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Tissue Distribution, Elimination, and Metabolism of Dietary Sodium [^{36}Cl]Chlorate in Beef Cattle

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Two steers (~195 kg) were each dosed with 62.5 or 130.6 mg/kg body weight sodium [^{36}Cl]chlorate for three consecutive days. All excreta were collected during the dosing and 8 h withdrawal periods. The apparent radiochlorine absorption was 62−68% of the total dose with the major excretory route being urine. Parent chlorate was 65−100% of the urinary radiochlorine; chloride was the only other radiochlorine species present. Similarly, residues in edible tissues were composed of chloride and chlorate with chloride being the major radiolabeled species present. Chlorate represented 28−57% of the total radioactive residues in skeletal muscle; in liver, kidney, and adipose tissues, chlorate ion represented a smaller percentage of the total residues. Chlorate residues in the low dose steer were 26 ppm in kidney, 14 ppm in skeletal muscle, 2.0 ppm in adipose tissue, and 0.7 ppm in liver. These data indicate that sodium chlorate may be a viable preharvest food safety tool for use by the cattle industry.

KEYWORDS: Sodium chlorate; food safety; residue; cattle; *E. coli* O157:H7

INTRODUCTION

Contamination of beef carcasses with human pathogens including *Escherichia coli* and *Listeria* during slaughter and processing has led to the recall of over 37.8 million pounds of beef during the past decade (*1*). Beef products containing undetected pathogens have contributed to an unquantified number of food-borne illnesses during the same time period. Although beef producers, packers, and retailers are actively seeking pre- and postharvest solutions to eliminate beef-borne pathogens, the problem of carcass contamination remains.

Recently, a new preharvest technology that greatly reduces or eliminates the numbers of pathogens inhabiting the gastrointestinal tracts of cattle (*2*−*4*), sheep (*5*), swine (*6*−*8*), and poultry (*9*, *10*) has been developed. The technology is based on the feeding of a sodium chlorate-containing product (ECP) 24−72 h prior to an animal's slaughter. 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^-; *11*). 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 (*12*, *13*). Use of chlorate does not adversely affect the commensal microflora of gastrointestinal tracts (*2*). Unlike many antibiotics, development of chlorate resistance seems to occur only in pure bacterial cultures and not in mixed culture (*14*).

Although the use of chlorate by the cattle industry seems to be a practical means of reducing the probability of pathogen contamination at slaughter, data demonstrating the absorption, distribution, metabolism, and excretion of chlorate in treated cattle do not exist. Furthermore, the safety of chlorate residues in edible tissues of cattle has not been demonstrated. In rodents, chlorate appears to be rapidly absorbed and excreted (*15*) and chlorate is apparently metabolized to chlorite and chloride ions. The overall goal of this study was to determine if further development of chlorate as a commercial product was warranted from the perspective of the magnitude of residues in edible tissues. The objective of this study was to determine the absorption, distribution, metabolism, and excretion of sodium chlorate in cattle. Because sodium chlorate disposition in ruminants has not been previously studied and because the cost of the test article ($Na^{36}ClO_3$) on a per animal basis was substantial, this communication describes results obtained from a preliminary study in which only two animals were dosed.

MATERIALS AND METHODS

Reagents and Chemicals. Unlabeled sodium chlorate (CAS no. 7775-09-9; 99.96% $NaClO_3$; 0.03% NaCl; 0.01% H_2O) was obtained from EKA Chemicals (Columbus, MS). Radiolabeled sodium chlorate

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($Na^{36}ClO_3$), 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 (<4 °C) until formulated for dosing.

Sodium nitrate was obtained from ICA TriNova, LLC (Marietta, GA). Sodium chloride (VWR, West Chester, PA); heparin, 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 (PerkinElmer Life and Analytical Sciences, Boston, MA); methylamine (The Matheson Co., East Rutherford, NJ); phenyl mercuric nitrate, phenyl mercuric chloride (Aldrich, St. Louis, MO), and acetonitrile and methanol [high-performance liquid chromatography (HPLC) grade; EM Science, Gibbstown, NJ] were obtained from well-known vendors.

General LSC Techniques. Background radiochlorine and limits of quantitation were determined for individual matrices (i.e., urine, liver, kidney, skeletal muscle, and adipose tissue) as described by Smith et al. (*16*). Individual samples within a matrix set were generally counted for 10−20 min each. Radiochlorine was quantified using Beckman LS1701 (Fullerton, CA) or Packard 2550 (Meriden, CT) liquid scintillation counters. Each instrument was calibrated using a sealed radiochlorine standard (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.) 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.

Test Article Preparation and Characterization. The radiochemical purity of stock sodium [^{36}Cl]chlorate was assessed using two chromatographic methods. Thin-layer chromatography (TLC) was performed on 5 cm × 20 cm, aluminum-backed Silica Gel 60 F_{254} plates using a solvent system composed of 90% acetonitrile and 10% (v/v) of a 33% (w/v) methylamine solution in water (*17*); radiochlorine was quantified using a Bioscan Imaging Scanner (Bioscan, Inc., Washington, DC).

Duplicate 10 μL injections of the diluted stock chlorate solution were made onto sequential Dionex (Sunnyvale, CA) AG- and AS-11 guard and analytical columns. Solvent (100 mM NaOH in a 60:40 [v/v] mixture of water and methanol) was delivered 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 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 into LSC vials at approximate 2 min intervals; Ultima Gold LSC cocktail was added to each vial, and vials were counted for a minimum of 10 min each. Radiochemical purity of sodium [^{36}Cl]chlorate formulated on its proprietary carrier was also assessed approximately 6 months after its formulation.

Specific Activity Determination. The specific activity of sodium [^{36}Cl]chlorate was determined chromatographically before and after dilution with unlabeled sodium chlorate. Briefly, unlabeled sodium chlorate (0.1370 g) was weighed (Mettler AE100 balance, Mettler Instrument Corp., Heightstown, NJ), dissolved in water, and transferred to 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 stock and formulated sodium [^{36}Cl]chlorate were made; peak areas were recorded, and the sodium [^{36}Cl]chlorate peaks were trapped into LSC vials and counted for a minimum of 10 min. The specific activity was determined by dividing the dpm of each trapped peak by its mass (μg), as determined by regression of its peak area on the standard curve generated from the standards.

Animals and Feeding. Two Loala steers (nos. 171 and 172; approximately 166 kg at purchase) were trained to metabolism crates (1.0 m × 2.1 m × 2.7 m; W × H × L) over a 7 week period. Steers were provided a mixture of alfalfa and grass hay (provided on an ad libitum basis) from delivery to the completion of the study. Beginning 21 days prior to study commencement, 0.5 kg of cracked corn was provided daily and this amount was gradually increased to 2.7 kg per day up to the time of the study.

Study Design. At 72, 48, and 24 h prior to sodium [^{36}Cl]chlorate dosing, a proprietary sodium nitrate premix was fed to each steer as a component of the grain. Sodium nitrate is hypothesized to render pathogenic bacteria more susceptible to sodium chlorate (*5*, *10*) and was fed in this study to mimic conditions under which bacterial nitrate reductase might be induced. Sodium nitrate was delivered in the diet at 31 mg/kg body weight before the commencement of chlorate dosing. At 0, 24, and 48 h, each animal was orally dosed with either 63 or 126 mg/kg body weight of sodium [^{36}Cl]chlorate. Time 0 was 24 h after the last sodium nitrate feeding. Fifty-four hours (T54) after the initial sodium [^{36}Cl]chlorate dose and 8 h after the last sodium [^{36}Cl]chlorate dose, each animal was slaughtered and tissues were collected.

The sodium chlorate dose was selected based on the anticipation that the maximum exposure to a chlorate product would be three consecutive days with a preslaughter withdrawal period of 0 days. Therefore, test animals were dosed orally with $Na^{36}ClO_3$ for three consecutive days starting approximately 24 h after the last administration of sodium nitrate. Animal 171 was dosed with 63 mg/kg body weight of sodium chlorate, while animal 172 was dosed with 126 mg/kg body weight of sodium chlorate. These doses represent 1.5× the maximal intended use levels of 42 and 84 mg/kg body wt per day, respectively. A 1.5× dosing level was used to comply with unpublished, but widely known, U.S. Food and Drug Administration Center for Veterinary Medicine (U.S. FDA CVM) guidelines for the conduct of residue studies for compounds having a 0 day withdrawal period (*16*). Approximately 8 h after the last dose administration, each animal was stunned and exsanguinated.

Dose Formulation. Stock sodium [^{36}Cl]chlorate (6.402 mCi; 1.185 g) was combined with 180 mL of water and 118.83 g of unlabeled sodium chlorate. The sodium chlorate was completely dissolved and mixed, and a 100 μL sample was removed for purity and specific activity determinations. Dissolved sodium [^{36}Cl]chlorate was added to a proprietary carrier (280.0 g), allowed to dry, and stored in a labeled amber glass bottle until use.

Capsule Preparation and Administration. Formulated sodium [^{36}Cl]chlorate was weighed into gelatin capsules. The amount of formulated material weighed was based on body weights of 191.8 and 197.7 kg for steers 171 and 172, respectively. Each gelatin capsule held roughly 20 g of the [^{36}Cl]chlorate formulation. Immediately prior to dosing, each capsule was lubricated with vegetable oil, placed in a balling gun, and dosed. On dosing day 2, capsule no. 4 broke in the throat of steer 172 prior to its being released from the balling gun. Some of the contents of the capsule spilled into the head area of the metabolism crate. The capsule and its remaining contents were recovered, repackaged into an additional capsule, and readministered to steer 172. The contents of the spilled capsule were recovered to the extent possible, and radiochlorine in the recovered fraction was quantified by liquid scintillation counting. Dosing was otherwise uneventful.

Collection of Excreta. Urine and feces were collected in intervals of 0−12, 12−24, 24−36, 36−48, and 48−56 h after the initial sodium [^{36}Cl]chlorate administration. Modified incontinent bags (*18*) were fitted to steers to ensure quantitative collection of clean urine. Urine was weighed and mixed thoroughly, and subsamples were collected and stored at −20 °C.

Slaughter and Tissue Collection. Animals 171 and 172 were stunned, elevated, and exsanguinated. Blood was collected into basins that contained approximately 64000 units of sodium heparin and was weighed. Steers were eviscerated, and blood, brain, liver, kidney, adipose tissue, skeletal muscle, lung, spleen, small intestine, large intestine, stomach complex (consisting of the rumen, reticulum, omasum, and abomasum), skin, eyes, heart, bone, diaphragm, remainder of carcass, bile, and adrenal glands were collected. A "remainder of the carcass" fraction was collected that contained tissue scraps and tissue not associated with any one organ; for animal 171, liquid and solid portions were separated and assayed separately. Edible tissues were ground the day of slaughter (before freezing), but other tissues were

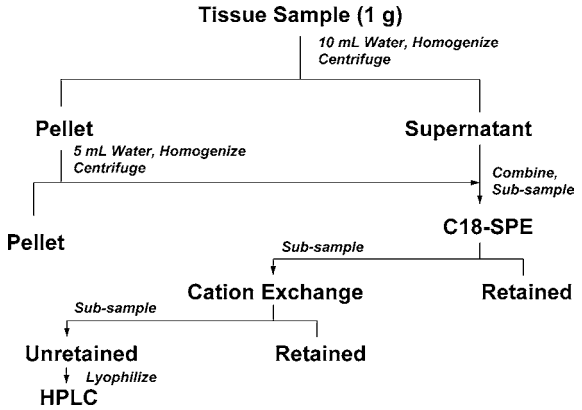

Figure 1. Schematic of tissue processing and analysis.

frozen, partially thawed, and then ground. The summed weights of tissues collected at slaughter were 97.3 and 98.1% of the live weights of steers 171 and 172, respectively.

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 LSC vials and diluting with 15 mL of Ultima Gold LSC fluid. Each sample was counted for 5 min on a LSC counter.

Total Radioactive Residues. *Practical Demonstration of Limit of Quantitation of Radioactive Residues.* Blank aliquots (2 g) of liver, kidney, skeletal muscle, and adipose tissue were fortified with $Na^{36}ClO_3$ so that 0.2 g samples would contain nominal levels of 0, 10, 20, 200, and 400 DPM, respectively. Kidney samples were inadvertently fortified with only 5, 10, 100, and 200 DPM per 0.2 g. After fortification, sample vials were vortex mixed at high speed for a minimum of 1 min. Quintuplicate 0.2 g aliquots of each fortified tissue were weighed into glass LSC vials. To each replicate, 8.0 mL of Carbosorb was added and tubes were incubated in a shaking water bath at 60 °C overnight. After the tubes were removed from the incubator and cooled to room temperature, 12 mL of Permafluor was added to each vial, and vials were placed on the LSC, dark adapted for a minimum of 1 h, and were counted overnight for 20 min each.

Liquid Samples. Subsamples from each animal and(or) each time point were thawed, and quintuplicate 1 mL aliquots were removed and weighed into 7 mL LSC vials. Six milliliters of Ultima Gold LSC cocktail was added to each vial, the vials were mixed, and samples were counted for 20 min (or an error rate of 0.2%) on a LSC counter. Background radiochlorine was determined by counting 1 mL blank samples.

Solid Samples. Quintuplicate 0.2−0.25 g aliquots were removed and placed into LSC vials. For fecal samples, 1 mL of water was added after the addition of feces. To each sample, 8.0 mL of Carbosorb E was added. Tubes were placed in a shaking water bath (Dubnoff, Chicago, IL) and were incubated overnight at 60 °C. After the tubes were removed and allowed to cool, 12 mL of Permafluor E was added to each tube. Samples were placed on the LSC and allowed to dark adapt for at least 1 h before the initiation of counting. For some fecal samples, resulting counts were too highly quenched for reliable quantitation, so 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. *Edible Tissues*. **Figure 1** illustrates the sample preparation scheme used for speciation of tissue metabolites. Edible tissue samples were run in sample sets consisting of duplicate 1 g subsamples of control tissues, fortified control tissues, tissues from animal 171, and tissues from animal 172. For adipose tissue samples, 2 g subsamples were analyzed. Control tissues were fortified with 30 μL of a standard solution containing 1 μg/μL each of $Na^{36}Cl$ and $Na^{36}ClO_3$ having specific activities of 100 dpm/μg. Samples were weighed into 50 mL polypropylene tubes (Sarstedt, Newton, NC), control tissues were fortified with radioactive standards, and 10 mL of nanopure water was added to each tube. Tissues were homogenized using a Tekmar (Cincinnati, OH) tissue homogenizer and centrifuged for 15 min at 31500*g* using a Sorvall RC-2 (Norwalk, CT) centrifuge equipped with a SS-34 rotor. Supernates were decanted into weighed glass LSC vials, and the resulting pellets were resuspended in 5 mL of water. The suspended pellets were mixed and centrifuged, and supernates were decanted and combined with their respective supernates. From each pooled supernate, a 2.5 mL aliquot was removed, weighed, and diluted with 15 mL of Ultima Gold LSC cocktail. A 5.0−7.5 mL aliquot of each supernate, depending upon the tissue, was removed and loaded on a previously conditioned (methanol followed by water) C18 Mega Bond Elut (2 or 5 g of sorbent depending upon tissue; Varian Assoc., Harbor City, CA) solid phase extraction (SPE) column. Each column was rinsed with water (5 mL), and the load and rinse fractions were collected into weighed glass LSC vials. A 1 mL aliquot of each "load/rinse" fraction was removed for quantitation of radiochlorine. Depending on the tissue, 5 mL or the entire remaining load/rinse fraction from the C18 SPE tube was loaded onto a preconditioned (methanol followed by water) cation exchange SPE tube (3 mL LC-SCX; Supelco, Bellfonte, CA). Loaded samples were allowed to pass through their respective SPE tubes, and tubes were rinsed with 2.5 mL of water. Aliquots (1 mL) were removed from the combined load/rinse fraction for LSC. The remaining fractions were frozen, lyophilized, reconstituted in 1 mL aliquots of water, and filtered (13 mm, 0.45 μm, PFTE syringe filter; Alltech, Deerfield, IL) in preparation for ion chromatographic analysis.

Ion chromatography was performed using the chromatographic equipment described previously. For speciation of the tissue metabolites, a gradient solvent system was used consisting of 10 and 100 mM NaOH, with Dionex AG- and AS-11 HC guard and analytical columns, respectively: isocratic 10 mM NaOH for 10 min; linear gradient to 50% 10 mM NaOH, 50% 100 mM NaOH from 10 to 30 min; isocratic for 2 min; linear gradient to 100% 10 mM NaOH from 32 to 40 min; reequilibrate for 30−40 min. Each day, prior to injection of samples from a tissue set, a standard containing $^{36}Cl^-$ and $^{36}ClO_3^-$ was injected onto the chromatograph so that recoveries and distribution of radioactivity could be determined. Individual samples were injected, and fractions were collected at approximate 3 min intervals for the first 15 min; thereafter, fractions were collected to minimize the splitting of radioactive peaks into separate vials. Fractions were quantified by LSC using Ultima Gold LSC cocktail. For chromatographic runs, the first four vials were used to assess background levels (initial 12 min of chromatographic run). The limit of quantitation for each run was defined as the mean dpm value of the first four vials plus three standard deviations of the mean. Chromatographic fractions that contained dpm values less than the limit of quantitation were said to have "0" counts.

Reaction with Phenyl Mercuric Nitrate. Phenyl mercuric nitrate (PMN) reacts with aqueous chloride ion under acidic conditions to form water insoluble phenyl mercuric chloride (*19*, *20*). The reaction was used in this study to quantify [^{36}Cl]chloride in tissues and to unambiguously verify the conversion of [^{36}Cl]chlorate to [^{36}Cl]chloride in tissues. Sample sets consisted of $Na^{36}Cl$ and $Na^{36}ClO_3$ fortified control tissues, and tissue samples containing incurred residues from steers 171 and 172. Duplicate 2.5 g samples were weighed into 50 mL polypropylene tubes, control tubes were fortified with [^{36}Cl]chloride or [^{36}Cl]chlorate (95.4% chlorate, 4.6% chloride), 10 mL of water was added to each tube, and tissues were homogenized, mixed, and centrifuged as described above. Respective supernates were combined and weighed, and aliquots were analyzed by liquid scintillation counting. Portions (5.0 mL) of the remaining supernates were placed in 100 mL separatory funnels and acidified to pH 1.5 with 1% nitric acid (~4 mL), and 25 mL of a 0.4 mg/mL aqueous solution of phenylmercuric nitrate was added. To each separatory funnel, 15 mL of chloroform was added (3×) and the layers were mixed. Respective chloroform extracts were removed and combined in 50 mL volumetric flasks; flasks were diluted to the mark with chloroform. Duplicate 5 mL aliquots were placed in LSC vials; the chloroform was allowed to evaporate, and the residue was reconstituted in 1 mL of methanol and then diluted with 15 mL of Ultima Gold LSC fluid. The remaining aqueous phases were placed in

volumetric flasks and diluted to the mark, and aliquots (5 mL) were removed for LSC. Radiochlorine in extracted samples was counted for 10 min each.

Confirmation of phenyl mercuric chloride in chloroform extracts of skeletal muscle was conducted by gas chromatography−mass spectrometry. Samples, dissolved in chloroform, were introduced onto an Autospec mass spectrometer (Micromass, Beverly, MA) using a Hewlett-Packard 5890 gas chromatograph equipped with a HP-7673A autosampler. Samples (1 μL) were cool-on column injected onto a 30 m × 0.25 mm i.d. DB5 MS column (J & W Scientific, Folsom, CA) with a 0.25 μm film thickness. A 1 m retention gap constructed of deactivated fused silica (0.53 mm i.d.) protected the column. Phenyl mercuric chloride eluted at a retention time of approximately 14.5 min using a temperature gradient starting at 150 °C, held for 2 min, followed by ramping to 200 °C at a rate of 5 °C per min, followed by ramping to 320 °C at a rate of 10 °C per min. Total ion chromatograms were generated, and mass spectra were evaluated at the retention time of the phenyl mercuric chloride standard; mass spectra of tissue extract samples were compared to the mass spectrum of the phenyl mercuric chloride standard.

Speciation of Urinary Radiochlorine. Urine was analyzed in sample sets corresponding to collection period and consisting of duplicate replicates each of control samples, fortified control samples, and samples from steers 171 and 172. Urine was thawed, 1 mL aliquots were removed for analysis, fortified samples were spiked with a mixture of [^{36}Cl]chloride and [^{36}Cl]chlorate, and 2 mL of nanopure 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) C18 SPE tubes (Bakerbond, 500 mg of sorbent, 3 mL tube; J. T. Baker, Phillipsburg, NJ). Sample loads from each column were collected and combined with a subsequent 1 mL water rinse of each tube. A 100 μL aliquot was removed, weighed, and subjected to LSC. The remaining load/rinse fractions collected from the C-18 SPE tubes were loaded onto preconditioned (5 mL of methanol followed by 5 mL of water) SCX tubes (LC-SCX, 3 mL; Supelco). Sample loads were collected and combined with 1.5 mL water rinses of each tube. An aliquot (0.25 mL) was removed from each tube and weighed, and radiochlorine was quantified by LSC. About 1 mL of each sample was filtered through a 0.45 μm PTFE syringe filter (17 mm; Alltech) in preparation for ion chromatographic analysis. Aliquots (20−160 μL, depending upon the concentration of radioactivity) were injected onto the HPLC system described for the tissue analysis, and radiochlorine was eluted using the gradient previously described for tissues.

RESULTS AND DISCUSSION

Radiochemical Purity. The radiochemical purity as assessed by HPLC and TLC was 94.5 and 94.3%, respectively. The radiochemical impurity was $Na^{36}Cl$, the starting material for the synthesis of sodium chlorate. During the formulation of the dosing material, the total amount of $Na^{36}ClO_3$ used was adjusted for radiochemical impurity. Radiochemical purity of the formulated $Na^{36}ClO_3$, 6 months after its formulation, was 96.8% as assessed by ion chromatography.

Specific Activity. Unformulated (stock) sodium [^{36}Cl]chlorate had a specific activity of 11573 dpm/μg. Formulated sodium chlorate had a specific activity of 114 dpm/μg.

Live Phase. Animal dosing was without event for steer 171. On dosing day 2, (24 h after the initial dose) capsule 4 broke in the mouth of steer 172 and a portion of the capsule was spilled onto the floor of the metabolism crate. Radioactivity recovered from the floor of the metabolism crate indicated that 139.4 μCi of radiochlorine was present, representing 8.3 g, or 40%, of the formulated material within capsule 4. The 8.3 g of lost material represented 10.0% of the total dose for dosing day 2, and 3.3% of the total 3-day dose of steer 172. Of greater concern was that Steer 172 stopped consuming food on dosing day 2, eating none of its daily grain allotment and only a little forage. Furthermore, steer 172 failed to consume grain on dosing day 3 but did consume some forage. Fecal output for steer 172 stopped entirely during hours 36−48 of the study (12−24 h after capsule breakage).

Table 1. Recoveries of Radiochlorine Fortified into Edible Tissues

	radiochlorine			
tissue	target (dpm/g)	theoretical[a] (dpm/0.2 g)	measured (dpm ± SD)	recovery % ± SD
liver	50	10.4	11.4 ± 1.0	110.3 ± 10.0
	100	20.4	21.4 ± 2.1	104.8 ± 8.2
	1000	202	197.2 ± 7.8	97.6 ± 0.8
	2000	415	392.6 ± 10.5	94.6 ± 1.4
kidney	25	5.2	4.9 ± 1.3	93.0 ± 24.2
	50	9.6	8.4 ± 0.90	86.9 ± 3.7
	500	106	107.5 ± 19.0	101.0 ± 4.3
	1000	205	213.5 ± 13.8	104.4 ± 1.0
skeletal muscle	50	9.7	8.9 ± 2.0	91.9 ± 20.1
	100	20.3	19.4 ± 2.6	95.9 ± 12.9
	1000	206.6	230.8 ± 54.1	111.8 ± 26.2
	2000	409.5	396.4 ± 58.3	96.9 ± 15.1
adipose tissue	50	8.6	10.6 ± 2.9	121.5 ± 17.6
	100	18.8	20.4 ± 5.7	106.6 ± 14.7
	1000	176.6	186.0 ± 54.7	104.6 ± 6.2
	2000	372.3	376.4 ± 66.5	103.4 ± 24.4

[a] Theoretical dpm calculated by: (control tissue wt/fortification dpm) × subsample wt.

Tissue Residues. Radiolabeled chlorate was used so that all chlorate-related residues (parent chlorate and metabolites) could be quantified, so that the degree of chlorate metabolism could be determined, and so that chlorate metabolites could be identified. Detection of 5 dpm of [^{36}Cl]chlorate fortified into blank tissues was possible using Carbosorb solubilization of tissues (**Table 1**). Recoveries for all tissues were between 91 and 121%. Variation was greatest for low level fortifications and for skeletal muscle and adipose tissue samples in which uniform mixing of the fortified radiolabel was most difficult. These data demonstrate that low levels of radiochorine can be reliably detected in edible tissues using Carbosorb and Permafluor as solubilizers and scintillants, respectively. Other techniques (combustion; Soluene 350 digestion; Soluene 350 with peroxide bleaching; Solubilization with Ultima Gold or Hionic Fluor scintillation fluid) led to low recoveries and greater variation than the technique used for this study (data not shown).

Distribution and Disposition of Radioactive Residues. The excretion of radiochlorine in urine and feces is shown in **Table 2**, and the overall distribution and recovery of radiochlorine are shown in **Table 3**. Urine was the major excretory route of radiochlorine with approximately 39−47% of the total administered dose being eliminated in urine by slaughter. Because the last one-third of the total dose was administered only 8 h prior to slaughter, these percentages suggest a fairly rapid excretion of radiochlorine. In contrast, fecal elimination of radiochlorine was minimal with steers 171 and 172 excreting only 1.7 and 0.4% of the total dose, respectively. At slaughter, the small intestine and large intestine collectively contained 8.2 and 5.2% of the total dosed radiochlorine, for steers 171 and 172, respectively, suggesting that absorption or resorption of radiochlorine was occurring in the lower tract. Edible tissues contained a significant fraction of the dosed radiochlorine at slaughter. By virtue of its large proportion of the carcass, skeletal muscle contained the largest fraction of the radiochlorine retained in the body. However, tissues with excretory function such as the liver (bile) and kidney (urine) contained higher concentrations of total residues, also suggesting that the total residue is eliminated rapidly. Total recovery of dosed radio-

Table 2. Excretion of Total Radioactive Residues in Urine and Feces of Steers 171 and 172

		urine radiochlorine		fecal radiochlorine	
animal	time period[a] (h)	concn[b] (ppm)	fraction (%)[c]	concn[b] (ppm)	fraction (%)[c]
171	0–12	1696	7.3	22	0.1
	12–24	1044	2.7	41	0.2
	24–36	4401	8.4	48	0.3
	36–48	3927	7.9	109	0.7
	48–56	4828	10.7	116	0.4
	slaughter	6080	1.6	NA	
		total urine	38.6		1.7
172	0–12	6720	12.8	9	0.0
	12–24	5595	7.0	30	0.0
	24–36	11920	12.5	55	0.1
	36–48	8157	6.0	0	0.0
	48–56	7029	8.4	151	0.2
	slaughter	4521	0.3	NA	
		total urine	47.0		0.4[d]

[a] Animals were dosed at 0, 24, and 48 h. [b] Data are expressed as ppm of sodium chlorate equivalents. [c] Animal 171 received 1993.4 μCi; animal 172 received a total dose of 3976.3 μCi; values are expressed as a percentage of the total dose. [d] Radioactivity excreted in feces (expressed as sodium chlorate equivalents) during time periods 0–12, 12–24, and 36–48 summed to a total of 0.1% of the dose.

Table 3. Concentrations of Radiochlorine, Recoveries of Radiochlorine in Tissues, and Total Recoveries of Radiochlorine in Steers 171 and 172

	steer 171			steer 172		
fraction	ppm	μCi	%	ppm	μCi	%
urine		796.4	38.6		1864.7	46.9
feces		34.8	1.7		14.4	0.4
		edible tissues[a]				
liver	69.6	9.7	0.5	80.7	10.0	0.3
kidney	226.0	5.6	0.3	235.5	6.2	0.2
skeletal muscle	52.9	185.2	9.3	46.9	163.3	4.1
adipose tissue	37.8	NA	NA	29.2	NA	NA
	totals	200.5	10.1		179.5	4.6
		inedible tissues[b]				
brain	76.4	1.4	0.1	59.3	1.2	0.0
lung	171.8	12.0	0.6	163.4	10.0	0.3
spleen	129.4	2.5	0.1	125.6	2.3	0.1
skin	140.7	121.3	6.1	149.2	141.7	3.6
heart	93.0	4.3	0.2	102.7	4.7	0.1
diaphragm	73.5	2.0	0.1	74.8	2.0	0.1
remains of C, solid[c]	73.0	24.7	1.2	116.2	57.8	1.5
remains of C, liquid[c]	99.2	14.9	0.7		0.0	0.0
bone	71.1	130.7	6.6	65.5	128.2	3.2
stomach complex[d]	140.1	215.8	10.8	460.4	836.9	21.0
small intestine	269.0	98.5	4.9	320.7	118.0	3.0
large intestine	245.0	65.5	3.3	312.3	87.5	2.2
blood	187.9	68.5	3.4	244.1	67.7	1.7
bile	190.6	0.3	0	240.3	1.9	0.0
	totals	762.4	38.2		1459.9	36.8
cage wash		4.6	0.2		49.4	1.2
	totals	1771.7	88.8		3567.9	89.9

[a] Traditionally edible tissues in the United States. [b] Traditionally nonedible tissues in the United States. [c] Remains of C, remains of carcass; a liquid portion was collected for animal 171 only. [d] The stomach complex consisted of the rumen, reticulum, omasum, and abomasum.

chlorine was 88.8 and 89.9% for animals 171 and 172, respectively.

For each animal, about 10% of the total radioactivity was not accounted for. It is conceivable that some radiochlorine could be lost through respiration, but this is unlikely because the only chlorine species likely to be volatile enough for loss in air would be Cl_2 and ClO_2. Studies by Abdel-Rahman (*15*) and unpublished studies conducted in our laboratory have clearly shown that chlorate and its metabolites are not excreted in expiratory gases of rodents. A more likely cause of the low recovery of radiochlorine was the manner in which the hide was sampled. At slaughter, a sample of the hide was removed from the center of the back were the animal could not lick and where contamination with urine or feces was not an issue. Areas of the hide that were contaminated with saliva, urine, and(or) feces were not sampled; the total amount of radiochlorine present on the hide is almost certainly underestimated.

Summation of the total amount of radiochlorine recovered in nongastrointestinal tissues and in the urine of the steers indicates that 62.1−67.9% of the dosed chlorate was absorbed by steers 172 and 171, respectively. Because cattle were slaughtered only 8 h after the last administration of chlorate (33% of the total dose), these data suggest that chlorate and-(or) its metabolite(s) are rapidly absorbed. Urinary radioactivity represented from 56.9 to 75.5% of the total absorbed radiochlorine in steers 171 and 172, respectively, indicating that radiochlorine was rapidly excreted after absorption. Furthermore, urine collected in the 24 h period after the initial dose contained 30 and 57% of the dosed radiochlorine for animals 171 and 172, respectively.

For edible tissues, total radioactive residues were greatest in kidney (226−236 ppm), followed by liver (70−81 ppm), skeletal muscle (53−47 ppm), and adipose tissue (29−38 ppm). For traditionally nonedible tissues, gastrointestinal tissues generally contained the greatest concentrations of radioactive residue. Ruminal concentrations of radioactivity in steer 172 were about 3.3 times more concentrated than the radioactivity in the rumen of steer 171. In contrast, concentrations of radioactivity in edible tissues of steer 172 matched the concentrations of radioactivity in steer 171 closely, despite the 2-fold dose of chlorate that steer 172 received. If an absorption threshold had occurred, then one might expect higher gastrointestinal concentrations and roughly equal tissue concentrations of radiochlorine between the two animals. However, animal 172 was not consuming normal amounts of feed during the latter portion of the study; if gastrointestinal motility in animal 172 were decreased, then a decreased rate of chlorate absorption would be expected.

Speciation of Tissue Residues. Figure 2 shows an example ion chromatogram of nonradioactive chlorite (ClO_2^-), [^{36}Cl]-chloride, and [^{36}Cl]chlorate and shows the distribution of radioactivity in fractions trapped as solvent eluted from the column. Baseline resolution of the three ions was readily obtained, and the chromatographic distribution of radiochlorine clearly indicates that if radioactive chlorite were present in a tissue sample, it could easily be resolved from either chloride or chlorate.

Figure 3 shows an example chromatogram and a radioprofile of a kidney extract from steer 171. Radioprofiles from other tissues were qualitatively similar to the example radioprofile shown for kidney. Radioprofiles generated from each tissue indicated the presence of [^{36}Cl]chloride and [^{36}Cl]chlorate, but no [^{36}Cl]chlorite-associated radioactivity was detected in any of the tissues.

The speciation of radioactive residues present in edible tissues of cattle is shown in **Table 4**. For steer 171 (low dose), parent chlorate represented from 1.3 to 28.4% of the total radioactive residue, depending on the tissue. Total radioactive residues in liver were comprised almost entirely of chloride, with the concentration of chlorate residue being 0.7 ppm. Chlorate residue concentrations in skeletal muscle, kidney, and adipose

Table 4. Speciation of Total Radioactive Residues in Edible Tissues of Cattle[a]

	steer 171						steer 172					
	TRR[b]	extractability[c]	chloride		chlorate		TRR[b]	extractability[c]	chloride		chlorate	
tissue	(ppm[d])	(%)	%	ppm[d]	%	ppm[d]	(ppm[d])	(%)	%	ppm[d]	%	ppm[d]
adipose tissue	37.8	99.4	94.9	35.9	5.2	2.0	29.2	74.6	59.8	17.5	40.2	11.7
skeletal muscle	52.9	100.4	73.3	38.8	26.7	14.1	46.9	100.7	56.0	26.3	44.9	21.1
liver	69.6	98.2	98.8	52.3	1.3	0.7	80.7	95.4	98.5	46.2	1.6	1.3
kidney	226	96.4	88.6	200	11.5	25.9	236	97.1	71.6	169	28.4	67.0

[a] Reported values are means from duplicate analyses. [b] TRR, total radioactive residues. [c] Percentage of total radioactive residue extracted into water. [d] Calculated as ppm of sodium chlorate equivalents.

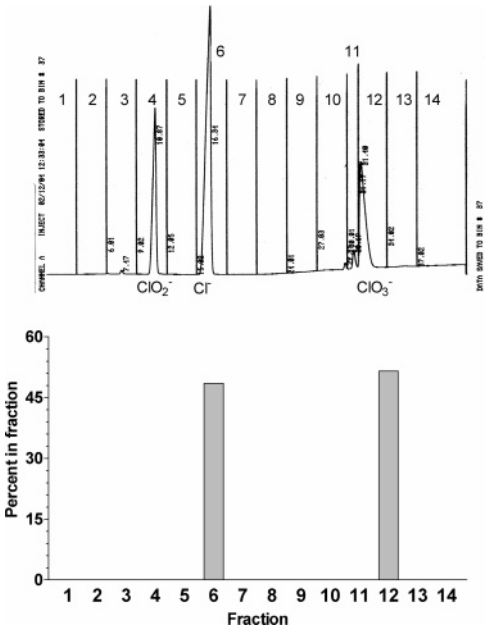

Figure 2. Ion chromatogram of chlorite, [^{36}Cl]chloride, and [^{36}Cl]chlorate standards. (**Top**) Ion chromatogram with vertical lines indicating beginning and ending points of numbered fractions. (**Bottom**) Distribution of radioactivity in collected fractions. Data are expressed as percentage of recovered radioactivity.

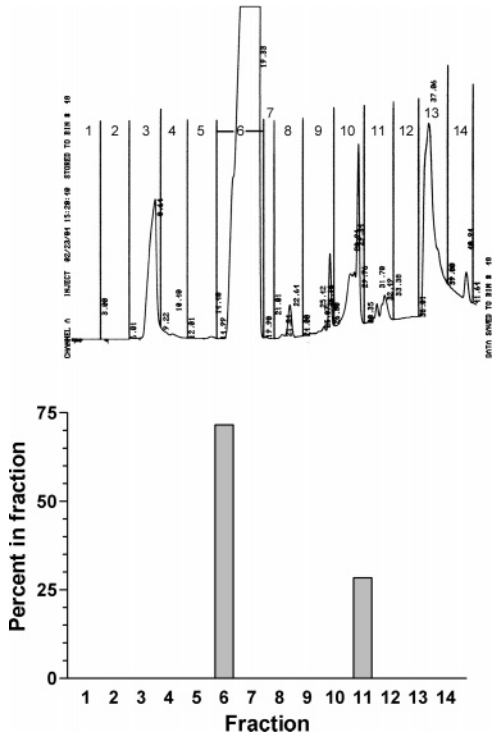

Figure 3. Representative ion chromatogram from a kidney extract of steer 172. (**Top**) Ion chromatogram with vertical lines indicating beginning and ending points of numbered fractions. (**Bottom**) Distribution of radioactivity in collected fractions. Data are expressed as percentage of recovered radioactivity.

tissue were 14.2, 25.9, and 2.0 ppm, respectively. For steer 172, chlorate concentrations ranged from 1.3 ppm in liver to 67.0 ppm in kidney. Skeletal muscle and adipose tissue had intermediate concentrations of chlorate at 21.1 and 11.7 ppm, respectively.

Results of the skeletal muscle, liver, and kidney chloride analysis using phenyl mercuric nitrate are presented in **Table 5**. Reaction with phenyl mercuric nitrate removed over 99% of the chloride from an aqueous extract of [^{36}Cl]chloride-fortified skeletal muscle. In contrast, reaction of phenyl mercuric nitrate with an aqueous extract of [^{36}Cl]chlorate-fortified skeletal muscle removed only 6.6% of the total radioactivity. Because 5.6% of the total radioactivity in the chlorate fortification was chloride ion, it was concluded that reaction of radiochlorine with phenyl mercuric nitrate was specific for chloride. Furthermore, reaction of phenyl mercuric nitrate with radiochlorine extracted from skeletal muscle of steers 171 and 172 indicated that the chloride ion represented 71.9 and 58.1% of the total residue, respectively. These values agree with ion chromatography data indicating that chloride represented 71.6 and 57.0% of the total radiochlorine in skeletal muscle of animals 171 and 172, respectively. In liver, chloride represented an average of 98.7% of the total radioactivity (for animals 171 and 172) as measured by ion chromatography; after reaction with phenyl mercuric nitrate, chloride in livers from animal 171 and 172 assayed at 95.5% of the total residue. Similarly, ion chromatographic analysis of steer 171 kidney indicated that chloride ion represented 88.6% of the radioactive residue, while reaction with PMN indicated that chloride represented 85.1%; the respective values for steer 172 kidney were 71.6 and 70.8%.

Radiochlorine extracted into the chloroform layers was positively identified as phenyl mercuric chloride as evidenced by comparison of mass spectra of authentic phenyl mercuric chloride and peaks eluting at the retention time of phenyl mercuric chloride present in chloroform extracts of animals 171 and 172 (**Figure 4**). Each mass spectrum shows a multiplet of peaks around *m*/*z* 314 [M^+]; the multiplet is due to the seven natural isotopes of mercury [^{196}Hg (0.2%), ^{198}Hg (10.1%), ^{199}Hg (16.9%), ^{200}Hg (23.1%), ^{201}Hg (13.2%), ^{202}Hg (29.7%), and ^{204}Hg (6.8%); (*21*)] in combination with the two natural isotopes of chlorine [^{35}Cl (75.77%), ^{37}Cl (24.23%); (*21*)]. The ion cluster around *m*/*z* 277 represents loss of chlorine, while the base peak at *m*/*z* 77 represents the loss of both mercury and chlorine. The ion at *m*/*z* 112 represents a rearrangement whereby mercury is lost and chlorine is retained.

Table 5. Distribution of Radiochlorine in Aqueous (Chlorate) and Chloroform (Chloride) Fractions after Reaction of Tissue Extracts with Phenyl Mercuric Nitrate and Extraction of Phenyl Mercuric Chloride into Chloroform

			recovery of radiochlorine			
sample no.	tissue ID	fortification[a]	aqueous extract[b] (%)	chlorofom extract[c] (%)	aqueous layer[d] (%)	total recovery (%)
			skeletal muscle			
1	control	$^{36}Cl^-$	98.9	98.5	1.1	99.6
2	control	$^{36}Cl^-$	100.2	99.6	0.7	100.3
3	control	$^{36}ClO_3^-$	109.9	7.3	93.7	101.0
4	control	$^{36}ClO_3^-$	111.3	5.9	91.8	97.7
5	steer 171	none	93.5	71.9	20.8	92.7
6	steer 171	none	98.5	71.9	23.0	94.9
7	steer 172	none	102.0	58.6	36.6	95.2
8	steer 172	none	97.2	57.5	36.5	94.0
			liver			
1	control	$^{36}Cl^-$	99.7	95.5	0.8	96.3
2	control	$^{36}Cl^-$	97.3	94.8	0.6	95.4
3	control	$^{36}ClO_3^-$	110.4	5.8	93.4	99.2
4	control	$^{36}ClO_3^-$	109.1	5.8	95.9	101.7
5	steer 171	none	101.2	94.4	2.5	96.8
6	steer 171	none	97.5	96.1	9.3	105.4
7	steer 172	none	99.0	94.5	0	93.5
8	steer 172	none	98.8	96.9	1.6	98.6
			kidney			
1	control	$^{36}Cl^-$	98.2	92.0	2.7	94.8
2	control	$^{36}Cl^-$	95.8	93.3	0	93.3
3	control	$^{36}ClO_3^-$	100.4	5.6	85.9	91.4
4	control	$^{36}ClO_3^-$	100.5	5.2	89.4	94.6
5	steer 171	none	100.6	82.1	3.9	85.9
6	steer 171	none	100.7	88.0	7.6	95.6
7	steer 172	none	97.2	75.6	13.2	88.2
8	steer 172	none	102.4	66.0	15.2	81.2

[a] Radiolabeled chlorate contained approximately 5.6% [^{36}Cl]chloride ion. [b] Percentage of total fortified or incurred radiochlorine extracted into water. [c] Percentage of total radiochlorine in aqueous extract removed by chloroform. [d] Percentage of total radiochlorine in aqueous layer not removed by chloroform. [e] Sum of percentage radiochlorine in chloroform and aqueous layers.

Table 6. Speciation of Radiochlorine Excreted into Urine of Steers 171 and 172

	steer 171		steer 172	
time period (h)	Cl^- (%)	ClO_3^- (%)	Cl^- (%)	ClO_3^- (%)
0–12	9.0	91.1	3.4	96.6
12–24	35.0	65.0	7.5	92.5
24–36	3.1	96.8	1.9	98.0
36–48	1.7	98.3	0.0	100.0
48–56	1.8	98.2	0.3	99.1

The reaction of tissue extracts with phenyl mercuric nitrate provides additional evidence that the very large peak present in ion chromatogram with a retention time similar to chloride was properly identified. Although quantitative data obtained from the chloride analysis using phenyl mercuric nitrate agree with data obtained from ion chromatographic analysis, the assay was executed to verify that the chloride ion is a major product of chlorate metabolism in ruminants.

Speciation of Urinary Residues. The composition of radiochlorine excreted into urine is shown in **Table 6**. Chlorate was the major radioactive species present in urine with the chloride ion being the only other chlorine species present. Chlorate ranged from 65.0 to 98.3% of the total urinary radioactivity for animal 171 and 92.5 to 100% of the urinary radioactivity for animal 172. For both animals, the largest amount of chloride was excreted during the 12−24 h period after the initial dose. Thereafter, the chloride ion represented a maximum of 3.1% of the urinary radioactivity for both animals. Abdel-Rahman et al. (*15*) reported that 72 h after an oral dose of [^{36}Cl]chlorate, rats had excreted 40% of the total radioactivity in the urine; of this, 62.8% was chloride ion, 25.1% was chlorate ion, and 12.1% was chlorite ion. In cattle, the vast majority of urinary radioactivity was chlorate with little chloride and no chlorite being present. The doses of chlorate administered to cattle in this study were much greater than the dose administered to rats (0.15 mg/kg body weight) by Abdel-Rahman et al. (*15*). In addition, their data were collected over a withdrawal period of 72 h after a single administration, whereas data in this study were collected after three doses and only an 8 h withdrawal period.

In conclusion, chlorate is rapidly absorbed and excreted in steers. Radiochlorine was present in edible tissues primarily as chloride ion, with lesser amounts of chlorate. The proportion of chloride and chlorate was highly tissue dependent. In contrast, chlorate was the major chlorine species present in urine of steers, indicating that the kidney actively excretes chlorate. Because there was a large difference between the proportion of the total residue present as chloride in tissue and urine, it can be concluded that chloride was actively retained, while chlorate was actively excreted.

This study was designed to generate tissue residues after a 3 day chlorate exposure with animals being slaughtered after a practical 0 day withdrawal period. Under this scenario, the doses were high because: (i) chlorate was administered over an extended period even though chlorate efficacy has been demonstrated after a single administration (*3*, *5*); (ii) steers were slaughtered with an 8 h withdrawal period, when efficacy has been measured 24 h after dietary exposure to chlorate (*5*); and (iii) steers were administered 150% of the target dose because it was anticipated that a 0 day withdrawal period might be most useful for cattle producers. With the extended dosing period,

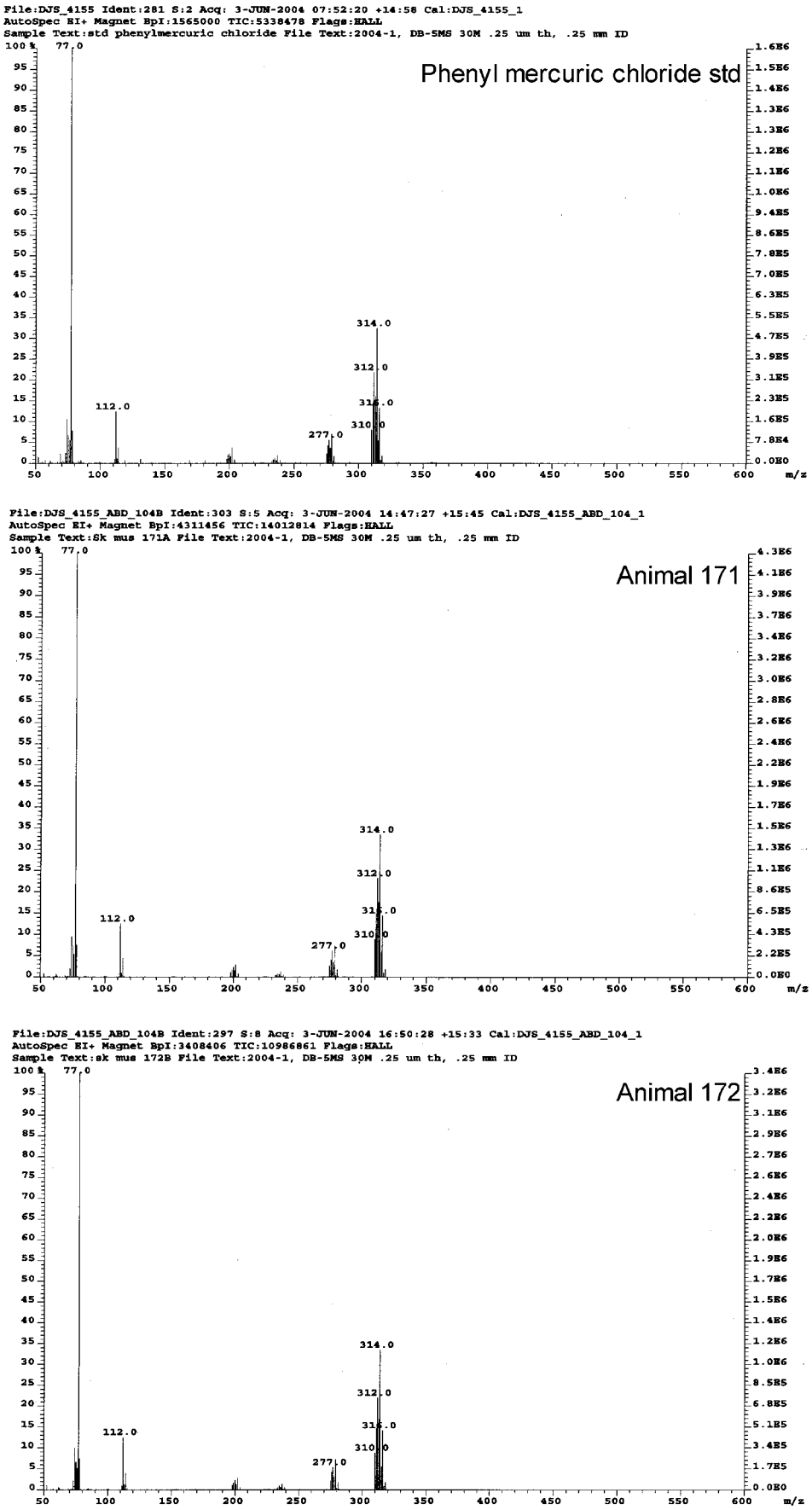

Figure 4. Mass spectra of authentic phenyl mercuric chloride and phenyl mercuric chloride present in chloroform extracts of aqueous homogenates prepared from skeletal muscle of steers 171 and 172.

short preslaughter withdrawal time, and an elevated dosing level, two of the four edible tissues (adipose tissue and liver) from the low dose steer (animal 171) contained chlorate residue levels that are thought to be favorable from a food safety point of view. Although no safe tissue concentrations for sodium chlorate have been established by the U.S. FDA CVM, chlorate residues in adipose tissue and liver fell below provisional estimates of safe tissue concentrations (unpublished) provided by the agency. Because chlorate appears to be rapidly metabolized and excreted in steers and because efficacy has been shown for chlorate at lower doses with extended withdrawal periods, further investigation of sodium chlorate at lower doses and a longer withdrawal period is warranted.

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