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Tissue Distribution, Elimination, and Metabolism of Sodium [^{36}Cl]Perchlorate in Lactating Goats

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Perchlorate has contaminated water sources throughout the United States but particularly in the arid Southwest, an area containing large numbers of people and few water sources. Recent studies have demonstrated that perchlorate is present in alfalfa and that perchlorate is secreted into the milk of cows. Studies in lactating cows have indicated that only a small portion of a perchlorate dose could be accounted for by elimination in milk, feces, or urine. It was hypothesized that the remainder of the perchlorate dose was excreted as chloride ion. The purpose of this study was to determine the fate and disposition of ^{36}Cl-perchlorate in lactating dairy goats. Two goats (60 kg) were each orally administered 3.5 mg (16.5 μCi) of ^{36}Cl-perchlorate, a dose selected to approximate environmental perchlorate exposure but that would allow for adequate detection of radioactive residues after a 72 h withdrawal period. Blood, milk, urine, and feces were collected incrementally until slaughter at 72 h. Total radioactive residue (TRR) and perchlorate concentrations were measured using radiochemical techniques and liquid chromatography mass spectrometry (LC−MS−MS). Peak blood levels of TRR occurred at 12 h (∼195 ppb) postdose; peak levels of parent perchlorate, however, occurred after only 2 h, suggesting that perchlorate metabolism occurred rapidly in the rumen. The serum half-life of perchlorate was estimated to be 2.3 h. After 24 h, perchlorate was not detectable in blood serum but TRR remained elevated (160 ppb) through 72 h. Milk perchlorate levels peaked at 12 h (155 ppb) and were no longer detectable by 36 h, even though TRRs were readily detected through 72 h. Perchlorate was not detectable in skeletal muscle or liver at slaughter (72 h). Chlorite and chlorate were not detected in any matrix. The only radioactive residues observed were perchlorate and chloride ion. Bioavailability of perchlorate was poor in lactating goats, but the perchlorate that was absorbed intact was rapidly eliminated in milk and urine.

KEYWORDS: Perchlorate; total radioactive residue; liquid chromatography mass spectrometry; lactating dairy goats; absorption, distribution, metabolism, and excretion (ADME)

INTRODUCTION

Perchlorate occurs naturally in Chilean nitrate deposits used for fertilizers (*1*, *2*) and in North American potashes, playa crusts, and hankites (*3*). However, the discovery that large volumes of ground and surface waters were contaminated by perchlorate used for the manufacture of solid rocket fuel prompted concern over chronic health affects of low-level perchlorate exposure in humans (*4*). Apprehensions regarding perchlorate contamination caused the U.S. Environmental Protection Agency (U.S. EPA) to include perchlorate on the Drinking Water Candidate Contamination List (*5*) and resulted in the monitoring of perchlorate throughout the United States (*6*).

During the past decade, perchlorate has also been detected in ground and surface waters not associated with industrial point sources (*7*, *8*). In addition, perchlorate has been measured in food sources, such as vegetables (*9*, *10*), and both human (*11*) and bovine milk (*11*, *12*) throughout North America. In water, perchlorate has been detected in at least 25 different states (*13*). Water concentrations of perchlorate are typically low (i.e., less than 4 ppb; *8*), but groundwater from contaminated sites may contain perchlorate concentrations measured in hundreds of parts per million (*14*). Of the 47 bovine milk samples analyzed from 11 states, 46 of the samples contained from 0.4 to 11 ppb perchlorate (mean = 2.0 ppb) (*11*). Of the 36 human milk samples collected in 18 states, all contained perchlorate at concentrations ranging from 1.4 to 92.5 ppb (mean = 10.5 ppb) (*11*). The source(s) of perchlorate in human and bovine milk are unknown.

Exposure to perchlorate is of potential health concern because large doses of perchlorate competitively inhibit the thyroidal sodium iodide symporter (NIS), which transports iodide into the thyroid (*15*). Inhibition of the NIS may reversibly lower the production of thyroid-stimulating hormone (TSH). In most populations, a lowering of TSH levels is uneventful (*16*), but it

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has been hypothesized that perchlorate could adversely affect the pituitary−thyroid axis in highly sensitive populations. Such populations are proposed to be developing fetuses, developing fetuses in marginally iodine-deficient women, and infants (*16*, *17*), with neurological abnormalities defining adverse outcomes.

In the United States, the greatest exposure to perchlorate has been hypothesized to be through drinking water and agricultural products, but prenatal dietary supplements were recently demonstrated to contain as much as 2420 ppb perchlorate (*18*). For infants, breast or dairy milk could be a source of perchlorate exposure (*11*, *12*).

Capuco et al. (*19*) estimated that dairy cattle biotransform 80% of the perchlorate that they are exposed to, thereby effectively reducing the amount of perchlorate transferred to milk after oral exposure. Because perchlorate is present in the environment, drinking water, animal forage, and milk of humans and animals and because milk could be a primary rout of perchlorate exposure for infants, we initiated an absorption, distribution, metabolism, and excretion (ADME) study in a lactating ruminant using ^{36}Cl-labeled perchlorate. The dose (3.5 mg; 16.6 μCi/goat) was selected on the basis of the minimal amount of radiochlorine that could reasonably be expected to be administered to goats and still have radiochemical detection in tissues after a 72 h study period.

MATERIALS AND METHODS

Chemicals. Unlabeled sodium perchlorate (CAS number 7601-89-0) was obtained from Sigma Chemical Co (St. Louis, MO). Sodium ^{18}O-perchlorate (99 atom %) was purchased from Icon Isotopes (Summit, NJ). Unlabeled sodium chlorate (CAS number 7775-09-9) was obtained from EKA Chemicals (Columbus, MS). Sodium chloride (VWR, West Chester, PA), heparin and sodium salt (Sigma Chemical Co., St. Louis, MO), sodium hydroxide (50% solution for ion chromatography; Fluka Chemical Corp., Milwaukee, WI), Ultima Gold liquid scintillation fluid, Carbosorb-E, and Permafluor-E (Perkin-Elmer Life and Analytical Sciences, Boston, MA), and acetonitrile and methanol [high-performance liquid chromatography (HPLC) grade; EM Science, Gibbstown, NJ] were obtained from well-known vendors as indicated.

Radiolabeled Chemicals. Sodium chlorate ($Na^{36}ClO_3$), having a specific activity of 0.575 mCi/mmol was purchased from Ricerca Biosciences (Concord, OH). The aqueous radioactive sodium chlorate stock solution was stored in a refrigerator (<4 °C). The purchased radiolabel was roughly 51 atom % ^{36}Cl. Radiolabeled sodium [^{36}Cl]perchlorate was synthesized and purified as described by Ruiz-Cristin (*20*) with some modification. Briefly, approximately 1000 μCi of an aqueous $Na^{36}ClO_3$ solution (3 mL) was transferred to a 20 mL Pyrex beaker, and the beaker was set within a Plexiglas box (approximately 16.5 × 16.5 × 17 cm; $l \times w \times h$). Inner (approximately 8 mm coil diameter) and outer (approximately 20 mm coil diameter) platinum electrodes were placed within the $Na^{36}ClO_3$ solution (**Figure 1**). The inner and outer electrodes were mounted onto a Plexiglas frame that set within the inner walls of the Plexiglas box and that rested lightly on the top of a Pyrex beaker. The Plexiglas box sat upon a stir plate, and a small stir bar was placed in the electrolysis beaker with sufficient clearance between the electrodes and the stir bar to prevent contact. The reaction chamber was sealed with a tight-fitting Plexiglas lid, and the chamber flushed lightly with air during the electrolysis. Air leaving the chamber was bubbled through a trap containing 1 N NaOH to trap any chlorine gas that formed. Electrolysis was allowed to proceed for approximately 22.5 h at 5 V; amperage ranged from 690 to 1480 mA. Temperature was not controlled, but additional water was added at 2, 9, and 15 h. The perchlorate content of the reaction mixture was 0.5, 6.5, 11.7, and 17.4% of the total radioactivity at 0, 9, 15, and 22.5 h, respectively, as determined by ion chromatography with radiochemical detection.

Electrolysis products were purified over a 2.5 × 63 cm column of Sephadex G-10. The reaction mixture was loaded onto the column head and eluted at a flow rate of 0.65 mL/min; eluent was collected into 265 fractions at 5.7 min intervals. Each fraction was assayed (5 μL) for radioactivity. Under these conditions, [^{36}Cl]chloride eluted in fractions 41−50 (3.2% of the total activity), [^{36}Cl]chlorate eluted in fractions 53−76 (79.9% of the total activity), and [^{36}Cl]perchlorate eluted in fractions 164−243 (16.9% of the total radioactivity). Perchlorate fractions were pooled; the solvent was evaporated; and the residue was dissolved in purified water and transferred to a 10 mL volumetric flask for storage. Radiochemical purity of the perchlorate was >99.9% as determined by ion chromatography with radiochemical detection. The specific activity of the [^{36}Cl]perchlorate was 10 420 dpm/μg as calculated from the specific activity of the [^{36}Cl]chlorate starting material.

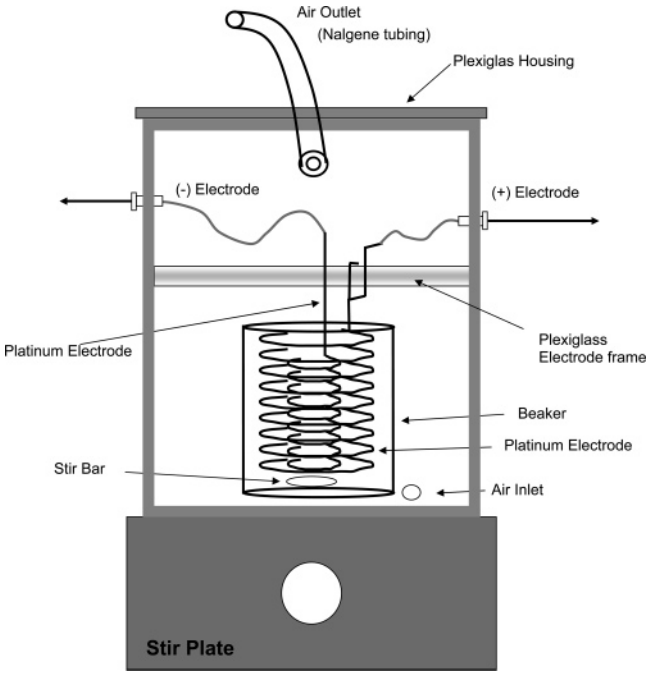

Figure 1. Design of the electrolysis cell used for the synthesis of [^{36}Cl]perchlorate.

General LCS Techniques. Background radiochlorine and limits of quantitation (LOQ) were determined for individual matrices (i.e., urine, liver, kidney, skeletal muscle, and adipose tissue) as described by Smith et al. (*21*) for [^{36}Cl]chlorate in beef cattle. Individual samples within a matrix set were counted for 10−20 min each. Radiochlorine was quantified using Beckman LS1701 (Fullerton, CA) or Packard 2550 (Meriden, CT) liquid scintillation counters, with energy windows set at 97−449.5 keV. This energy window was used after running an instrument-programmed optimization procedure using a ^{36}Cl standard. Sample quench was corrected using using the H# (Beckman) or tSIE (transformed spectral index of the external standard; Packard) options for each instrument. Each instrument was calibrated weekly using sealed radiochlorine standards (Analytics, Inc., Atlanta, GA) prepared in Ultima Gold liquid scintillation fluid.

Radiochemical Purity Determinations and Dose Preparation. Duplicate injections of a dilution of the purified [^{36}Cl]perchlorate solution were made onto sequential Dionex (Sunnyvale, CA) AG- and AS-16 guard and analytical columns. The solvent (50 mM NaOH) was delivered at a flow rate of 1.0 mL/min using a Waters (Milford, MA) model 600E pump and controller equipped with Teflon pump heads and a Rheodyne (Cotati, CA) model 97251 PEEK injector. Samples were injected using a Hamilton (Reno, NV) 50 μL syringe. Radioactivity eluting from the column was detected with a Packard Model 500 TR flow scintillation analyzer (10−790 keV energy window; Packard, Meriden, CT) using UltimaFlow AP scintillation fluid at a flow rate of 2 mL/min. The progress of the [^{36}Cl]perchlorate synthesis was determined using the same column conditions as described above.

Animals. Two lactating Alpine goats (60 kg), purchased from Poplar Hill Dairy Goat Farm (Scandia, MN), were delivered to the barn

facilities and allowed to acclimate for 20 days prior to dosing. Animals were allowed ad libitum access to an alfalfa-grass hay mixture and water from delivery to completion of the study. In addition, each goat received approximately 250 g of a mixture of ground cob corn and rolled oats (50:50) at each milking. Goats were milked daily at 0700 and 1900; mean daily milk production during the 20 day adaptation period was 1.8 ± 0.1 and 1.9 ± 0.2 kg for goats 141 and 142, respectively. During the adaptation period, each goat was trained to metabolism crates that allowed the separation of urine and feces (22; 0.5 × 1.0 × 1.2 m; $w \times h \times l$). On the morning of dosing, the jugular vein of each goat was catheterized, and catheters were filled with a 100 units/mL solution of sodium heparin until sampling. Control milk samples were obtained prior to dosing goats. A nonlactating female goat (22.2 kg) was used as a source of control tissue for determination of background activities for individual tissue matrices.

Dose Preparation and Dosing. For each goat, sodium [^{36}Cl]-perchlorate (1080 μL, 16.6 μCi, 3.53 mg) was transferred into a gelatin capsule containing ground cob corn and oats, the capsule was capped, and each capsule was frozen. Goats 141 and 142 weighed 59.3 and 60.7 kg at dosing, resulting in sodium perchlorate doses of 59.5 and 58.1 μg/kg of body weight, respectively. Immediately prior to dosing, each goat was milked. At dosing, each gelatin capsule was lubricated with vegetable oil, placed in a balling gun, and administered to the appropriate goat. Dosing was uneventful.

Collection of Excreta, Milk, and Blood. Urine was collected in intervals of 0−4, 4−8, 8−12, 12−24, 24−36, 36−48, 48−60, and 60−72 h after dosing. Milk and feces were collected in 12 h intervals. Control milk was collected from each goat prior to dosing. Blood samples were collected into heparinized tubes from an indwelling jugular catheter prior to dosing and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 24, 36, 48, 60, and 72 h postdosing. One aliquot (10 mL) of blood was collected into a heparanized tube, mixed, and frozen, while another aliquot (10 mL) was collected in a glass tube and allowed to clot, and serum was collected after centrifugation.

Slaughter and Tissue Collection. At 72 h postdosing, each goat was stunned, elevated, and exsanguinated. Blood was collected into basins in which a heparin solution had been added. Goats were eviscerated, and blood, brain, liver, kidney, adipose tissue, skeletal muscle, lung, spleen, mammary gland, heart, thyroid, skin, bone, bile, and the remainder of the carcass were harvested and weighed. The gastrointestinal tract was dissected into ruminal (rumen, reticulum, omasum, and abomasums) and intestinal (duodenum, jejunum, ileum, large intestine, and cecum) segments. Ruminal and intestinal contents were separated from the tissues and were weighed separately. A "remainder of the carcass" fraction was collected that contained tissue scraps and tissue not associated with any one organ or tissue type. Skeletal muscle was dissected from the carcass, pooled and weighed. The summed weights of tissues collected at slaughter were 97.6 and 96.5% of the live weights of goats 141 and 142, respectively. Subsamples of the skin, bone, and ground skeletal muscle were removed and frozen.

Cage Wash. Each metabolism crate was washed with water, and the water was collected. Radiochemical analyses were conducted by weighing quintuplicate 1 mL aliquots of cage wash samples into glass liquid scintillation counting (LSC) vials and diluting with 15 mL of Ultima Gold LSC fluid. Each sample was counted for 20 min on an LSC counter.

Tissue Processing. All tissues, except the thyroid, skin, and bone, were ground twice the day of slaughter and were promptly frozen. The thyroid was diced, placed in a mortar, and subsequently homogenized in liquid nitrogen with a pestle. Bone was broken into small pieces with a hammer; small pieces were placed in a mortar and subsequently homogenized in liquid nitrogen with a pestle. Skin was digested for 3 days at 50 °C in 1 M sodium hydroxide using a 1:3 ratio of skin/NaOH (wt/wt).

Measurement of Total Radioactive Residues. Total radioactive residues were measured in tissues (except for skin), whole blood, bile, and urine as described by Smith et al. (*21*). The sample size for radiochemical analyses of tissues was 0.2 g, except for skin, ruminal fluid, GI contents, and bile, which had 0.08, 0.1, 0.1, and 0.05 g, respectively. Background radioactivity was determined for each matrix, and all samples were allowed to dark-adapt for a minimum of 1 h prior to counting on the LSC. Feces (1 g) were weighed into vials; 10 mL of water were added; and the feces were extracted into water overnight on a rolling mixer (Denley Spiramix 10, Essex, U.K.). Tubes were centrifuged (27000*g*) for 15 min, and the supernatants were decanted onto conditioned (methanol followed by water) C18 Bond Elut columns (2 g sorbent; Varian, Harbor City, CA) to remove highly quenching pigments. Aqueous eluents were collected into a vial along with a 5 mL water rinse of each column, and 0.5 g aliquots were assayed for radioactivity.

Radioactive residues in skin were measured by pouring digested skin samples into 500 mL volumetric flasks, diluting the flasks to the mark with water, and mixing thoroughly. Aliquots (1 mL) of the diluted skin digests (equivalent to 0.08 g of intact skin) were placed in glass LSC vials, diluted with 15 mL of LSC fluid, and counted for 20 min each. Background radioactivity was determined by digesting, diluting, and LSC of skin samples from the control goat. Serum and bile samples (0.1 and 0.05 mL, respectively) were weighed into glass LSC vials, diluted with 15 mL of LSC fluid, and counted (20 min) directly.

Milk samples (1 mL) were weighed directly into glass LSC vials and counted for 20 min each after the addition of 15 mL of Ultima Gold LSC fluid. To ensure that total radioactive residue (TRR) in milk could be directly counted in LSC fluid, sample sets containing 0, 0.1, 0.250, 0.5, and 1.0 mL of control goat milk were each fortified with 50 μL of solutions containing 5, 10, 25, 50, 100, 500, 1000, or 2000 dpm of radiochlorine (as $^{36}Cl^-$). Recoveries of radioactivity for each milk volume radiochlorine-fortification combination were then calculated after background radioactivity was subtracted. Recoveries of radiochlorine at the 5 and 10 dpm fortifications (across all milk volumes) were 90 and 87.5%, respectively; all other recoveries were 95% or greater, indicating that total residues in milk could be quantified to quite low levels. The calculated limit of detection for milk total radioactive residues was 0.4 ppb. The detection of low levels of radiochlorine in other matrices (urine and tissues) was validated previously (*21*) and, for this study, averaged to 2.4 ± 0.9 ppb perchlorate equivalents for solid tissue samples (0.08−0.2 g sample sizes), 13 ppb for ruminal fluid (0.1 g sample), 8 ppb for GI contents (0.1 g sample size), and 6 ppb for bile (0.05 g sample size). Because of the highly quenching nature of fecal material, only 0.05 g samples were analyzed for total radioactive residues, with a resulting LOQ of 12.0 ± 1.8 ppb perchlorate equivalents.

Speciation of Residues. Determination of urinary and milk perchlorate was initially attempted using ion chromatography with radiochemical detection (i.e., a Dionex AS 16 HC column eluted with 50 mm NaOH at 1 mL/min, with detection via a Packard 500 TR flow analyzer). At early time points (12 h time points and earlier), radiochemical peaks corresponding to ^{36}Cl-chloride and/or ^{36}Cl-perchlorate were clearly present; however, the integration of perchlorate peaks was highly variable because of the inherent insensitivity of flow-through detectors when fairly low counts are introduced. Although chloride peaks were easily detected at later time points (after 12 h), perchlorate signals were too low to reliably quantify. Thus, total radioactive residues are reported on the basis of LSC, and perchlorate residues are reported on the basis of LC−MS−MS analyses.

LC−MS−MS Analyses. Perchlorate in milk, serum, and urine was determined by the $Cl^{18}O_4^-$ internal standard method of Krynitskey et al. (*23*) as described by Capuco et al. (*19*), except that C18 Bond-Elut SPE columns were used in place of Supleclean Envi-Carb columns and a tandem quadrapole time-of-flight (QTOF) mass spectrometer (Micromass, Milford, MA) was used for detection of perchlorate (negative ion elecrospray; 35 V cone voltage; 5.0 eV collision energy; 120 °C source temperature, 400 °C desolvation temperature; 40 L/h cone gas flow; 500 L/h desolvation gas flow). For the serum analysis, 0.5 mL samples were used instead of 5 mL samples and the final dilution volume was 8 mL rather than 40 mL. The source [^{36}Cl]chlorate from which [^{36}Cl]perchlorate was synthesized had a specific activity of 0.575 mCi/mmol and was not 100 atom % ^{36}Cl, but it was of sufficient specific activity that the chlorine-36 isotopic peak (*m*/*z* 99.95) was approximately 2 times the size of the chlorine-35 and -37 isotope peaks (**Figure 2**). Although perchlorate ions arising from chlorine-35, -36, and -37 were all collected, quantitation was based on the chlorine-

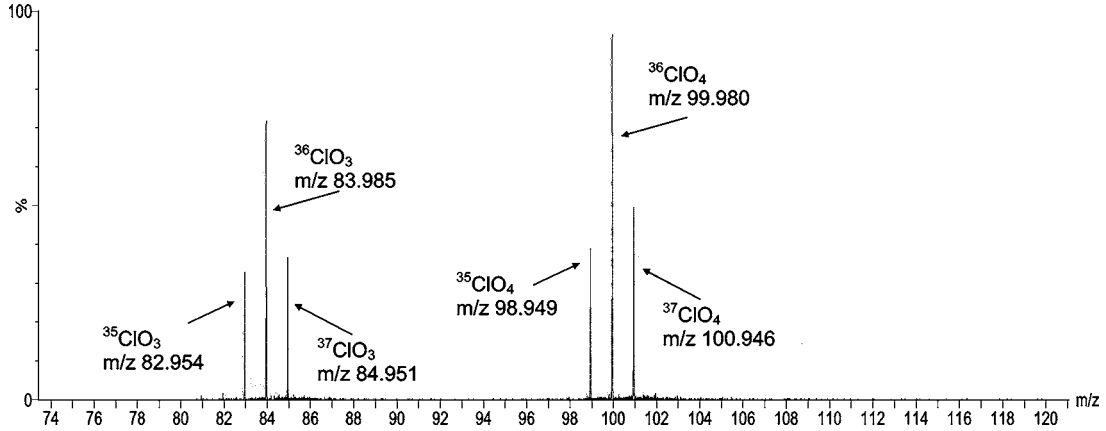

Figure 2. Mass spectrum of ^{36}Cl-labeled perchlorate.

36 isotope peak, by virtue of increased selectivity and sensitivity (relative to the chlorine-35 and -37 isotopes).

RESULTS

Disposition of Radiochlorine. **Table 1** shows the recovery of radiochlorine in the urine, feces, milk, and tissues of goats after oral administration of [^{36}Cl]perchlorate. Milk was a major route for the elimination of radioactivity (12.6−15.6% of the dose), surpassing the excretion of radioactivity in urine (8.1−14.8% of the dose) and feces (<1% of the dose). Fecal radioactivity was detected only during the 12−24 h collection period.

Milk always contained greater than 1% of the dosed radioactivity, regardless of the collection period (**Table 1**). Roughly 53% of the radioactivity present in milk was excreted during the initial 24 h period. Excretion of radioactivity in milk (as a percentage of the dose) was relatively constant after 24 h; likewise, most (73−93%) of the radioactivity excreted in urine was excreted during the 24 h period immediately following dosing, with an almost constant excretion of radioactivity thereafter.

The majority of the dosed radiochlorine (55.3%) remained in tissues at the 72 h slaughter time. Major fractions of radioactivity were present in blood, carcass remains, skeletal muscle, and skin. These tissue compartments contained large amounts of radioactivity by virtue of their relatively large masses rather than because of high tissue concentrations of radioactivity. Tissues with the greatest concentrations of radiochlorine were blood, kidney, and skin (1422, 1108, and 927 dpm/g, respectively).

Total radioactive residues in whole blood and in serum are shown in **Figure 3**. Absorption of radioactivity was rapid and increased in a near linear manner until peak levels were reached at about 12 h; thereafter, total radioactive residues decreased very slowly, with a only 13% drop in TRR from 12 to 72 h. Linear regression of the 12−72 h serum TRR data from both animals [serum TRR = −0.53(hours) + 200 ppm; $R^2 = 0.69$] suggested a serum half-life for TRR of 196 h. At each time point, the serum always contained greater concentrations of radioactivity than the whole blood. The ratio of TRR in serum relative to whole blood was very consistent across all time frames (1.16 ± 0.04, mean ± standard deviation), suggesting that the chemical composition of the radioactivity in the blood was consistent across time.

Speciation of Total Residues. Figure 4 shows serum levels of parent perchlorate in goats. The absorption of perchlorate was rapid because perchlorate concentrations had peaked by 2 h after dosing. Depletion of parent perchlorate was rapid, with concentrations near LOQ (0.5 ppb) by 24 h. When the perchlorate depletion curves (ie., data from 2 h and later) were fit to a monoexponential decay curve ($C = C_2e^{-Kt}$, where C is the perchlorate concentration, C_2 is the perchlorate concentration at 2 h, K is the rate constant, and t is time), the 2−72 h perchlorate depletion curve had a half-life of 2.3 h ($R^2 = 0.944$; 95% confidence interval of 1.7−3.7 h).

Concentrations of perchlorate in urine and milk are shown in **Table 2**. Urinary perchlorate concentrations ranged from nondetectable (<0.5 ppb) to 698 ppb, with the greatest concentrations being present in the initial urine collection after dosing. Goat 142 did not urinate during the initial 8 h of the study; perchlorate concentrations in the urine collected 8−12 h after dosing contained 698 ng/mL perchlorate. Greater than 80% of the radioactive residue eliminated in urine during the first 12 h of the study was perchlorate, and greater than 50% of the residue eliminated in the 12−24 h period was perchlorate; thereafter, urinary perchlorate declined very rapidly to levels near or below levels of detection. For both goats, urinary perchlorate concentrations, as measured by mass spectrometry, during the initial 12 h of the study were greater than urinary TRR. Because the error (expressed as percentage coefficient of variation, % COV) associated with the replicate radiochemical analysis of the urine was 1% or less, and the average error associated with the mass spectral analysis of the same samples was 14.3%; urinary concentrations of perchlorate residues were adjusted to 100% of the total radioactive residue for goat 141 at 0−4 and 4−8 h and goat 142 at 8−12 h. The total mass of perchlorate excreted in urine was 5.5 and 14.1% of the dose for goats 141 and 142, respectively.

Perchlorate in milk was depleted within 24 and 36 h, respectively, for goats 142 and 141, respectively. Peak milk concentrations (155 ng/mL) were present in milk collected 12 h after dosing. Expressed in terms of the total dose, 3.9 and 4.6% of the dose was excreted in milk as parent perchlorate by goats 141 and 142, respectively. In contrast to urine, milk consistently contained some residue present as ^{36}Cl-chloride, even at the earliest time points.

DISCUSSION

Total recovery of radioactivity was only about 81% of the administered dose. Normally, one would expect greater recoveries in a balance-excretion study of this type. In the present study, it is likely that the poor recoveries are somewhat artifactual. Unlike studies employing tritium or 14-carbon, radiochlorine in feces, intestinal fluids, bone, and muscle could not be determined using combustion analysis because of the incomplete volatilization of inorganic chlorine salts (*24*) at temperatures

Table 1. Disposition and Recovery of Radioactivity in Goats Orally Dosed with 60 μg/kg of Body Weight of [^{36}Cl]Perchlorate[a]

fraction	time (h)	goat 141 (%)	goat 142 (%)	average (%)
milk	0–12	4.4	6.1	5.2
	12–24	2.2	2.0	2.1
	24–36	1.6	2.0	1.8
	36–48	1.5	1.9	1.7
	48–60	1.4	1.7	1.5
	60–72	1.5	1.9	1.7
totals		12.6	15.6	14.1
urine	0–4	2.5	*b*	1.3
	4–8	1.4	*b*	0.7
	8–12	1.0	12.2	6.6
	12–24	1.0	1.5	1.3
	24–36	0.4	0.3	0.4
	36–48	0.5	0.3	0.4
	48–60	0.6	0.2	0.4
	60–72	0.7	0.3	0.5
totals		8.1	14.8	11.5
feces	0–12	NDR[c]	NDR	0.0
	12–24	0.2	0.4	0.3
	24–36	NDR	NDR	0.0
	36–48	NDR	NDR	0.0
	48–60	NDR	NDR	0.0
	60–72	NDR	NDR	0.0
totals		0.2	0.4	0.3
tissue				
adipose		0.1	0.2	0.2
bile		0.1	<0.1	<0.1
blood		9.1	7.6	8.4
bone		3.9	3.7	3.8
brain		0.2	0.2	0.2
carcass remains		8.6	3.4	6.0
GI tissue		2.7	2.5	2.6
GI contents		2.6	1.7	2.1
heart		0.5	0.5	0.5
kidney		0.5	0.6	0.5
liver		1.0	0.9	0.9
lungs		1.3	1.9	1.6
mammary gland		3.5	2.5	3.0
rumen tissue		2.7	2.0	2.3
rumen contents		1.3	1.8	1.6
skeletal muscle		8.4	13.4	10.9
skin		10.5	10.2	10.4
spleen		0.2	0.2	0.2
thyroid		<0.1	<0.1	<0.1
totals		57.2	53.4	55.3
cage wash		0.0	0.0	0.0
total recovery		78.0	84.3	81.1

[a] LOQ (in ppb of perchlorate equivalents) of total radioactive residues for milk, urine, and tissues were 0.4, 2.0, and 2.4, respectively. LOQs for radioactive residues in ruminal fluid, GI contents, and bile were 13, 8, and 6 ppb, respectively. [b] No urine was excreted during this time period. [c] NDR, no detectable residue, detection limit for radiochlorine in feces was 12.0 ± 1.8 ppb of perchlorate equivalents.

typical of tissue oxidizers. Radiochlorine in tissues present in this study was lightly digested in a basic quarternary alkyl-amine and assayed in a compatible liquid scintillation fluid. These techniques were validated for ^{36}Cl using cattle tissues (*21*), and use of this technique has led to acceptable recoveries of radiochlorine in studies using [^{36}Cl]chlorate in cattle (*21*) and swine (*35*), albeit with much greater doses of radiochlorine. For this study, it was necessary to use the smallest mass of perchlorate possible, while maintaining an acceptable detection limit for total radiochlorine. Because the highest specific activity of perchlorate available was only about 10.4 dpm/ng, the total amount of radioactivity dosed was only about 16 μCi, which is a limited quantity of radioactivity for a 60 kg goat (about 592 dpm/g tissue or 56.8 ng/g perchlorate, assuming even distribution with no elimination). Additionally, for many tissue matrices (i.e., blood, GI contents, ruminal fluid, and feces), the maximum

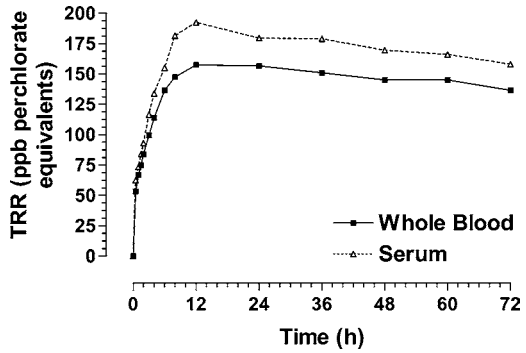

Figure 3. Average total radioactive residues in blood (—) and serum (- - -) of goats 141 and 142 during the 72 h study period. Total radioactive residues are expressed as sodium perchlorate equivalents.

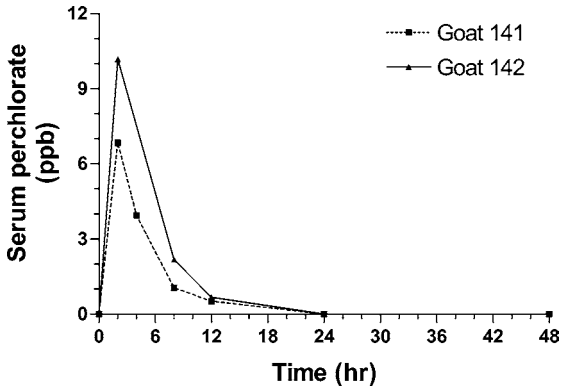

Figure 4. Concentrations of ^{36}Cl-perchlorate (in ppb) in blood serum of goats dosed with [^{36}Cl]perchlorate.

Table 2. Concentrations of Total Radioactive and Perchlorate Residues and Total Mass of Perchlorate Eliminated in the Urine and Milk of Goats Dosed with 60 μg/kg of Body Weight [^{36}Cl]Perchlorate

		goat 141			goat 142		
fraction	time (h)	TRR[a] (ppb)	perchlorate (ppb)	perchlorate (μg)	TRR[a] (ppb)	perchlorate (ppb)	perchlorate (μg)
urine	0–4	157	157	94	NU[b]	NU	
	4–8	145	145	50	NU	NU	
	8–12	56	45.4	28	698	698	431
	12–24	19	9.8	18	54	45.9	46
	24–36	10	1.6	3	17	12.6	9
	36–48	10	0.9[c]	2	8	7.6	8
	48–60	14	NDR[d]		9	2.7	2
	60–72	16	NDR		8	1.0	1
			total = 195			total = 497	
milk	0–12	199	155.2	121	216	155.5	154
	12–24	91	16.9	15	85	11.9	10
	24–36	7.6	1.6	1	7.6	NDR	
	36–48	7.2	NDR		7.6	NDR	
	48–60	7.1	NDR		7.2	NDR	
	60–72	6.4	NDR		7.3	NDR	
			total = 137			total = 164	

[a] TRRs are expressed in perchlorate equivalents. [b] NU, no urine, was excreted during the indicated time period. [c] Perchlorate was detected in only one of two aliquots. [d] NDR, no detectable residue, at limits of quantification of 0.5 ppb for urine and milk, respectively.

sample mass that could be used without excessive quenching was 50−100 mg. Collectively, the error associated with interpolating the radiochemical analysis of small tissue aliquots, containing low amounts of activity, to a whole animal basis is likely the reason for the poor recoveries. This is easily illustrated by noting that, for the analysis of TRR, replicates of milk samples (1000 mg) had COVs less than 2%, replicates of urine

samples (250 mg) had COVs from 1 to 9% (even when total residues were relatively low), and replicates (50−100 mg) of GI contents, ruminal fluid, and feces (12−24 h) had COVs ranging from 9 to 18%.

Perchlorate is essentially depleted from lactating goats within 24 h of a bolus dose. Elimination occurred mainly through the milk and urine. Perchlorate was not detected (1 ppb detection limit) in tissues at slaughter, and there was no evidence that TRR preferentially remained in the thyroid by 72 h after dosing. For example, across all tissues, TRR averaged 51 ± 29 ppb perchlorate equivalents, with residues in kidney being the greatest at 107 ppb (perchlorate equivalents) and residues in adipose tissue being the least at 10 ppb. In comparison, TRR residues in thyroid were 42 ppb (perchlorate equivalents). The biological half-life of thyroid radiochlorine dosed as [^{36}Cl]-perchlorate has been estimated at 3 (*25*) and 7.6 (*26*) h in rats. Because of the relatively short half-life of perchlorate in the thyroid, it is not surprising that there was no evidence for the accumulation of TRR in thyroids of goats killed 72 h (10−20 half-lives) after dosing.

Because our mass spectral analysis found no evidence for the presence of chlorate in any matrix measured, we conclude that the balance of the radioactive residue remaining in tissues, milk, and excreta was present as chloride ion. This conclusion was supported by radiochromatographic analysis of urine and milk samples in which large quantities of radiochlorine eluted at the retention time of chloride. For most samples, detection of a radioactive peak with a retention time consistent with chloride was not a problem but the quantification of radioactivity at the retention time of perchlorate was problematic; thus, for perchlorate analysis, we used the more sensitive mass spectral analysis. Aside from the direct chromatographic evidence, our conclusion that chloride ion was the only transformation product of perchlorate is supportable for the following circumstantial reasons: (1) Total radioactive residues in blood had an estimated depletion half-life of almost 200 h, a half-life more similar to chloride than chlorate or perchlorate. The half-life of chloride reported for nonlactating rats and dogs is 52 and 63 h, respectively (*27*, *28*), and the half-life of chlorate in cattle was reported to be 7.7 h (*29*). (2) Radioactive residues eliminated in milk and urine plateaued after perchlorate was depleted, suggesting that the radiolabel had equilibrated into the large physiologic pool of chloride. (3) Ruminal and gastrointestinal tissues and contents contained detectable levels of radiochlorine 72 h after dosing, whereas fecal levels of radioactivity were detectable only during one time period (12−24 h). Chloride ion is excreted as HCl into the stomach of nonruminants and into the abomasum of ruminants to aid in digestion. Absorption of chloride from the GI tract is nearly quantitative (*28*, *30*), consistent with results of this study in which radiochlorine was present in all gastrointestinal fractions but was mostly absent from feces. (4) Previous studies have unambiguously identified chloride as the sole metabolite of chlorate in ruminants (*21*, *31*) and nonruminants (Hakk et al., manuscript submitted). (5) Other studies have demonstrated that microbial metabolism of perchlorate results in its quantitative conversion to chloride (*32*, *33*). (6) Previous metabolism studies in nonruminants have found no evidence that perchlorate is metabolized to chlorate or chlorite (*26*, *34*).

Goats in this study were provided a dose (3.53 mg; 60 μg/kg of body weight) of perchlorate that likely exceeds most environmental exposures but which fell within (on a mg/kg of body weight basis) the dose range of perchlorate given by Capuco et al. (i.e., 0−75 μg/kg of body weight; *19*). Residues of perchlorate in milk from our goat study ranged from 155 ppb during the first 12 h to nondetectable after 36 h. Levels of residues measured in 0−12 h goat milk are consistent with milk perchlorate residues from the study by Capuco et al. (*19*), in which milk residues in cattle dosed with 75 μg/kg of body weight perchlorate were 80−100 ppb. The apparent greater relative excretion of perchlorate by goats may be explained by the fact that cattle in the study by Capuco et al. (*19*) were producing greater amounts of milk (55 g of milk/kg of body weight) than goats in this study (28 g/kg of body weight). Alternatively, the goats in this study were provided a bolus dose of perchlorate that could have caused greater relative blood perchlorate concentrations than in cows of Capuco, which were ruminally infused with perchlorate. Presumably, greater blood concentrations of perchlorate would lead to greater rates of perchlorate transfer into milk.

Previous research has indicated that humans excreted an oral [^{36}Cl]perchlorate exclusively as parent compound (*34*). Perchlorate concentrations in bovine milk sampled from retail outlets have ranged from nondetectable levels to 11 ppb (*11*, *12*), but human breast milk collected in the United States has contained average concentrations of 11 ppb, with a high value of 92 ppb (*12*). In the Atacama Desert of Chile, where concentrations of perchlorate in drinking water are often greater than 100 ppb, breast milk concentrations of perchlorate averaged from 18 ± 18 to 95 ± 55 ppb, depending upon the region of collection (*17*). In the same study, average urine perchlorate levels ranged from 18 to 49 ppb, suggesting that the mammary gland of nonruminants can be the major route of perchlorate elimination.

Our data also show that in ruminants the mammary gland is a major source of perchlorate elimination relative to urine and feces. However, in contrast to humans, ruminants extensively metabolize perchlorate to chloride ion prior to elimination in milk or urine or retention into the endogenous chloride pool.

Our data support the data of Capuco et al. (*19*) who indicated that, in lactating ruminants, perchlorate is rapidly absorbed and eliminated and that a portion of the dose that is eliminated is unchanged in mammary secretions. These data indicate that, in lactating ruminants, perchlorate residues in milk could quickly be eliminated by removing animals from the source of perchlorate.

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