking, collection, or processing.

Known biological effects of perchlorate stem from its action as a competitive inhibitor of the sodium iodide symporter (NIS), which transports iodide into the thyroid gland for synthesis of thyroid hormones (9). As such, it has been used medically to reduce production of thyroid hormones in patients with Graves' disease (10). Sensitivity to perchlorate is heightened by concurrent iodine deficiency and physiological periods of thyroid hormone demand, such as pregnancy and lactation. Because of the importance of thyroid hormones for neurological development (10), there is concern about the intake of goitrogenic molecules, such as perchlorate, during pregnancy and early infant development (11, 12).

In addition to the thyroid, the mammary gland expresses the NIS and actively transports iodide (13). Consistent with existing knowledge of mammary gland physiology, increased circulating blood levels of perchlorate after possible uptake from the gastrointestinal tract may lead to elevated levels of perchlorate and decreased iodine in milk. However, the microbial activity and reducing environment of the rumen may ameliorate the delivery of dietary perchlorate to the circulation and peripheral organs. Conversely, generation of perchlorate by biological processes, analogous to generation of hypochlorite by phagocytic neutrophils (14), may provide an endogenous source of perchlorate in the absence of environmental input. Because thyroid hormones are galactopoietic and may alter mammary gland function (15), there is potential for perchlorate to influence milk production. The objective of this study was to evaluate the impact of ruminally administered perchlorate on concentrations of perchlorate in bovine milk and to assess the impact on animal health and productivity.

Materials and Methods

Cows and Experimental Design. Sixteen clinically healthy, lactating Holstein cows were used in this study, approved by the Beltsville Agricultural Research Center's Animal Care and Use Committee. The cows were surgically fitted with permanent 10-cm rumen cannulae (Bar Diamond Parma, ID) to permit rumen administration of perchlorate in a fully controllable manner. Cows were balanced with regard to stage of lactation and milk production across four treatment groups. Treatments consisted of continuous ruminal infusion of 1 liter of distilled water per day containing 0, 0.4, 4, or 40 mg of perchlorate, supplied as $NaClO_4$, over a 20-hr infusion period (2:00 p.m. to 10:00 a.m.). Perchlorate treatments were designed to provide daily quantities of perchlorate that equated to consuming 40 kg per day of a diet containing 0, 10, 100, or 1,000 ppb of perchlorate. Body weight of the cows averaged 537 ± 13 kg, and perchlorate dosages equated to 0, 0.7, 7.4, and 74.5 μg/kg of body weight per day.

The cows were permitted to recover from surgical implantation of the rumen cannulae and acclimate to the housing facility and management for 2 weeks. Thereafter, data and samples were collected during (*i*) a 2-week pretreatment period to establish basal levels for parameters of interest, (*ii*) a 5-week perchlorate-infusion period, and (*iii*) a 2-week posttreatment period. Aliquots of perchlorate solutions used for rumen infusion were collected daily and the concentration of perchlorate confirmed according to method-

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Abbreviations: NIS, sodium iodide symporter; ppb, parts per billion; RfD, reference dose; SCC, somatic cell count; T4, thyroxine; T3, triiodothyronine.

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ology described below. Concentrations were confirmed to be within 3% of predicted values.

The cows were housed in an environmentally controlled, tie-stall facility. Cows were fed, *ad libitum*, a diet (total mixed ration) that was formulated to meet National Research Council requirements for lactating cows (16). Water was made freely available by automated watering cups. The cows were provided exercise for ≈2 h daily, during the 4-hr noninfusion interval (10:00 a.m. to 2:00 p.m.), after blood, urine, and feces samples were collected.

While restrained in tie stalls, the cows were milked twice daily, at 7:00 a.m. and 6:00 p.m. Teats were dipped pre- and postmilking in an iodine-based teat dip (A&L Laboratories, Minneapolis). Milk weights were recorded at each milking. Cows were weighed weekly and fed daily at 9:00 a.m. Feed consumption was monitored by weighing feed offered and refusals. All cows consumed the same diet.

Blood, Urine, and Feces Samples. The experiment was initiated on a Monday, the infusions began 2 weeks later (Monday at 2:00 p.m.), and infusions were terminated 5 weeks after initiation (Monday at 10:00 a.m.). Blood and urine samples were collected twice weekly, on Tuesdays and Thursdays between 9:00 a.m. and 11:00 a.m., during the entire study. Additional blood and urine samples were collected 8 h after termination of perchlorate infusion. Blood was collected from the cocygeal vein into heparin, EDTA, and serum Vacutainer tubes (Becton Dickinson). Heparin-treated blood was used for perchlorate determination. EDTA-treated blood was used for blood differential cell counts, and EDTA-treated plasma for total thyroxine (T4) and triiodothyronine (T3) RIA. Serum was used for evaluation of blood electrolytes and clinical immunoassay of T4, T3, and free T4. Plasma and serum were collected after low-speed centrifugation for 15 min and either analyzed immediately or stored at −20°C until RIA of T4 and T3.

Fecal samples were collected on days 25 and 30 of the infusion period and on days 4 and 9 of the posttreatment period, during an a.m. bowel movement that sometimes fell outside the normal 9:00 a.m. to 11:00 a.m. sampling period. Fecal dry weight was determined after heating at 65°C until constant weight. Dried feces were ground and used for perchlorate and lignin analyses.

Feed Samples and Analyses. Samples of the total mixed ration were taken daily throughout the study. Equal masses of daily feed samples were combined, and the composition of weekly composite feed samples was determined (Cumberland Valley Analytical Services, Maugansville, MD). Iodine content of the diet was included in the analysis, and perchlorate content was determined as described below. Ingredients and composition of the diet are summarized in Tables 3 and 4, which are published as supporting information on the PNAS web site.

Milk Sampling and Analyses. Composite milk samples were obtained from each cow twice weekly at the a.m. milking. The milk obtained from the four mammary glands (composite milk) was thoroughly mixed and aliquoted for milk somatic cell count (SCC), composition, and perchlorate analyses. For SCC analysis, milk samples were heated for 15 min at 60°C and maintained at 40°C until assayed (Somacount 150, Bentley Instruments, Chaska, MN) (17). Milk composition was determined by Environmental Systems Service (Laurel, MD).

To monitor udder health, quarter milk samples were obtained twice weekly during the pretreatment period and once weekly for the remainder of the study. Before milking, ≈2 ml of milk was collected aseptically from each mammary gland for bacteriological analysis and ≈35 ml for SCC analysis. Bacteriological analysis was performed according to standard methods (18). Each sample was plated at 0.05-ml volume onto trypticase soy blood agar. Plates were incubated at 37°C for 48 h and examined to determine bacteriological status. If present, bacteria were categorized according to Gram staining, catalase test, and coagulase test.

To explore the relationship between udder health and perchlorate concentrations in milk, perchlorate concentration and SCC were assessed on quarter milk samples obtained once weekly during the pretreatment period, once per week during the final 2 weeks of the infusion period and once weekly during the posttreatment period. Additional quarter milk samples were obtained for perchlorate analysis when an increase in the SCC of quarter milk samples was detected. When the SCC for a quarter averaged ≤100,000 cells per ml during the pretreatment period, the milk from all quarters of a cow was assayed when SCC of that quarter exceeded 300,000 cells per ml on any sampling day during the study. If the pretreatment average SCC for a quarter was ≥100,000, milk from all quarters of the cow was assayed if SCC in that quarter increased at least 3-fold.

Blood Analyses. Total (free plus bound) concentrations of T4 and T3 were determined by RIA using ^{125}I-labeled solid-phase kits (MP Biomedicals, Orangeburg, NY). Hormones in EDTA-treated plasma were assayed according to protocols described by the manufacturer, except that the assays were incubated at 22°C for 16 h. By using the modified incubation protocol, time-related assay drift was prevented, and recovery of added T3 and T4 averaged 100% and 103%, respectively. For each hormone, all samples were analyzed in a single assay with coefficients of variation <5%. Free T4 concentrations in serum were assayed by using the Immulite 1000 system (Diagnostic Products, Los Angeles) according to the

Table 1. Perchlorate intake and partitioning in milk, blood, urine, and feces

Perchlorate intake, mg per day				Perchlorate, ppb, ln-transformed				Partition coefficient		
Infusion	Feed	Water	Total*	Blood†	Milk†	Urine†	Feces†	Milk/blood‡	Urine/blood§	Feces/blood†
0	0.46	0.03	0.50	−1.43 ± 0.09 (0.24)	1.47 ± 0.05 (4.37)	1.30 ± 0.07 (3.68)	1.77 ± 0.10 (5.84)	2.90 ± 0.20 (18.2)	2.74 ± 0.15 (15.4)	2.88 ± 0.12 (17.7)
0.4	0.43	0.03	0.87	−1.05 ± 0.05 (0.35)	2.02 ± 0.04 (7.51)	1.67 ± 0.08 (5.31)	1.83 ± 0.10 (6.25)	3.07 ± 0.20 (21.5)	2.72 ± 0.15 (15.2)	2.67 ± 0.12 (14.5)
4	0.44	0.03	4.47	0.51 ± 0.05 (1.66)	2.92 ± 0.06 (18.5)	3.23 ± 0.09 (25.2)	2.02 ± 0.09 (7.53)	2.41 ± 0.20 (11.1)	2.72 ± 0.15 (15.2)	1.54 ± 0.12 (4.67)
40	0.44	0.03	40.47	2.28 ± 0.07 (9.78)	4.45 ± 0.06 (85.3)	4.79 ± 0.12 (119.9)	3.55 ± 0.18 (35.0)	2.17 ± 0.20 (8.7)	2.51 ± 0.15 (12.3)	1.17 ± 0.12 (3.23)

Water intake was calculated (see *Supporting Text*). Correlation coefficients for blood, milk, urine, and feces vs. total perchlorate intake are provided. Perchlorate concentrations and partition coefficients are means ± SE of ln-transformed data (four cows per treatment) over the infusion period. Perchlorate concentrations are ng/ml for blood, milk, and urine and ng/g dry matter for feces. Numbers in parentheses are back-transformed (anti-ln) means.

Linear correlation coefficients of ln-ln-transformed data, r^2, were 0.909, 0.930, 0.922, and 0.805 for perchlorate, ppb, ln-transformed in blood, milk, urine, and feces, respectively.

*Total perchlorate intakes per unit body weight for the four treatments were 0.9, 1.62, 8.26, and 76.1 μg/kg per day.

†Treatment significance, $P = 0.0001$.

‡Treatment significance, $P = 0.002$.

§Treatment significance, $P = 0.67$.

manufacturer's protocols after an initial heat-inactivation step (30 min at 56°C).

EDTA-treated blood was mixed for at least 15 min on a platform rocker and analyzed for hemoglobin, hematocrit, and blood total and differential cell counts by using a Hemavet 3700 (CDC Technologies, Oxford, CT). Electrolytes and routine blood chemistry analyses were performed by using the VetACE clinical chemistry system (Alfa Wassermann, West Caldwell, NJ). These analyses were performed the day of sampling.

Lignin Analyses. Lignin in feed and feces was analyzed as described in ref. 19, with minor modification for use with the ANKOM 200 fiber unit (ANKOM Technology, Fairport, NY).

Perchlorate and Iodide Analyses. Milk, blood, urine, feces, water, and feed samples were prepared for perchlorate analysis by using modifications of previous extraction methods (20, 21). To analyze milk iodide content, milk was processed and extracted as for perchlorate analysis. For detailed sample preparation methods, see *Supporting Text*, which is published as supporting information on the PNAS web site.

All perchlorate and iodide analyses used a modification of the ion chromatography (IC)/triple quadrupole mass spectrometry (MS/MS) method (20). For detailed description of perchlorate and iodide IC/MS/MS protocols, see *Supporting Text*. Percent recoveries for perchlorate in water, blood, milk, urine, feed, and feces were as follows (mean ± SE): 98.3 ± 3.0, 101.7 ± 1.9, 96.9 ± 2.0, 93.3 ± 1.3, 93.7 ± 3.0, and 95.8 ± 2.6, respectively. Duplicates were within 5.5%. Detection limits for water, blood, milk, urine, feed, and feces were as follows: 0.06 ng/ml, 0.18 ng/ml, 0.28 ng/ml, 0.28 ng/ml, 0.53 ng/g dry weight, and 0.53 ng/g dry weight, respectively. Spike recovery for iodide in milk ($n = 9$) was 60 ± 11, and the relative percent difference for duplicates was 26%.

Experimental Quality Control. The experiment was performed in accordance with good laboratory practice guidelines (22). Data are available (http://bfgl.anri.barc.usda.gov/perchlorate/).

Statistical Analyses. Data were analyzed with SAS statistical analysis software (SAS 9, SAS Institute, Cary, NC) by using the mixed-linear-model procedures. For all data analyzed, animals were considered as random effects. For perchlorate concentrations in blood, milk, urine, and feces, the model also included fixed treatment by time effects, and data were log-transformed to stabilize residual variances. For blood chemistry and blood differential counts, the model also included fixed treatment by time effects; however, nontransformed data were analyzed, because variances were homogeneous. Because milk production and feed intake were measured daily and were expected to have a time-dependent correlation, a first-order autoregressive residual correlation was assumed for these data. For milk yield, a covariate for days in milk was included within first and multiparous lactations to account for the normal lactation-curve drop in production postpeak. Additional fixed effects for treatment and regression of time on treatment nested within treatment were included. For feed intake, fixed effect for treatment and a covariate for daily milk yield were included with the random animal effect.

Results

Perchlorate in Milk. The total perchlorate intake per day was calculated as the sum of dietary intake (feed plus water) and infused dose (Table 1). Feed intake was directly monitored, and water intake was estimated based on milk production, ambient temperature, and sodium consumption (23). Perchlorate concentration in the diet averaged 22.4 ppb and, in the water, averaged 0.34 ppb. During the pretreatment period, concentrations of perchlorate in milk averaged 4.3 ng/ml. Upon initiation of perchlorate infusion, the concentration in milk rose to a steady-state concentration within 1 day (Fig. 1), averaging 4.4, 7.5, 18.5, and 85.3 ng/ml for the 0-, 0.4-, 4-, and 40-mg-per-day treatment groups, respectively (Table 1). The steady-state concentration in milk was nonlinearly correlated with total perchlorate intake ($r^2 = 0.93$, Table 1). Concentrations of perchlorate in other compartments were similarly correlated ($P < 0.0001$) with intake (Table 1) and present in the following order: urine > milk > feces > blood. Upon termination of infusion, perchlorate concentrations in body fluids rapidly declined (Fig. 1). Clearance half-lives for perchlorate in blood and urine were estimated as 9 and 12 h, respectively, and for perchlorate in milk as 22 h. The longer half-life of perchlorate in milk vs. blood or urine is likely because of the 12-hr milking interval. If cows are milked more often than twice daily, it is likely that clearance can be accelerated. Should contamination cause an unacceptable concentration of perchlorate in milk, a conservative withdrawal time would be 72 h.

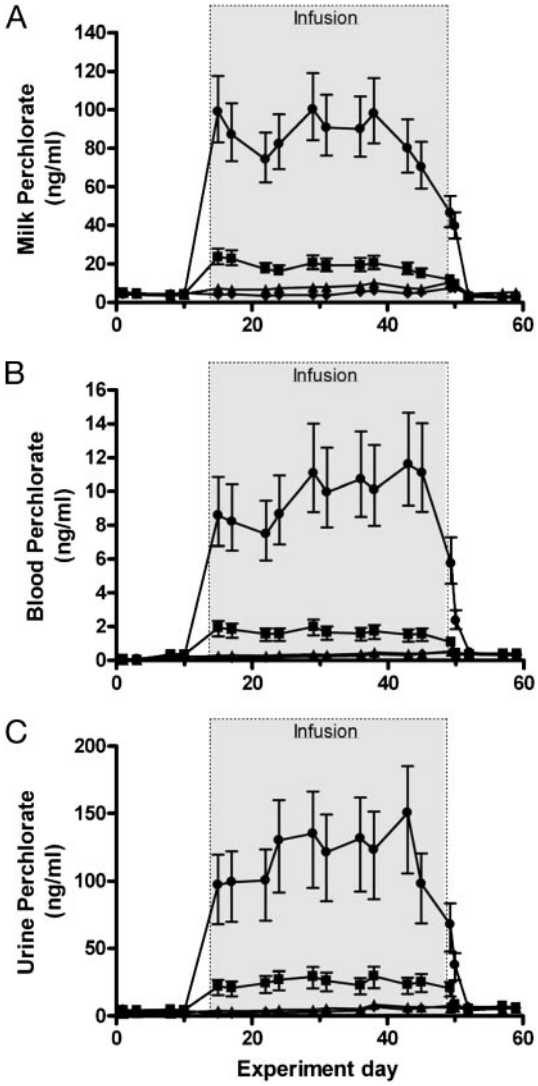

Fig. 1. Perchlorate concentrations in blood, milk, and urine of lactating cows infused ruminally with 0, 0.4, 4, or 40 mg of perchlorate per day. (*A*) Milk. (*B*) Blood. (*C*) Urine. The shaded box indicates period of perchlorate infusion. Values are means ± SE. ◆, 0 mg of perchlorate per day; ▲, 0.4 mg of perchlorate per day; ■, 4 mg of perchlorate per day; ●, 40 mg of perchlorate per day.

Partitioning and Fate of Perchlorate. The partitioning of perchlorate among the blood, milk, urine, and fecal compartments was evaluated by calculating the ratio of perchlorate concentration in each

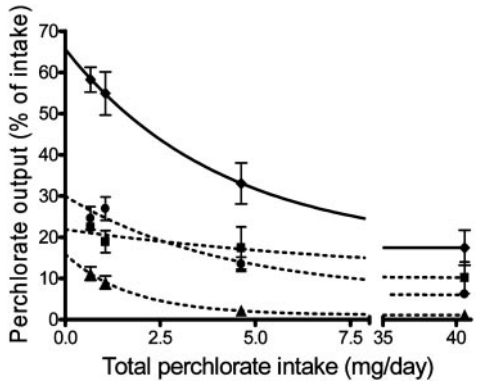

Fig. 2. Perchlorate output relative to perchlorate input. Daily output of perchlorate in milk, urine, and feces are reported relative to the total daily perchlorate load (perchlorate in feed plus water plus infusion). Water intake was estimated (23) and urine volume calculated as 21% of total water intake (16). Fecal mass was calculated based on the content of nondigestible fiber in the diet and in the feces. Values are means ± SE. ♦, total perchlorate output; ●, milk perchlorate output; ■, urinary perchlorate output; ▲, fecal perchlorate output.

compartment to that in blood (Table 1). This partitioning coefficient for urine was unaffected by the perchlorate load; however, the partitioning coefficients for milk and feces declined as the perchlorate load increased. Total output of perchlorate in milk, urine, and feces fell far short of total intake (Fig. 2). Total perchlorate output was 58, 55, 33, and 17% of the perchlorate load for cows in the mg per day 0-, 0.4-, 4-, and 40-mg-per-day infusion groups, respectively. The relationship ($r^2 = 0.999$) between perchlorate load (x) and total perchlorate output (y, as percent of x) was curvilinear and described by $y = 47.89 \times e^{(-0.238x)} + 17.41$. Similarly, daily perchlorate output for each compartment (milk, urine, and feces) declined in curvilinear fashion as perchlorate load increased, after exponential decay functions.

Perchlorate and Animal Health. Reduction in uptake of iodide by the thyroid reduces biosynthesis of thyroid hormones. In this study, we saw no effect of perchlorate infusion on circulating concentrations of total T4, total T3, and free T4 (Fig. 3). Additional parameters of animal health were evaluated and found to be unaffected by perchlorate treatment. Feed consumption (see Table 5, which is published as supporting information on the PNAS web site), blood chemistry profiles (see Table 6, which is published as supporting information on the PNAS web site), and blood cell differential counts (see Table 7, which is published as supporting information on the PNAS web site) were unaffected ($P > 0.05$) by perchlorate treatment.

Perchlorate's inhibition of NIS has the potential to compromise iodine and thyroid hormone regulation of mammary gland function. However, perchlorate infusion did not significantly impact lactation. Milk iodide concentrations were not affected ($P > 0.1$) by perchlorate infusion and averaged 794 ± 65, 747 ± 65, and 737 ± 66 μg/liter for cows in the 0-, 4-, and 40-mg-per-day infusion groups, respectively, during the last week of infusion (data not shown). We found that milk yield (Table 5) was unaffected by perchlorate. Although minor reductions in milk fat and solids were observed (Table 2), these are of little biological relevance, because milk composition still fell within the normal range. Although the number of animals in the study was not suitable for rigorously evaluating the impact on udder health, there was no effect ($P > 0.05$) of perchlorate treatment on the SCC in milk (Table 2). The numerical increase in SCC in the 0.4- and 40-mg-per-day perchlorate-treatment groups during the posttreatment period was attributed to the development of clinical mastitis in a single mammary gland in one cow in each of these treatments.

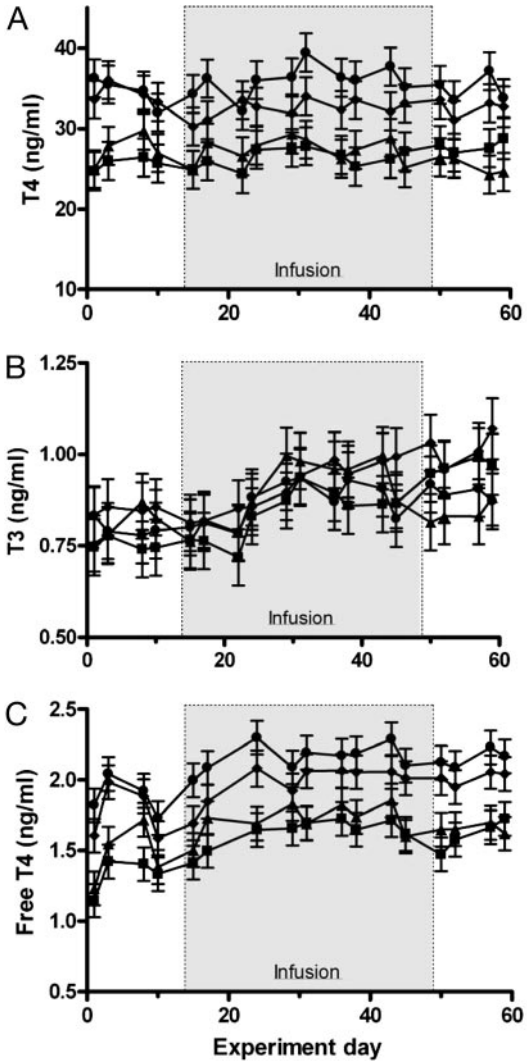

Fig. 3. Concentrations of total T4, total T3, and free T4 in the circulation. Each point represents the mean for four cows. (*A*) Total T4. (*B*) Total T3. (*C*) Free T4. The shaded box indicates period of perchlorate infusion. Perchlorate infusion did not reduce concentrations of thyroid hormones ($P > 0.05$). Values are means ± SE. ♦, 0 mg of perchlorate per day; ▲, 0.4 mg of perchlorate per day; ■, 4 mg of perchlorate per day; ●, 40 mg of perchlorate per day.

Discussion

The rapid transfer of dietary perchlorate to milk is consistent with existing knowledge of mammary gland biology. Like the thyroid, the mammary gland exhibits a capacity to transport and concentrate iodide (24). In the lactating mammary gland, this transport occurs through action of the NIS (25), although there is evidence for another active transporter of iodine in lactating and nonlactating mammary gland (26). Because perchlorate serves as a competitive inhibitor of iodide transport by the NIS, with both iodide and perchlorate being transported into the mammary secretory cell, concentrations of perchlorate and iodine in milk may be influenced by the relative consumption of iodine and perchlorate. The quantity of iodine in the diet exceeded the National Research Council requirement (16) for lactating cows (0.6 ppm), which is ≈160 times the concentration of perchlorate in the diet. However, the K_M for perchlorate transport by the NIS is an order of magnitude lower than that for iodine, and serum concentrations of iodine are typically several orders of magnitude below the K_M for iodide. Therefore, iodine concentrations in the diet may not influence

Table 2. Effect of perchlorate on milk composition

Period	Perchlorate, mg per day	Fat, %	Protein, %	Lactose, %	Total solids, %	SCC × 10^3 per ml
Pretreatment						
	0	3.80 ± 0.22	2.80 ± 0.07	4.82 ± 0.03	12.13 ± 0.28	25.3 ± 4.1
	0.4	3.89 ± 0.23	2.81 ± 0.11	4.56 ± 0.10	11.99 ± 0.42	89.3 ± 41.1
	4	3.21 ± 0.18	2.55 ± 0.07	4.59 ± 0.06	11.08 ± 0.22	84.4 ± 30.0
	40	3.55 ± 0.20	2.79 ± 0.04	4.58 ± 0.07	11.65 ± 0.11	148.9 ± 56.3
Treatment						
	0	4.12 ± 0.08	2.92 ± 0.07	4.80 ± 0.02	13.19 ± 0.17	31.6 ± 3.1
	0.4	3.96* ± 0.16	2.96 ± 0.08	4.63 ± 0.05	12.37 ± 0.28	144.1 ± 48.0
	4	3.73 ± 0.12	2.71 ± 0.05	4.60 ± 0.04	11.86 ± 0.18	90.0 ± 15.7
	40	3.62 ± 0.06	2.86 ± 0.04	4.53 ± 0.05	11.84 ± 0.10	201.0 ± 45.1
Posttreatment						
	0	4.12 ± 0.17	3.17 ± 0.06	4.99 ± 0.07	12.77 ± 0.23	27.1 ± 8.2
	0.4	3.94 ± 0.36	3.09 ± 0.17	4.62 ± 0.10	12.57 ± 0.57	568.3 ± 356.9
	4	3.29 ± 0.18	2.86 ± 0.07	4.82 ± 0.06	11.86 ± 0.28	53.5 ± 12.3
	40	3.57 ± 0.20	2.98 ± 0.02	4.72 ± 0.10	12.18 ± 0.22	262.9 ± 112.2

Values are for four cows per treatment group during three experimental periods. Pretreatment means ($n = 8$ per treatment) are for 2 weeks prior to perchlorate infusion. Treatment means ($n = 20$ per treatment) are means for the 5-week infusion period. Posttreatment means ($n = 8$ per treatment) are for 2 weeks after perchlorate infusion. Effect of perchlorate infusion was evaluated as a compositional change from the pretreatment to treatment period relative to the change for control cows. Data are means ± SE.
*Significance, $P < 0.05$.

perchlorate transport (27), but dietary perchlorate may significantly reduce iodide transport into milk (24).

Despite this potential effect, we did not detect an effect of perchlorate intake on the iodide content of milk ($P > 0.05$). This finding is consistent with earlier radiotracer studies in cows indicating that impaired iodide transfer to milk did not occur at perchlorate exposures <0.1 mg/kg per day (28). Additionally, the abundance of dietary iodine or use of an iodine-based teat dip as a mastitis prophylactic may have precluded an effect of perchlorate on milk iodide in our study. The teat dip may contribute to milk iodine and minimize potential effects of perchlorate. Prevalent use of iodine teat dips in commercial dairies may largely protect against a potential perchlorate-induced reduction in the iodine content of commercial milk.

Evaluation of perchlorate mass balance suggests that it is metabolized within the gastrointestinal tract. The total output of perchlorate was as low as 17% of total intake. There is no evidence for the accumulation of perchlorate in tissues or for the organification or metabolism of perchlorate by animal tissues (29, 30). However, within the anaerobic environment of the cow's rumen, microbes can likely reduce perchlorate through a series of enzymatic conversions from (ClO_4^-) to chlorate (ClO_3^-) to chlorite (ClO_2^-) to chloride (Cl^-). The existence of anaerobic bacteria expressing perchlorate reductase and capable of these metabolic conversions is well documented (31), and it seems likely that the complex microbial flora of the rumen would have this capacity.

Further support for this concept is obtained by evaluation of the partitioning of perchlorate between the blood and milk, urine, or feces. As with iodine, the renal clearance capacity for perchlorate is likely to be limited only by the glomerular filtration rate, and, therefore, the kidney is capable of high rates of clearance and maintenance of a consistent partitioning coefficient between blood and urine, despite increasing perchlorate loads. However, the mammary gland's inability to maintain the same ratio of perchlorate concentration across the blood–milk barrier as the perchlorate load increased may be due to limitations imposed by the V_{max} of membrane transport or down-regulation of the NIS at higher perchlorate concentrations (analogous to the inhibition of iodide uptake and its organification by the thyroid that occurs with high iodide intake) [Wolff–Chaikoff effect (10)]. Although the partitioning coefficients for milk and feces are both reduced with increasing perchlorate load, mechanistically, these effects differ because ion transport is from the blood in the first instance and to the blood in the latter. Therefore, a decline in the feces partitioning coefficient would not reflect reduced membrane transport of perchlorate out of the gut. We suggest that the decline reflects a reduced supply of perchlorate to the lower gastrointestinal tract due to perchlorate metabolism in the rumen.

With significant metabolism of perchlorate in the rumen, we infer that a lactating woman or monogastric animal exposed to the same dietary levels of perchlorate as a ruminant animal would secrete more perchlorate into maternal milk, because they lack the appropriate microbial flora to reduce perchlorate. This inference is consistent with data from a survey of cow and human milk that indicated that concentrations of perchlorate in human milk were five times greater than concentrations in bovine milk (10 ppb vs. 1.8 ppb), although neither perchlorate nor iodide intakes were reported (32). Thus, the rumen of the dairy cow may serve as a biological filter to reduce transfer of perchlorate to milk and reduce transfer to the environment by decreasing urinary and fecal output of perchlorate.

Even at the highest dose of perchlorate (76.1 μg/kg per day), more than 100-fold greater than the National Research Council reference dose (RfD) (11), concentrations of thyroid hormones in the circulation were not reduced. Certainly, mammalian species exhibit differing sensitivities to perchlorate, based largely on the thyroid's storage capacity. Although the exposure period in this study was limited to 5 weeks, this should have been ample time to observe a decline in thyroid hormone concentrations, were iodide uptake by the thyroid impaired. A significant decline in the concentration of thyroid hormones was evident in the serum of cattle 3 weeks after treatment with low doses of another goitrogen, propylthiouracil (PTU) (33). Because PTU inhibits iodide uptake by the thyroid and conversion of T4 to T3, the decline in serum T4 due to inhibition of iodide uptake by the thyroid was actually preceded by increased serum T4 due to inhibition of T4 to T3 conversion (33). We conclude that the maximum perchlorate exposure in our study was insufficient to reduce synthesis of thyroid hormones.

When the relationship between SCC and perchlorate levels in milk was evaluated by using composite milk (combined from the four mammary glands of the udder) and milk from each mammary gland, the Pearson correlation coefficients were −0.005 ($P = 0.89$) for composite samples and −0.00572 ($P = 0.8822$) for individual mammary gland milk samples, suggesting that somatic cells do not increase perchlorate concentrations in the milk by virtue of their

metabolic activities or affect milk concentrations by disrupting the blood–milk barrier during diapedesis of leukocytes from the blood into the milk. However, because the observable range of SCC was limited, this conclusion is tentative.

Because of its potential impact on tissue iodine and thyroid hormone concentrations, possible effects of perchlorate on mammary gland physiology are worthy of investigation. Iodide may itself play a role in mammary gland health and physiology because of its antioxidant function (34). Additionally, thyroid hormones are important regulators of general body metabolism and mammary gland function. *In vivo* studies as early as 1934 demonstrated that thyroid hormone administration can increase milk production in dairy cows, and *in vitro* studies have shown that T3 potentiates the activity of other lactogenic and galactopoietic hormones (35, 36). Organ-specific changes in thyroid hormone metabolism occur during lactogenesis that may facilitate adaptation to a lactational state by promoting differential rates of energy utilization and partitioning of nutrients to the mammary gland (15, 37, 38). However, mammary gland function and health were not affected by perchlorate intake.

Reduced uptake of iodide by the thyroid ultimately causes a reduction in the biosynthesis of thyroid hormones (10). Through negative feedback, decreased concentrations of thyroid hormones in the circulation cause an increase in thyroid stimulating hormone (TSH). The potential impact of perchlorate on thyroid hormones has raised concern about perchlorate in the food and water supply and has precipitated a recent review by the National Academy of Science (11). Because thyroid hormones are essential for normal brain development, exposure of pregnant women and young children to perchlorate should be kept below conservative limits that are derived from scientific data (11). Additionally, individuals who are iodine-deficient have reduced reserves of iodinated hormones and are more sensitive to perchlorate inhibition of thyroid hormone synthesis. Although some have expressed concern about potential risk from increased concentrations of TSH, it has generally been deemed a very low risk (39).

Our experiment establishes a relationship between perchlorate intake and perchlorate concentrations in milk. Equilibration of dietary perchlorate among body fluids was rapid, as was clearance after perchlorate withdrawal. At the dosages evaluated, perchlorate did not affect animal health, including thyroid hormone concentrations, hematological profile, and mammary gland health. Particularly noteworthy is strong suggestive evidence that the rumen metabolizes a substantial proportion of the perchlorate consumed, suggesting that ruminants may serve as biofilters. Finally, perchlorate intake did not decrease iodine content of bovine milk. These experimental findings provide information necessary to determine the impact of dietary perchlorate on concentrations of perchlorate and iodine in bovine milk and provide an appropriate milk clearance time after significant perchlorate exposure of dairy cows.

Our data also serve as a resource for assessing environmental influences on milk perchlorate levels and provide a context for assessing the potential for impacting human health. In its recent review of health implications of perchlorate ingestion, the National Research Council determined an RfD of 0.7 μg of perchlorate per kg of body weight per day. This RfD was derived to protect the most at-risk populations, pregnant women and fetuses, infants, and adults with low iodine intake and borderline thyroid function. Applying this RfD, a 10-kg child would not be at any risk from ingestion of 7 μg of perchlorate daily. Based on measures in our study, this quantity of perchlorate would only be reached in 1,600 ml of milk (4.4 ng of perchlorate per ml) obtained from cows consuming a total of 0.9 μg of perchlorate per kg per day (diet and water without infusion) or 636 ml of milk containing the highest quantity of perchlorate assayed in commercial milk (11.3 ng/ml; for 101 samples, mean $\pm$ SE was 5.8 $\pm$ 0.6, www.cfsan.fda.gov/~dms/clo4data.html). Because $\approx$400 ml of milk would be required to fully satisfy the daily protein and calcium requirement of a 10-kg child (www.bcm.edu/cnrc/consumer/archives/factsanswers.html), the child's nutritional needs for calcium and protein could be met without exceeding the RfD for perchlorate. A 60-kg pregnant woman could consume in excess of 3.7 liters of milk containing 11.3 ng of perchlorate per ml without exceeding the RfD, and this quantity of milk would be well in excess of average daily consumption. By limiting perchlorate content of the dairy cow's diet, human exposure to perchlorate in milk can be kept to a minimum.

When deriving the RfD for perchlorate, lack of available data did not permit accounting for perchlorate exposure in the human diet (11). Recent studies suggest that a basal level of perchlorate in the environment may be expected (5), which could result in detectable quantities of perchlorate in the human diet. Indeed, perchlorate has been detected in human milk and urine (6, 32). The extent to which this may be a result of natural exposure rather than environmental contamination may be assessed by monitoring perchlorate content of human urine and its isotopic composition (5, 6).

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