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Effect of Sodium [^{36}Cl]Chlorate Dose on Total Radioactive Residues and Residues of Parent Chlorate in Beef Cattle†

D. J. SMITH,*,‡ C. E. OLIVER,§ J. S. CATON,§ AND R. C. ANDERSON||

United States Department of Agriculture Agricultural Research Service, Biosciences Research Laboratory, 1605 Albrecht Boulevard, Fargo, North Dakota 58105-5674, Department of Animal and Range Sciences, North Dakota State University, Fargo, North Dakota, 58105, and United States Department of Agriculture Agricultural Research Service, Food and Feed Safety Research, 2881 F&B Road, College Station, Texas 77845

The objectives of this study were to determine total radioactive residues and chlorate residues in edible tissues of cattle administered at three levels of sodium [^{36}Cl]chlorate over a 24-h period and slaughtered after a 24-h withdrawal period. Three sets of cattle, each consisting of a heifer and a steer, were intraruminally dosed with a total of 21, 42, or 63 mg of sodium [^{36}Cl]chlorate/kg of body weight. To simulate a 24-h exposure, equal aliquots of the respective doses were administered to each animal at 0, 8, 16, and 24 h. Urine and feces were collected in 12-h increments for the duration of the 48-h study. At 24 h after the last chlorate exposure, cattle were slaughtered and edible tissues were collected. Urine and tissue samples were analyzed for total radioactive residues and for metabolites. Elimination of radioactivity in urine and feces equaled 20, 33, and 48% of the total dose for the low, medium, and high doses, respectively. Chlorate and chloride were the only radioactive chlorine species present in urine; the fraction of chlorate present as a percentage of the total urine radioactivity decreased with time regardless of the dose. Chloride was the major radioactive residue present in edible tissues, comprising over 98% of the tissue radioactivity for all animals. Chlorate concentrations in edible tissues ranged from nondetectable to an average of 0.41 ppm in skeletal muscle of the high-dosed animals. No evidence for the presence of chlorite was observed in any tissue. Results of this study suggest that further development of chlorate as a preharvest food safety tool merits consideration.

INTRODUCTION

Contamination of beef carcasses with pathogens such as *Escherichia coli* and *Listeria* during slaughter and processing have led to the annual recall of over 800 000 kg of beef during the past decade (*1*); this average excludes a recall of 10 000 000 kg of beef in 2002. Food-animal products containing undetected pathogens continue to contribute to an unquantified number of foodborne illnesses. In beef cattle, it has been established that hides are a major source of carcass contamination (*2*) and that hide-washing intervention steps effectively reduce subsequent pathogen loads on carcasses (*3*, *4*). Although postharvest sanitation techniques are becoming increasingly efficient, they are in use because no practical methods exist for removing pathogens from live animals prior to slaughter. It has been suggested (*5*) that intervention techniques that eliminate pathogen loads in live animals could have a greater relative impact on food safety than any postharvest intervention strategy known, aside from cooking. In reality, a combination of both pre- and postharvest intervention strategies will likely be employed to minimize risks associated with pathogen-contaminated meats.

Recently, a new preharvest technology that greatly reduces or eliminates the numbers of pathogens inhabiting gastrointestinal tracts of cattle (*6*−*8*), sheep (*9*), swine (*10*−*12*), and poultry (*13*, *14*) has been developed. The technology is based on the feeding of an experimental sodium chlorate-containing product (ECP) 24−72 h prior to the slaughter of an animal. During the chlorate exposure period, bacterial species containing intracellular respiratory nitrate reductase are thought to metabolize chlorate (ClO_3^-) to the bacterial toxin chlorite (ClO_2^-; *15*). Chlorate toxicity is specific to nitrate-reductase-containing bacteria that have the ability to intracellularly convert chlorate to chlorite but which lack chlorite dismutase enzymes capable of rapidly metabolizing chlorite to the chloride ion (*16*, *17*). Use of chlorate does not adversely affect the commensal microflora of gastrointestinal tracts (*6*). Unlike many antibiotics,

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* To whom correspondence should be addressed. Telephone: 701-239-1238. Fax: 701-239-1430. E-mail: smithd@fargo.ars.usda.gov.

‡ United States Department of Agriculture Agricultural Research Service, Biosciences Research Laboratory.

§ North Dakota State University.

|| United States Department of Agriculture Agricultural Research Service, Food and Feed Safety Research.

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development of chlorate resistance seems to occur only in pure bacterial cultures and not in mixed culture (*18*).

Previously Smith et al. (*19*) determined that sodium [^{36}Cl]-chlorate administered to ruminating cattle [63 or 126 mg (kg of body weight)$^{-1}$ day^{-1} for 3 consecutive days; slaughtered with an 8-h withdrawal period] is transformed only to the chloride (Cl^-) ion, although residues of parent chlorate ion were present in edible tissues. Because the chloride ion is a nutrient that is essential for life in mammalian and avian species, the presence of chloride residues in animal tissues does not represent a food-safety risk. Chlorite (ClO_2^-) ions, which could be a food-safety concern, were absent from edible tissues and urine of chlorate-treated cattle. In the study of Smith et al. (*19*), chlorate residues present in skeletal muscle and kidney were greater than estimated safe tissue concentrations of chlorate. However, because cattle were exposed to chlorate for 3 consecutive days, with a dose representing 150% of the target dose, and because the cattle were slaughtered following an 8-h withdrawal period, relatively high chlorate residues in skeletal muscle and kidney were not believed to represent insurmountable obstacles for further development of sodium chlorate as a feed additive.

The purpose of this study was to determine the effect of the sodium chlorate dose on chlorate residues in edible tissues of cattle slaughtered following 24-h feeding and withdrawal periods and to determine specifically if the magnitude of chlorate residues in skeletal muscle and kidney would be prohibitive for further development of chlorate as a possible preharvest pathogen intervention strategy. The feeding and withdrawal periods were chosen to mimic potential feeding and withdrawal periods used under commercial settings. An additional objective was to measure the elimination of chlorate and its metabolites in the urine of dosed animals. Only two animals per dosing level were used because of the cost of the radiolabeled material, carcass disposal costs, and the preliminary nature of the study.

MATERIALS AND METHODS

Reagents and Chemicals. *Chemicals.* Unlabeled sodium chlorate [CAS number 7775-09-9; 99.96% $NaClO_3$, 0.03% NaCl, 0.01% H_2O] was obtained from EKA Chemicals, Columbus, MS. Sodium chlorate was stored dry at room temperature until use. Radiolabeled sodium chlorate ($Na^{36}ClO_3$) having a specific activity of 0.575 mCi/mmol was purchased from Ricerca Biosciences (Concord, OH). The aqueous sodium [^{36}Cl]chlorate stock solution was stored refrigerated until formulated for dosing.

Sodium chloride (VWR; West Chester, PA), heparin, sodium salt (Sigma Chemical Co., St. Louis, MO); sodium chlorite (Fluka Chemical Corp., Milwaukee, WI), sodium hydroxide (50% solution for ion chromatography; Fluka Chemical Corp.), Ultima Gold liquid scintillation fluid, Carbosorb-E, and Permafluor E (Perkin−Elmer Life and Analytical Sciences, Boston, MA), methylamine (The Matheson Co., East Rutherford, NJ), and acetonitrile and methanol (HPLC grade; EM Science, Gibbstown, NJ) were used in the study.

Test Article Preparation and Characterization. Radiochemical purity of stock sodium [^{36}Cl]chlorate was assessed using two chromatographic methods. Thin-layer chromatography (TLC) was performed on 5 × 20 cm, aluminum-backed Silica Gel 60 F_{254} plates using 10−15 mL of a solvent system composed of 90% acetonitrile and 10% (v/v) of a 33% (w/v) methylamine solution in water. Aliquots (1 μL) of the diluted radioactive stock solution were spotted about 1 cm from the bottom of triplicate plates. The solvent was allowed to travel for 15−18 cm; plates were removed from tanks; and radioactivity was quantified over a 15-min scan time using a Bioscan Imaging Scanner (Bioscan, Inc., Washington, DC).

Duplicate 20 μL injections of the chlorate-dosing solution were injected onto sequential Dionex (Sunnyvale, CA) AG- and AS-11-HC guard and analytical columns. The solvent [100 mM NaOH in a 60:40 (v/v) mixture of water and methanol] was delivered to the column at a flow rate of 0.5 mL/min using a Waters (Milford, MA) model 600E pump and controller equipped with Teflon pump heads and a Rheodyne (Cotati, CA) model 9725I PEEK manual injector. Samples were introduced using a Hamilton (Reno, NV) 50 μL syringe. Ions were detected using a Dionex CD 25 conductivity detector (100 mA) equipped with a Dionex ion suppression unit (ASRS Ultra-4 mm) operated in the external water mode. A Waters model 746 data module set at 0.5 cm/min was used to record chromatographic data. Fractions were collected in liquid-scintillation-counting (LSC) vials at approximate 4-min intervals for a total of 60 min; Ultima Gold LSC fluid was added to each vial; and vials were counted for 10 min each.

Radiochemical purity as assessed by TLC and HPLC was 94.1 and 94.3%, respectively, for an average radiochemical purity of 94.2%. The radiochemical impurity consisted of [^{36}Cl]chloride, which was the starting material for the [^{36}Cl]chlorate synthesis. Test [^{36}Cl]chlorate was not purified further because chloride is a natural product common to known life forms. Under the TLC and HPLC chromatographic conditions employed at the initiation of the study, a 0.5% radiochemical impurity was not detected. However, the presence of approximately 0.5% perchlorate in the stock material was subsequently verified by the presence of radioactivity at the retention time of perchlorate using ion chromatography, when chromatographing undiluted (high specific activity) stock solutions of [^{36}Cl]chlorate.

Specific Activity Determination. Specific activity of sodium [^{36}Cl]chlorate was determined chromatographically before and after dilution with unlabeled sodium chlorate. Briefly, unlabeled sodium chlorate (0.0999 g) was weighed (Mettler AE100 balance; Mettler Instrument Corp., Heightstown, NJ) and diluted to the mark with water in a 100-mL volumetric flask. Triplicate 4, 8, 12, and 16 μL aliquots of the chlorate solution were injected onto the ion chromatography system described for the determination of radiochemical purity, and the respective peak areas were recorded. Quadruplicate injections (10 and 25 μL, respectively) of diluted stock and formulated sodium [^{36}Cl]chlorate were made; peak areas were collected, and the sodium [^{36}Cl]chlorate peaks were trapped in LSC vials as they eluted from the column. Trapped [^{36}Cl]chlorate peaks were diluted in LSC fluid and counted for a minimum of 10 min each. Specific activity was determined by dividing the disintegrations per minute of each trapped peak by its mass (μg), as determined by regression of its peak area on the standard curve generated from the standards. Specific activities of the stock and formulated sodium [^{36}Cl]chlorate were 11 997 and 202 dpm/μg, respectively.

General LSC Techniques. Background radiochlorine was determined in quintuplicate for individual matrixes; blank matrixes in which background determinations were made were prepared in the same manner as test matrixes. Average background values were calculated for each matrix and were subtracted from gross DPM values obtained for individual test samples. The limit of quantitation (LOQ) was defined as the average DPM value of the control matrix plus three standard deviations (*20*). Individual replicates having DPM values below the LOQ were considered as having "no detectable residue" (NDR). For cases of sample sets ($n = 5$) having replicates above and below the LOQ, the mean DPM value of all replicates was determined; sample sets having a mean value above the LOQ were considered to have detectable residues, but sample sets having the average of all replicates below the LOQ were considered to have no detectable residues.

Samples were generally counted for 20 min each or for a time period specifically indicated for a given matrix. Radiochlorine was quantified using Beckman LS1701 (Fullerton, CA) or Packard 2550 (Meriden, CT) liquid scintillation counters. Each instrument was calibrated regularly using sealed ^{36}Cl-standards (Analytics, Inc., Atlanta, GA) prepared in Ultima Gold LSC fluid. A series of nitromethane-quenched vials, constructed with 0.1 μCi of $^{36}Cl^-$ in 15 mL of Ultima Gold, was purchased (Analytics, Inc., Atlanta, GA) and used to construct quench curves for each instrument. Quench was corrected using the H# (Beckman) or tSIE (transformed spectral index of the external standard; Packard) options for each instrument. Both corrections are based on the degree to which the sample matrix being counted quenches the Compton spectrum of an external source.

Animal Handling. Prior to conduct of the study, approvals were granted from the Institutional Animal and Use Committee and the

USDA Radiation Safety Committee. Three-quarter blood Loala (Angus/Loala × Loala) heifers ($n = 3$; 103.5 ± 4.9 kg) and steers ($n = 3$; 159.7 ± 23.0 kg) were purchased from Auction Effertz, Ltd. (Bismarck, ND) and transported to Fargo, ND, on May 10, 2004. Animals were housed in concrete-floored pens (3.5 × 3.1 m; room A112) covered with wood shavings to absorb urine and feces. All cattle were given ad libitum access to an alfalfa and grass hay mixture and water for the pre-study and study periods and were provided 454 g of cracked corn daily from 14 days after delivery to the USDA facilities until the end of the study. The cattle were adapted to the USDA barn facilities for 18 days prior to ruminal cannulation. Animals were trained to stand in head gates and then metabolism crates (1.0 × 2.1 × 2.7 m; $W \times H \times L$) during the 4−6-week period after surgery. Cattle were ruminally cannulated using a 1-step cannulation procedure on May 28, 2004. At the end of the surgery, each animal was turned back to its pen and given access to food and water. Surgeries were uneventful.

Feed and water were removed from animals 24 and 12 h prior to surgery, respectively. The left side of each animal was clipped, washed thoroughly, disinfected with iodine scrub, and anesthetized by subcutaneous and cutaneous injection of lidocaine (3−5 mL per injection; 30−40 mL total; Abbott Labs, Chicago, IL) in an reverse "7" pattern following the lumbar vertebrae and the 13th (last) rib. Animals were rescrubbed with iodine. At a point located midway between the hip and the last rib and 2.5 cm below the lumbar vertebrae, a 7−10-cm vertical incision was made through the skin; the skin and the subcutaneous fascia were separated by blunt dissection. The superficial muscle wall below the skin was opened by blunt dissection with the end of a blunt scissors; the space was enlarged by blunt dissection of the muscle so that a medium-sized fist would fit through it snuggly. The underlying peritoneum was grasped with rat tooth forceps, and a hole was clipped and enlarged with scissors so that the opening of the peritoneum was of equal size as the opening of the superficial muscle layer. At this point, 10 000−15 000 units of penicillin G (Sigma, St. Louis, MO) diluted in 500 mL of physiologic saline was poured into the peritoneal cavity. The ruminal wall was pulled through the opening and clamped to the side of the animal so that the ruminal wall was exposed. Using a scalpel, a small hole was made in the rumen wall, and the hole was enlarged using a scissor so that the opening roughly matched the length of the original incision. The ruminal wall was folded over the skin opening, and the rumen was clamped with a Backhaus towel clamp at the dorsal and ventral aspects of the orifice so that the ruminal lumen was exposed. The ruminal cannula (Bar Diamond #4C, Parma, ID), having been soaked in hot water, was folded so that the inner flange, lubricated with iodine/betadine paste, was passed through the fistula formed by the skin, muscle layer, and ruminal wall. The inner and outer aspects of the cannula were folded back so that the inner flange was entirely encompassed within the rumen and the outer flange entirely encompassed the exterior orifice of the ruminal fistula. Each animal was then given a bolus dose of sulfamethazine (60 g; Duravet, Inc., Blue Springs, MO) via the ruminal cannula; the cannula was capped; and each animal was given a subcutaneous injection of 13 000 units of penicillin G.

At 1−1.5 h before dosing, permanent jugular catheters (0.050 ID × 0.090 OD × 54 inch length) were placed using an 11-gauge needle. Catheters were secured with a single stitch 2−3 cm proximal to the catheter exit point. When not in use, catheters were filled with saline containing 35 or 100 units/mL of heparin. Steer number 173 (high dose steer) was initially catheterized but pulled its catheter before dosing. For safety reasons, no attempts were made to recatheterize the jugular of Steer 173. Blood data will be presented as a separate report.

Study Design. One steer and one heifer each were randomly selected to receive nominal daily doses of 21, 42, or 63 mg/kg of body weight of sodium [^{36}Cl]chlorate. The dosing levels and withdrawal period were chosen to mimic potential commercial feeding and withdrawal practices. To prevent the radiochemical contamination of the study facilities and to ensure quantitative dosing, sodium [^{36}Cl]chlorate was administered through ruminal cannulas. Ruminal cannulation was chosen in lieu of oral gavage to avoid spillage of radiolabel in the event that a gelatin capsules broke during gavage (*19*) or in the event that an animal eructated a capsule soon after gavage. In an effort to mimic exposure of cattle to the ECP product over a 24-h feeding period, the total daily ECP dose for each animal was divided into four equal aliquots that were administered at 0, 8, 16, and 24 h. Although delivery of the dose as equal aliquots over a 24-h period cannot mimic feeding, we believed our approach would be "conservative", from a residue standpoint, relative to feeding because each animal received a full 25% of its dose at the initiation of the withdrawal period (i.e., 24 h prior to slaughter). Sodium nitrate is hypothesized to render pathogenic bacteria more susceptible to sodium chlorate (*7*, *14*) by inducing the expression of nitrate reductase. Because it may be incorporated into a commercial ECP product, a total dose of 14 mg/kg of body weight sodium nitrate was intraruminally delivered to each animal in aliquots of 3.5 mg/kg of body weight at 0, 8, 16, and 24 h.

Table 1. Target and Actual Doses of Sodium Chlorate and Radiochlorine Delivered to Steers and Heifers[a]

			target dose		actual doses		
			chlorate		chlorate		^{36}Cl
animal	sex	animal weight[b] (kg)	(g)	(mg/kg)	(g)	(mg/kg)	(μCi)
175	♀	110	2.31	21.0	2.35	21.4	214
178	♂	148	3.12	21.0	3.16	21.4	288
177	♀	136	5.71	42.0	5.80	42.7	528
174	♂	195	8.19	42.0	8.32	42.7	757
176	♀	123	7.75	63.0	7.85	63.8	714
173	♂	195	12.29	63.0	12.45	63.9	1133

[a] Formulated material was delivered in 4 equal aliquots at 0, 8, 16, and 24 h. [b] Animals were weighed the day prior to dosing.

During the dosing and withdrawal periods, urine and feces were collected in 12-h intervals, and blood was collected through jugular catheters at regular intervals from all animals except steer 173. For steer 173, urine was collected at 12-h intervals, but feces and blood were not collected for safety reasons. Feces (0−48 h) were pooled and sampled after animal 173 was slaughtered. Animals were housed in metabolism crates throughout the dosing and withdrawal periods. Cattle were slaughtered 24 h after the last administration of sodium chlorate, and edible tissues were collected for the analysis of total residues and chlorate residues.

Capsule Preparation and Administration. Sodium [^{36}Cl]chlorate formulated onto a proprietary carrier was weighed into 19 (outer diameter) × 50 mm (length) gelatin capsules. The amount of formulated material weighed into capsules was based on the body weight of each animal and the fact that the total dose was administered as 4 equal aliquots. Target and actual total doses of chlorate are shown in **Table 1**. Sodium nitrate formulated onto a proprietary carrier was weighed into 19 × 50 mm gelatin capsules so that each animal received a total of 14 mg/kg of body weight during the 24 h dosing period.

Immediately prior to the administration of each aliquot of the dose, a 2.5 × 45 cm (width × length) poly(vinyl chloride) (PVC) pipe was inserted between the wall of the ruminal cannula and the cannula cap into the rumen and through the ruminal fiber mat. Capsules (chlorate and nitrate) were then dropped into the protruding end of the pipe and were gently placed in the rumen using a plunger constructed from a piece of a smaller diameter PVC pipe. Heifer 175 inadvertently received 2 nitrate capsules at the 16-h aliquot administration; the same heifer was administered chlorate and its normal aliquot of nitrate at the 24-h aliquot administration. Otherwise, dosing was without event for all animals. Dose aliquots were administered to steer 178 on June 28 and 29, heifer 175 on June 29 and 30, steer 174 on July 6 and 7, heifer 177 on July 7 and 8, steer 173 on July 12 and 13, and heifer 176 on July 13 and 14, 2004. Cattle receiving the low dose were dosed the last week of June; the intermediate dose was administered to animals the week of July 6; and the high dose animals received chlorate the week of July 12.

Collection of Excreta. Urine and feces were collected in 12-h intervals until slaughter at 48 h, with time zero being defined as the time of the initial sodium [^{36}Cl]chlorate administration. Modified incontinent bags (*21*) were fitted to steers to ensure that urine collection was quantitative and that the urine remained free from fecal contamination. Urine was collected in stainless steel basins, of known weights,

nested in plastic basins. Urine from heifers was collected in stainless steel basins at various times by inducing micturition. Some urine excreted from heifers was collected in fecal trays; this urine was collected but kept separate from clean urine samples. At 12-h sampling intervals, all urine was weighed; clean urine was mixed thoroughly; and 150−400 g of subsamples were collected.

Slaughter and Tissue Collection. Animals were stunned using a captive bolt stun gun, elevated with a hoist, and exsanguinated. Cattle were eviscerated, and liver, kidney, skeletal muscle (biceps femoris), and adipose tissue (kidney, heart, and pelvic fat) samples were removed, ground, and frozen. No attempt was made to collect inedible tissues for the conduct of a mass-balance study. For skeletal muscle, aliquots of the ground sample were either frozen immediately after grinding or were stored for 2 weeks at 3.1 ± 0.3 °C to determine if storage at refrigerated temperatures would affect chlorate concentration. After the 2-week refrigeration period, tissues were frozen. After the collection of edible tissues, animals were skinned, boned, and boxed for disposal.

Total Radioactive Residues. *Edible Tissues.* A practical demonstration of the detection of fortified ^{36}Cl in beef tissues has been reported (*19*). Tissues were partially thawed, and quintuplicate 0.2 g of subsamples was placed in glass LSC vials. To each vial, 8.0 mL of Carbosorb E was added. Vials were placed in a shaking water bath (Dubnoff, Chicago, IL) and incubated overnight at 60 °C. Vials were removed from the incubator, allowed to cool, and to each vial, 12 mL of Permafluor E was added. Samples were placed on the LSC and allowed to dark adapt for at least 1 h before the initiation of counting.

Urine. Urine subsamples were thawed, and quintuplicate 0.25-mL aliquots were removed and weighed in 20-mL glass LSC vials. A total of 15 mL of Ultima Gold LSC fluid was added to each vial; the vials were mixed; and samples were counted for 20 min (or an error rate of 0.2%) on an LSC counter. Background radiochlorine was determined by counting 0.25-mL aliquots of urine collected from each animal prior to dosing with sodium [^{36}Cl]chlorate.

Feces. Fecal matter was partially thawed, and quintuplicate 0.1-g aliquots were removed, placed in 20-mL glass LSC vials, and processed as described for tissues. Resulting counts were too highly quenched for reliable quantitation; therefore, 2.0 mL aliquots of each sample were removed and placed in new LSC vials, and 10.8 mL of Permafluor and 7.2 mL of Carbosorb were added. Samples were mixed by hand and recounted. Quench values of the recounted samples were well within the limits defined by the quench curve.

Speciation of Residues. *Edible Tissues.* **Figure 1** illustrates the strategy used for speciation of radiochlorine. Tissues were analyzed in sets consisting of 5-g duplicates each of control, fortified control, and test-animal tissues. Control tissues were fortified with 25 μL of a standard solution containing 1 μg/μL each of Na^{36}Cl and Na^{36}ClO$_3$ having specific activities of approximately 200 dpm/μg. Samples were extracted essentially as described by Smith et al. (*19*), except that ice-cold acetonitrile was added to precipitate protein to the aqueous supernatant, following the initial extraction and centrifugation. Acetonitrile was evaporated under N_2 prior to C18 solid-phase extraction; otherwise, the procedure used was described previously.

Ion chromatography is described below. For each chromatographic run, fractions were collected at 2−4 min intervals and the presence of chloride or chlorate was determined by the presence of radiochlorine in trapped fractions. Resolution of chlorite, chloride, and chlorate was described by Smith et al. (*19*). Limits of quantitation of radioactive peaks were determined for each chromatographic run by multiplying the standard deviation of quadruplicate background determinations by 3 and adding the product to the mean background value.

Urine. Urine was analyzed in sample sets corresponding to the collection period. Sample sets consisted of duplicates of control (blank) urine, fortified, and incurred samples. Urine was thawed; 1-mL aliquots were removed for analysis; fortified samples were spiked with 25 μL of a mixture of [^{36}Cl]chloride and [^{36}Cl]chlorate; and 1 mL of purified water was added to each tube. Tubes were vortex-mixed, and their contents were loaded onto preconditioned (5 mL of methanol followed by 7.5 mL of water) C-18 SPE tubes (Bakerbond, 500 mg of sorbent, 3-mL; J. T. Baker, Phillipsburg, NJ). The nonretained aqueous eluent was collected in preweighed glass liquid scintillation vials. Each tube

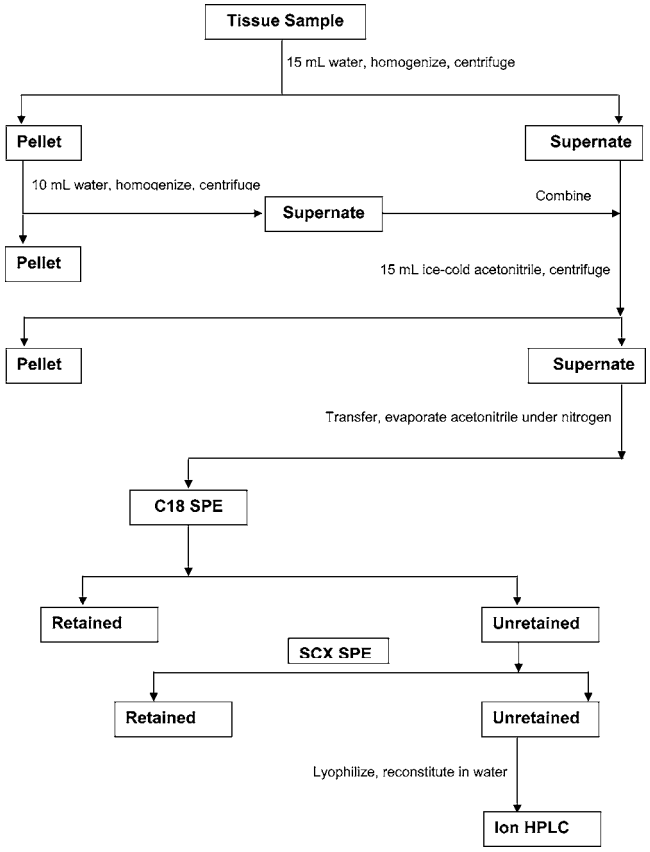

Figure 1. Speciation strategy for edible tissues.

was subsequently rinsed with 1 mL of water, and this "rinse" fraction was collected into the same vial as the "load" fraction. Vials were weighed, and a 100-μL aliquot was removed, weighed, and subjected to LSC after the addition of LSC cocktail. The entire "load/rinse" fraction, collected from the C-18 SPE tubes, was loaded onto preconditioned (5 mL of methanol followed by 5 mL of water) SCX tubes (LC-SCX, 3-mL; Supelco, Bellfonte, CA), and the nonretained eluent passing through each tube was collected into a weighed glass vial. Cation-exchange tubes were rinsed with 1.5 mL of water, and the "rinse" was collected in the same vial as the "load" fraction. A 0.25 mL aliquot was removed from each tube and weighed, and radiochlorine was quantified after the addition of LSC fluid. About 1 mL of each sample was filtered through a 0.45 μM PTFE syringe filter (17 mm; Alltech, Deerfield, IL) in preparation for ion chromatographic analysis. Aliquots of each sample were injected onto the HPLC system described for the tissue analysis, and radiochlorine was eluted using the gradient described below. Fractions of HPLC eluent were collected in LSC vials during each chromatographic run, and radiochlorine in each fraction was quantified by LSC. Limits of quantitation were calculated as described for chromatographic analyses of the tissue extracts.

The ion chromatographic system was described under "Test Article Preparation and Characterization." Tissue and urine extracts were injected onto Dionex AG-11 HC and AS-11 HC guard and analytical columns, respectively, and eluted with a sodium hydroxide gradient consisting of 10 and 100 mM NaOH. Each run was initiated with 10 mM NaOH being held isocratically for 10 min followed by a linear gradient to 50% 100 mM NaOH at 30 min; 50% 100 mM NaOH was held for 2 min, and then the gradient was linearly returned to starting conditions by 40 min. Flow rates were 0.5 mL/min for tissue analyses and 1 mL/min for urine analyses.

RESULTS

Excretion of Radiochlorine. *Total Radioactive Residues.* The excretion of radiochlorine in urine and feces of cattle is shown in **Table 2**. On the average, low-, medium-, and high-dosed animals eliminated about 20, 33, and 48% of the administered

Table 2. Elimination of Radiochlorine (Percent of Total Dose[a]) in Urine and Feces of Cattle Dosed with Sodium [^{36}Cl]Chlorate

			collection period				
animal	sex	fraction	0–12 h	12–24 h	24–36 h	36–48 h	totals
			21 mg/kg Dose				
175	heifer	urine	1.1	2.4	5.6	5.0	14.1
		feces	0.0	1.5	2.9	2.5	6.9
		totals	1.1	3.9	8.5	7.5	21.0
178	steer	urine	1.7	5.0	4.8	4.6	16.1
		feces	0.0	0.0	1.0	1.0	2.0
		totals	1.7	5.0	5.8	5.6	18.1
			42 mg/kg Dose				
177	heifer	urine	5.3	7.8	3.5	3.7	20.3
		feces	1.1	4.0	4.5	3.1	12.7
		totals	6.4	11.8	8.0	6.8	33.0
174	steer	urine	2.2	9.6	6.2	7.1	25.1
		feces	0.0	0.4	3.5	3.6	7.5
		totals	2.2	10.0	9.7	10.7	32.6
			63 mg/kg Dose				
176	heifer	urine	20.2	10.6	10.0	6.3	47.1
		feces	0.3	3.1	4.2	3.3	10.9
		totals	20.5	13.7	14.2	9.6	58.0
173	steer	urine	1.6	8.2	6.0	8.2	24.0
		feces	NA[b]	NA	NA	NA	14.6
		totals	1.6	8.2	6.0	8.2	38.6

[a] Doses of radiochlorine: heifer 175, 214 μCi; steer 178, 288 μCi; heifer 177, 528 μCi; steer 174, 757 μCi; heifer 176, 714 μCi; and steer 173, 1133 μCi. [b] NA = not applicable. For safety reasons, feces excreted from times 0 to 48 h were collected and pooled after steer 173 had been removed from the metabolism crate for slaughter.

radiochlorine by the end of the 48-h study period. Radiochlorine balance was not conducted during this study; therefore, the total recovery of radiochlorine was not calculated. It is apparent from the excretory data that the percentage of radiochlorine excreted during the study period was dose-dependent and the excretion of radiochlorine in urine and feces was generally consistent with that reported for our previous mass-balance study (*19*). Urine was the primary route of radiochlorine excretion containing roughly 78, 69, and 72% of the total radiochlorine excreted by the low-, medium-, and high-dosed animals, respectively.

Speciation of Urine Residues. The composition of urinary radiochlorine is shown in **Table 3**. Urine residues were composed of chlorate and chloride ion. Chromatographic evidence of chlorite in urine was not encountered for any of the urine samples. Chlorate represented between 13.9 and 89.4% of the urinary radioactivity depending on the collection time and dose. Regardless of the dosing level, the fraction of radioactivity present as chlorate decreased with time. At the last collection period, chlorate represented less than one-half of the urine radioactivity, regardless of dose. Because chlorate residues in tissues (see below) were, on the average, less than 1.5% of the total radioactive residue, the higher chlorate composition of urine collected in the 12 h prior to slaughter clearly demonstrates the active excretion of chlorate by the kidney.

Tissue Residues. *Extraction Strategy.* The overall efficiencies of radiochlorine extraction were 92.3, 90.3, 86.8, and 74.7 for liver, kidney, skeletal muscle, and adipose tissue, respectively. In addition to the overall recovery for each tissue, recoveries were measured after each major extraction step: extraction of tissue radioactivity in water, C-18 solid-phase extraction (SPE), and strong cation exchange (SCX) SPE. Homogenization of tissues in water followed by centrifugation was 99.7, 97.8, 95.9, and 76.1% effective at removing radiochlorine from liver, kidney, skeletal muscle, and adipose tissue, respectively. Recoveries of radiochlorine after C-18 SPE were 93.2, 93.4, 92.5, and 99.7% for liver, kidney, skeletal muscle, and adipose tissue, respectively. As might be expected for an anion being applied to a cation-exchange column, recoveries of radiochlorine after SCX SPE were high, being 99.3, 98.9, 97.9, and 98.5 for liver, kidney, skeletal muscle, and adipose tissue, respectively.

With the use of [^{36}Cl]chlorate- and [^{36}Cl]chloride-fortified control tissues, the stability of chlorate in urine and tissue samples during the extraction process was demonstrated. For each set of tissue and urine extractions, control samples (tissues or urine) were fortified with a fortification standard of known composition. Fortified tissues were carried through the extraction process, and the extracted radiochlorine was analyzed by ion chromatography. As demonstrated in **Table 4**, the radiochemical composition of tissue and urine extracts was consistent with the radiochemical composition of the fortification solution.

Residues in Edible Tissues. **Table 5** shows total radioactive residues (TRR), chloride residues, and chlorate residues in edible tissues of cattle. Total radioactive residues increased with dose for all edible tissues ranging from a low of 6 ppm (chlorate equivalents) in skeletal muscle of the low-dose cattle to a high of 79.6 ppm (chlorate equivalents) in kidneys of the high-dose animals. Total radioactive residues were generally the lowest in adipose tissue and skeletal muscle and were the greatest in kidneys. Speciation of the radioactivity using ion chromatography revealed that total radioactive residues were composed nearly exclusively of chloride (Cl^-) ion. Average chlorate residues ranged from a low of 0.02 ppm in adipose tissue of low-dose animals to approximately 0.4 ppm in kidneys and

Table 3. Chemical Composition of Radiochlorine Excreted into Urine of Cattle Dosed with 21, 42, or 63 mg/kg of Body Weight of [^{36}Cl]Chlorate[a]

		collection period (h)							
		0–12		12–24		24–36		36–48	
dose	animal	chloride (%)	chlorate (%)	chloride (%)	chlorate (%)	chloride (%)	chlorate (%)	chloride (%)	chlorate (%)
low	175 heifer	10.5	89.6	39.4	60.7	40.2	59.4	83.2	15.8
	178 steer	31.4	68.6	47.9	56.4	50.8	49.2	88.1	11.9
	average	21.0	79.1	43.7	58.6	45.5	54.3	85.7	13.9
medium	177 heifer	16.7	83.3	31.0	69.1	43.8	56.2	74.2	25.8
	174 steer	4.6	95.5	37.5	65.7	42.7	57.3	79.6	20.2
	average	10.7	89.4	34.3	67.4	43.3	56.8	76.9	23.0
high	176 heifer	11.5	88.5	19.5	80.1	20.5	79.5	59.7	40.3
	173 steer	21.6	78.4	32.8	73.7	34.4	65.3	52.7	47.2
	average	16.6	83.5	26.2	76.9	27.5	72.4	56.2	43.8

[a] Results are expressed as a percentage of the total radioactivity excreted during the indicated time period.

Table 4. Radiochemical Composition of a [^{36}Cl]Chlorate and [^{36}Cl]Chloride Fortification Standard and Composition of the Same Standard after Fortification into Blank Tissue and Urine Matrices with Subsequent Extraction and Analysis by Ion Chromatography

		percentage composition			
		standard[a]		fortified blank[b]	
item	fraction	chloride	chlorate	chloride	chlorate
liver		47.9 ± 0.3	52.1 ± 0.3	51.5	48.5
kidney		47.6 ± 0.2	52.3 ± 0.2	49.2	51.7
adipose tissue		47.8 ± 0.3	52.3 ± 0.3	48.1	51.9
skeletal muscle	frozen	48.5 ± 1.6	51.5 ± 1.6	49.5	50.6
	refrigerated	48.0 ± 0.2	52.0 ± 0.2	49.9	50.1
urine	0–12 h	47.9 ± 0.2	52.2 ± 0.2	48.8	51.3
	12–24 h	47.6 ± 0.4	52.4 ± 0.4	47.2	52.8
	24–36 h	48.1 ± 0.5	51.9 ± 0.5	47.2	52.8
	36–48 h	48.1 ± 0.3	51.9 ± 0.3	46.9	49.1

[a] Prior to each day's analytical run, the chlorate/chloride standard was injected onto the ion chromatography system and radiochemical composition was determined by trapping the chloride and chlorate peaks in liquid scintillation vials; radiochlorine was quantified using LSC. Data are presented as means ± standard deviations. [b] Means of duplicate determinations.

skeletal muscle of the medium- and high-dose animals, respectively. Limits of radiochlorine quantitation averaged 0.051, 0.036, 0.028, 0.035 ppm in liver, kidney, skeletal muscle, and adipose tissue, respectively. As reflected in **Table 5**, chlorate concentrations in many of the tissues approached the limits of quantitation.

Stability of Incurred Chlorate Residues with Refrigeration. Incurred chlorate residues present in skeletal muscle refrigerated at 3.1 ± 0.3 °C for 14 days are shown in **Table 6**. For each of the animals dosed with the low or medium level of chlorate, chlorate residues fell from detectable to nondetectable levels. Chlorate residues in skeletal muscle of the high-dose animals fell from an average of 0.41 to 0.1 ppm during the 2-week refrigeration period. For the low-dose animals, residues of chlorate fell to nondetectable without a concomitant increase in chloride. Concentrations of chlorate residues present in these tissues were not sufficient to increase the total reportable chloride value. The limit of chlorate quantitation for the refrigerated skeletal muscle analyses was 0.028 ppm.

DISCUSSION

On a qualitative basis, results of this study are in agreement with Smith et al. (*19*) who showed that the only measurable metabolite of chlorate present in cattle tissues is chloride. From a quantitative point of view, chlorate residues in edible tissues of this study were substantially lower than reported by Smith et al. (*19*) for two primary reasons. First, cattle in this study received single doses of 21, 42, or 63 mg/kg of body weight administered in four equal aliquots. Cattle in the previous study received daily doses of 63 or 126 mg/kg of body weight administered in bolus doses on each of 3 consecutive days. Second and perhaps more importantly, cattle in this study were slaughtered 24 h after their last exposure to [^{36}Cl]chlorate, whereas cattle in the previous study were slaughtered only 8 h after the last dose of chlorate.

In agreement with the previous cattle study (*19*), no [^{36}Cl]-chlorite was detected in urine or tissues samples analyzed for this study. The only other reports on the disposition and metabolism of [^{36}Cl]chlorate in experimental animals were published by Abdel-Rahman et al. in the 1980s (*22*−*24*). In their 1982 paper (*23*), Abdel-Rahman's group reported that chlorite was a major urinary metabolite after oral chlorate administration, comprising approximately 12.1% of the urinary radiochlorine or about 3.95% of the original chlorate dose. According to their 1984 paper (*24*), rats dosed with chlorate excreted about 10% of the total dose in urine as the chlorite ion. In a forensic analysis, Eysseric et al. (*25*) reported the presence of high concentrations (1500 mg/L) of chlorite in urine of a human exposed to overtly toxic quantities of chlorate. Criteria used to identify chlorite in the forensic investigation were not discussed and are not readily apparent to a reader of the paper.

Abdel-Rahman's group measured [^{36}Cl]chlorite indirectly after precipitation of chlorite and chloride with silver nitrate and subsequent differential solubilization methods described in a 1980 paper (*22*). Their analysis was based on the premise that both chloride and chlorite are precipitated from aqueous extracts with silver nitrate while leaving chlorate salts in solution. Using ammonium hydroxide to solvate precipitated silver salts of chlorite and chloride in one set of tubes and using sodium thiosulfate to selectively solvate silver chloride in another set of tubes, the presence of chlorite was calculated by the difference in solvated radiochlorine between the two sets

Table 5. Total Radioactive Residues, Chloride Residues, and Chlorate Residues in Edible Tissues of Cattle

			tissue											
			liver			kidney			skeletal muscle			adipose tissue		
dose (mg/kg)	animal	sex	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)
21	175	heifer	12.4	12.4	NDR[d]	30.0	29.4	0.54	6.4	6.3	0.06	7.0	7.0	NDR[d]
	178	steer	10.6	10.3	0.26	33.6	33.6	NDR[d]	5.5	5.4	0.04	6.0	6.0	0.03
		average[e]	11.5	11.4	**0.13**	31.8	31.5	**0.27**	6.0	5.9	**0.05**	6.5	6.5	**0.02**
42	177	heifer	20.2	20.1	0.08	48.8	48.4	0.37	9.8	9.6	0.15	9.1	9.0	0.06
	174	steer	21.6	21.4	0.11	49.3	48.8	0.42	10.1	9.7	0.25	9.0	8.8	0.19
		average[e]	20.9	20.8	**0.10**	49.1	48.6	**0.40**	10.0	9.7	**0.20**	9.1	8.9	**0.13**
63	176	heifer	30.0	29.9	0.06	76.8	76.7	0.08	13.7	13.4	0.20	17.9	17.8	0.15
	173	steer	34.1	34.0	0.09	82.4	82.4	NDR[d]	17.0	16.4	0.61	12.6	12.3	0.26
		average[e]	32.1	32.0	**0.08**	79.6	79.6	**0.04**	15.4	14.9	**0.41**	15.3	15.1	**0.21**

[a] TRR = total radioactive residues expressed in parts per million of chlorate equivalents [(total dpm/g of tissue)/202 dpm/μg = μg of chlorate equivalents/g of tissue = parts per million]. [b] Chloride residue calculated by multiplying the percentage of chloride in the extracted sample by the parts per million of the total radioactive residue. The concentrations of chloride do not reflect the physiological concentration of chloride in tissues, only that fraction of the total residue present as the radioactive chloride ion. [c] Chlorate residue calculated by multiplying the percentage of chlorate in the extracted sample by the parts per million of the total radioactive residues. [d] NDR = no detectable residue. [e] Averages for replicates in which one animal contained "NDR" were calculated by using a value of "0" for NDR.

Table 6. Chlorate and Chloride Residues in Refrigerated Skeletal Muscle Samples

			frozen skeletal muscle			refrigerated skeletal muscle		
dose	animal	sex	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)	TRR[a] (ppm)	chloride[b] (ppm)	chlorate[c] (ppm)
low	175	heifer	6.4	6.3	0.06	6.4	6.3	NDR[d]
	178	steer	5.5	5.4	0.04	5.5	5.5	NDR[d]
		average[e]	6.0	5.9	0.05	6.0	5.9	NDR[d]
medium	177	heifer	9.8	9.6	0.15	9.8	9.7	NDR[d]
	174	steer	10.1	9.7	0.25	10.1	9.9	NDR[d]
		average[e]	10.0	9.7	0.20	10.0	9.8	NDR[d]
high	176	heifer	13.7	13.4	0.20	13.7	13.3	0.10
	173	steer	17.0	16.4	0.61	17.0	16.8	0.10
		average[e]	15.4	14.9	0.41	15.4	15.1	0.10

[a] TRR = total radioactive residues expressed in parts per million of chlorate equivalents [(total dpm/g of tissue)/202 dpm/μg = μg of chlorate equivalents/g of tissue = parts per million]. [b] Chloride residue calculated by multiplying the percentage of chloride in the extracted sample by the parts per million of the total radioactive residue. The concentrations of chloride do not reflect the physiological concentration of chloride in tissues, only that fraction of the total residue present as the radioactive chloride ion. [c] Chlorate residue calculated by multiplying the percentage of chlorate in the extracted sample by the parts per million of the total radioactive residues. [d] NDR = no detectable residue. [e] Averages for replicates in which one animal contained "NDR" were calculated by using a value of "0" for NDR.

of tubes. Aside from the weakness of the indirect measurement of chlorite, the basic assumption of Abdel-Rahman et al. (*22*) that silver nitrate will precipitate low concentrations of silver chlorite is an error. The solubilities of silver chloride, silver chlorite, and silver chlorate in water at room temperature are 0.000 89, 4.5, and 100 mg/mL, respectively (*26*). Thus, the solubility of silver chlorite is approximately 5000 times that of silver chloride, and the assumption that silver chlorite will coprecipitate with silver chloride at μg or ng/mL concentrations appears to be an error. To precipitate *any* chlorite using silver nitrate, chlorite would need to be highly concentrated in urine or tissue samples. Further, Abdel-Rahman et al. (*22*−*24*) failed to provide evidence (either through literature citations or data) supporting two other assumptions central to their analytical method: (1) that ammonium hydroxide actually solvates silver chlorite and (2) that sodium thiosulfate *selectively* solvates silver chloride in the presence of silver chlorite.

Other studies specifically investigating the metabolic fate of chlorate in laboratory or food animals do not exist. Chlorate was measured in the urine of a suicidal human exposed to a chlorate-based weed killer (*27*), and chlorate was measured in urine of dogs experimentally intoxicated with sodium chlorate (*28*). Lacking are studies that would confirm or refute the specific findings of chlorite by Abdel-Rahman; to this end, scientists at the USDA-ARS, Biosciences Research Lab, Fargo, ND, have initiated a balance excretion and metabolism study of [^{36}Cl]chlorate in rats. To date, no data have been generated suggesting that chlorite is excreted in urine or that it is present in rat tissues.

In cattle, two plausible explanations exist for the complete metabolism of chlorate to chloride without the formation of detectable quantities of a chlorite intermediate. The first is that chlorate could be rapidly reduced to chloride in the gastrointestinal tract. The rumen, small intestine, and large intestine are anaerobic vessels, and a negative oxidation−reduction potential from −250 to −450 mV (*29*) is normally present, indicating a dearth of oxygen and an excess of reducing equivalents. In such an environment, a fairly strong oxidant such as chlorate would be a natural target for reduction. Indeed, anaerobic bacteria capable of reducing chlorate and/or perchlorate [i.e., (per)-chlortate respiring bacteria] are nearly ubiquitous in the environment. For example, Coats et al. (*30*) reported the isolation of (per)chlorate-reducing bacteria from such diverse environments as a swine waste lagoon, a "pristine aquatic environment", Mississippi River sediment, "pristine soil", drainage sediment from a gold mine, petroleum-contaminated sediment, water from Pohic Bay, and a Florida swamp. Bacterial isolates respired perchlorate and chlorate, with chloride being "the sole product of this reductive metabolism". The bacterial isolates had chlorite dismutase activities of 1928 μmol of chlorite (mg of protein)$^{-1}$ min^{-1}. Chlorite dismutase converts chlorite quantitatively into chloride ion and oxygen; it functions in the cell to destroy chlorite as rapidly as it is formed. Anaerobic microbes have been selected for remediation of perchlorate-contaminated soils because they are capable of completely reducing (per)chlorate to chloride ion without the accumulation of intermediate oxidation states (*31*). Although the capacity of ruminal bacteria to respire chlorate has not been investigated, the likelihood that such bacteria exist is good given the diversity of ruminal microbial populations and the fact that (per)chlorate-respiring bacteria have been isolated from farm-animal waste lagoons (*30*).

The second likely site of chlorate reduction is in the blood and/or in myoglobin-containing tissues. One of the major clinical diagnostic tools used to identify acute chlorate poisoning is the presence of extremely high levels of methemoglobin in blood of exposed animals (*27*, *32*). Methemoglobin formation is extensive enough in some cases that the blood and tissues (*33*, *34*) of affected individuals assume a brown color. Affected individuals characteristically excrete "muddy urine" that is literally turbid with methemoglobin. As proposed by Jung (*35*) and as described by Steffen and Wetzel (*32*), chlorate reacts directly with hemoglobin to form a complex that "slowly disintegrates into methemoglobin, chloride, and oxygen radicals." Although it has been established that chlorate acts on hemoglobin in a dose- and time-dependent manner in the formation of methemoglobin (*32*), the conversion of chlorate to chloride or other intermediates has not been directly measured. Because cattle have between 8 and 15 mg of hemoglobin/mL of blood, with four iron atoms per hemoglobin molecule, the capacity for chlorate reduction is great.

Data from this study provide anecdotal evidence of the capacity of chlorate to be reduced by animal tissues themselves. For example, chlorate was transformed to chloride when skeletal muscle samples were stored for 14 days at 3.1 °C; however, when skeletal-muscle-containing chlorate residues were stored frozen for 6 months, no chlorate degradation was observed (D. J. Smith, unpublished observations). Skeletal muscle contains two sources of heme iron, hemoglobin in red blood cells and myoglobin in muscle fibers, and each could presumably contribute to the reduction of chlorate. Unpublished data from our laboratory indicate that about 10% of chlorate fortified in

fresh skeletal muscle is from cattle that is converted to chloride within about 1 h; by 4 days, over 25% of the fortified chlorate was converted to chloride, indicating the capacity of the tissue to reduce chlorate to chloride. Oliver et al. (*36*) indicated that 20−86% of the chlorate fortified in human blood, brain, or liver was destroyed after a 60-h period. On the basis of these data, we hypothesize that globin-containing tissues have the capacity to reduce chlorate. A further study will be required to test this hypothesis.

Data reported in this study indicate that the vast majority of chlorate-related residues present in edible tissues of cattle are present as chloride ion and that chlorate residues remaining in edible tissues are measured in parts per billion rather than parts per million. Because *E. coli* O157:H7 contamination of beef products remains a persistent problem in the United States and other nations and because of the related acute risk that *E. coli* O157:H7 represents to consumers, further research and development of chlorate as a preharvest intervention technique for the beef industry seems warranted.

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