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Effect of Sodium [^{36}Cl]Chlorate Dose on Total Radioactive Residues and Residues of Parent Chlorate in Growing Swine

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An experimental chlorate-based product has been shown to be efficacious in eliminating economically important, Gram-negative human pathogens in the gastrointestinal tracts of food animals. Prior to the commercial marketing of such a product, the magnitude and chemical nature of residues remaining in edible tissues must be determined. Thus, the objective of this study was to determine the tissue distribution and elimination of sodium [^{36}Cl]chlorate in orally dosed swine. Three sets of pigs, each consisting of a barrow and a gilt, were orally dosed with a total of 20, 40, or 60 mg of sodium [^{36}Cl]chlorate per kg body weight via the drinking water. Urine and feces were collected throughout the 30 h study. Twenty-four hours after the last exposure to [^{36}Cl]chlorate, each pig was harvested and both edible and inedible tissues were collected. Urine and tissue samples were analyzed for total radioactive residues and for chlorate metabolites. Elimination of radioactivity in urine averaged 81.6, 83.7, and 83.9% of the total dose for the low, medium, and high doses, respectively. Fecal elimination of radioactivity averaged 1.1% of the dosed radiochlorine across all doses. Parent chlorate always represented greater than 97.4% of the urinary radiochlorine with the remaining radiochlorine being excreted as chloride ion. Chlorate represented 39−77% of fecal radioactivity, depending upon dose. Chlorate concentrations in edible tissues ranged from 0.01 to 0.49 ppm, with residues in liver and skeletal muscle generally lower than those in kidney and adipose tissue. Chlorate residues were concentrated in thyroid tissues (7.7−25.4 ppm) relative to edible tissues. No evidence for the presence of chlorite was observed in excreta or in tissues. Results of this study suggest that further development of chlorate as a preharvest food safety tool in swine merits consideration.

KEYWORDS: Sodium chlorate; food safety; pathogens; swine

INTRODUCTION

Respiratory nitrate reductases function in facultatively anaerobic bacteria to capture energy during the conversion of nitrate to nitrite (*1*). Because chlorate (ClO_3^-) is cometabolized by respiratory nitrate reductases to chlorite (ClO_2^-) and because chlorite is toxic to bacteria (*1*), Anderson et al. (*2*) recognized that the metabolism of chlorate by nitrate reductase in Gram-negative pathogens might be exploited for food safety purposes. The vast majority of bacteria present in food animals do not possess nitrate reductase activity; however, economically significant pathogens such as *Escherichia coli* O157:H7 and *Salmonella Typhimurium* express the enzyme when growing under anaerobic conditions (*1*, *3*). Anderson et al. (*2*) hypothesized that when sufficient levels of chlorate are present in the alimentary tract of food animals, pathogens containing nitrate reductase will generate "suicidal" levels of chlorite and will die; those organisms that do not express nitrate reductase were proposed to be unaffected by chlorate.

In vivo studies in both ruminants and nonruminants have validated this hypothesis. For example, chlorate significantly reduced *E. coli O157:H7* populations in gastrointestinal (GI) tracts of cattle and sheep (*4*, *5*) but had little effect on bacterial counts of total culturable anaerobes in ruminal fluid (*2*). Market-age broilers given access to a chlorate-containing product during the 48 h prior to slaughter had significant reductions (40−99%) in crop and cecal *Salmonella* populations (*6*).

In swine, treatment with chlorate is highly effective at reducing populations of both *E. coli* (*7*) and *S. Typhimurium* (*8*−*10*). GI concentrations of *E. coli* were decreased 1.03−2.9 log units (a 62−99.9% reduction, depending on tissue) when sodium chlorate was administered to experimentally infected pigs (*7*) and when the pigs were euthanized 8 h after the last chlorate administration. In weaned pigs artificially infected with *S. Typhimurium* (*8*), animals treated with chlorate contained only about three colony-forming units (CFU) of *S. Typhimurium* per gram of cecal contents, whereas control animals contained

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approximately 1400 CFUs of the pathogen. Consistent with these results are those of Anderson et al. (*9*) who demonstrated that chlorate significantly reduced the incidence of *S. Typhimurium* in lymph, ceca, and recta of finishing pigs.

Collectively, these data suggest that a chlorate-containing product could have several commercial applications, with the preslaughter elimination or reduction of both *E. coli* and *Salmonella* species being of primary importance. The purpose of this study was to determine the magnitude of total and chlorate residues remaining in edible tissues of swine after oral administration, to determine the metabolism of chlorate in swine, and to determine the absorption and elimination of chlorate in tissues and excreta of swine after oral administration.

MATERIALS AND METHODS

Chemicals. Unlabeled sodium chlorate (CAS no. 7775-09-9; 99.96% $NaClO_3$, 0.03% NaCl, and 0.01% H_2O) was provided by EKA Chemicals (Columbus, MS). Sodium chlorate was stored dry at room temperature until use. 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); and acetonitrile and methanol (high-performance liquid chromatography grade; EM Science; Gibbstown, NJ) were also used in the study.

Radiolabeled sodium chlorate ($Na^{36}ClO_3$) having a specific activity of 0.575 mCi/mmol and a radiochemical purity of approximately 95% was purchased from Ricerca Biosciences (Concord, OH). Stock sodium [^{36}Cl]chlorate was purified to a radiochemical purity of 99.9% essentially as described by Ruiz-Cristin et al. (*11*). Briefly, a 2.5 cm × 63 cm column of Sephadex G-10 was equilibrated with 0.1 M ammonium acetate (pH 7.0); stock sodium chlorate (0.8 mL aqueous solution; ~1 mCi) was loaded onto the column and was eluted with ammonium acetate at a flow rate of approximately 0.85 mL per min for approximately 24 h. Fractions were collected every 4.7 min (4 mL), and radiochlorine within each fraction was assessed by liquid scintillation counting of 5 μL aliquots. Under the chromatographic conditions used, [^{36}Cl]chloride eluted in fractions 39−46, [^{36}Cl]chlorate eluted in fractions 47−70, and [^{36}Cl]perchlorate eluted as a broad peak in fractions 229−270. Radiochemical purity of the purified [^{36}Cl]chlorate peak was assessed by ion chromatography as described by Smith et al. (*12*).

Dose Preparation. Purified [^{36}Cl]chlorate was diluted with nonradioactive sodium chlorate to a specific activity of 399 ± 1 dpm/μg. The specific activity was determined as described by Smith et al. (*12*). Three dosing solutions (1 L each) containing 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate, respectively, were prepared in aqueous solutions of 2.5 mM sodium nitrate. Sodium nitrate has been shown to increase the efficacy of chlorate in reducing pathogen numbers in live animals (*13*), presumably by inducing respiratory nitrate reductase in nitrate respiring bacteria. The actual chlorate concentrations were 99.9−100.3% of target values. Each dosing solution was transferred to duplicate 1 L plastic water bottles (Kaytee; Kaytee Products, Chicago, IL) so that water bottles contained 498 ± 0.5 g of dosing solution. Sipper tubes, supplied with the water bottles, were attached, and the bottles were stored frozen until dosing.

Animals and Animal Dosing. Three crossbred barrows (9.2 ± 0.4 kg) and gilts (8.2 ± 0.8 kg) were purchased from the North Dakota State University swine herd. Animals were ear tagged and housed by gender in concrete-floored pens during an 18−25 day acclimation period. Pigs were provided with ad libitum access to a swine starter ration (21.6% crude protein, 3.3% fat, and 2.6% fiber; 3204 kcal/kg metabolizable energy; 77.7% total digestible nutrients; North Dakota State University Feed Mill), which they received for the duration of the study. During the acclimation period, pigs were trained to metabolism crates (*14*) and to drink out of 1 L water bottles equipped with stainless steel "sipper tubes".

Table 1. Target and Actual Doses of Sodium [^{36}Cl]Chlorate and Radiochlorine, Dissolved in Drinking Water Delivered to Barrows and Gilts

animal	sex	dose weight (kg)	target dose[a] (mg/kg)	actual dose mg/kg	actual dose μCi	dose duration[b] (h)	withdrawal period[c] (h)
388	gilt	19.1	20	20.8	71	7.7	23.9
390	barrow	19.9	20	19.6	72	5.6	24.0
387	gilt	17.2	40	46.0	143	5.6	24.0
393	barrow	23.1	40	34.4	143	6.0	24.1
389	gilt	19.7	60	60.8	214	5.5	24.0
392	barrow	18.3	60	65.0	215	6.4	24.1

[a] Doses were delivered in approximately 500 mL of 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate in drinking water. [b] Time from initial exposure to [^{36}Cl]chlorate-containing drinking water to complete consumption of the fortified water. [c] Time between the last exposure to [^{36}Cl]chlorate-containing drinking water and slaughter.

Low, medium, and high chlorate doses were each administered to a single barrow or gilt in each of two periods (i.e., within period, a low, medium, and high dose was administered to three swine). Doses were administered by placing the appropriate water bottle containing the frozen chlorate solution on the metabolism cage immediately above the feed tray. Drips from the sipper tube fell directly into the feed and were thus consumed. The pigs drank the chlorate-containing water as it thawed such that the total dose was delivered to pigs within 6.1 ± 0.8 h instead of the 24 h as originally planned. Nevertheless, a 24 h withdrawal period was maintained, and pigs were harvested at 24.0 ± 0.1 h. The target and actual doses, length of exposure to the dosing solutions, and actual withdrawal times are summarized in **Table 1**.

Collection of Excreta. Swine were housed in metabolism crates that enabled the separate collection of urine and feces (*14*). Urine and feces excreted in the 0−12, 12−24, and 24−30 h time periods were pooled within excreta type for each animal, were weighed, and frozen. At collection, urine and feces were collected as quantitatively as possible. On one occasion, urine from a barrow was excreted beyond the confines of the metabolism crate and urine was recovered from the plastic-backed paper floor covering by placing the contaminated paper in an Erlenmeyer flask, diluting with a known mass of water, and allowing the paper to soak with occasional stirring. Radiochlorine in the water fraction was quantified using liquid scintillation counting.

Animal Harvest and Tissue Collection. Pigs were harvested at the appropriate time by captive-bolt stunning followed by exsanguination into a weighed basin containing 4 mL of heparin (6000 U/mL in physiological saline; Sigma). Pigs were then washed and eviscerated. Traditionally edible tissues (adipose tissue, skeletal muscle, liver, and kidney) and traditionally nonedible tissues (blood, bone, brain, diaphragm, GI contents, GI tract, heart, lung, skin, spleen, thyroid gland, and remainder of the carcass) were collected. Bile was removed using a hypodermic syringe. The GI tract, from the esophagus to the anus, was removed (with pancreatic tissues attached); the GI contents were removed, and the GI tissues and contents were each weighed, the GI contents were subsampled, and both contents and tissues were frozen. Visceral organs, the brain, and the thyroid gland were removed, weighed, and frozen intact. The skin was removed and weighed, and a subsample was removed from the center of the back. Pigs were boned, the bones were weighed, and the scapula was removed as the bone sample. The total muscle was weighed, and a subsample was removed from the *longissimus dorsi*. The remainder of the carcass fraction contained the reproductive tract, trachea, connective tissue, and various tissues not associated with other tissue fractions.

Partially thawed tissues of masses sufficient to pass through a grinder with greater than 50% recovery (brain, diaphragm, GI tract, heart, kidney, liver, lung, and skeletal muscle) were ground; the spleen and thyroid gland were homogenized on dry ice as described by Benville and Tindle (*15*). Adipose tissue was ground with a mortar and pestle after the addition of liquid N_2. Processed tissues were stored frozen. Skin was prepared for total residue analysis by placing 10 ± 0.1 g aliquots into a glass container, adding 90 mL of 1 N NaOH, weighing,

and incubating at 46 °C for approximately 60 h. Under these conditions, the skin was dissolved. Bone was prepared by dissolving approximately one-half of the scapula in 350 mL of 1 N NaOH over 72 h at 90 °C. The solubilized bone solution tended to gel upon cooling; therefore, bone solutions were reheated prior to analysis by liquid scintillation counting (LSC) (described below).

Analytical Methods. LSC techniques, determination of background radiochlorine, and speciation (determination of identity) of total radioactive residues were conducted as described by Smith et al. (*16*) with the following exceptions. Total radioactive residues in skin and bone were determined by placing 0.5 (bone) or 2.0 mL (skin) aliquots of the dissolved tissue in a LSC vial, adding Ultima Gold LSC fluid (15 mL), and counting for 20 min each. The SCX solid-phase extraction step, described by Smith et al. (*16*), was eliminated from the tissue extraction procedure. Briefly, samples were homogenized in water and centrifuged, protein in the resulting supernatant was precipitated with ice-cold acetonitrile, the acetonitrile was evaporated, and the resulting aqueous phase was evaporated under N_2. The remaining aqueous layer was then passed through a C-18 solid-phase extraction cartridge, and the unretained aqueous layer was lyophilized. The dry residue was redissolved in 1 mL of water, and the concentrate was filtered (13 mm, 0.45 μm, PTFE), and subsequently analyzed by ion chromatography as described by Smith et al. (*16*). Urine and tissue sample sets were run with both blanks and blanks fortified with known amounts of [^{36}Cl]chloride and [^{36}Cl]chlorate to determine recovery.

RESULTS

Disposition of Radiochlorine. Table 2 shows the distribution of radiochlorine among edible tissues, nonedible tissues, urine, and feces of dosed swine. Urine contained the greatest portion of the dosed radioactivity. Urine excreted during the first 12 h of the study contained a greater fraction of dosed radiochlorine than any other compartment measured. By the time of slaughter, the cumulative excretion of radiochlorine in urine was 83.1 ± 2.6% of the total dosed activity. Across all doses, feces contained a cumulative 1.1 ± 1.8% of the administered radiochlorine, an amount equal to the 1.0 ± 0.1% of the dosed radiochlorine remaining in edible tissues at slaughter. Nonedible tissues contained an average of 3.9 ± 0.7% of the dosed activity, with bone, skin, and blood retaining the largest percentage of radiochlorine, largely due to the fairly large masses of these fractions.

Concentrations of total residues are shown in **Table 3**. As expected from the recovery data, urine contained high concentrations of total residues, ranging from 62 to 2627 ppm depending upon the animal and excretion period. Concentrations of urinary radiochlorine dropped continuously with time periods for all animals. Concentrations of fecal radiochlorine ranged from nondetectable to 524 ppm in gilt 387. Radioactivity in gilt feces was generally greater than concentrations of radiochlorine in barrow feces due to contamination of feces from gilts with urine. In barrows, fecal radiochlorine concentrations ranged from nondetectable to 102 ppm. At slaughter (i.e., 24 h after the last exposure to chlorate containing water), total radioactive residues in feces were 13−215 ppm.

Total radioactive residues in edible tissues generally fell into the following rank order: kidneys > adipose tissue > liver > skeletal muscle. Concentrations of total residues in edible tissues generally showed an apparent dose−response relationship, except for adipose tissue in which residues in tissue of the low and medium dose animals did not appear to differ. Because the pigs were only 17−23 kg, carcasses contained only a small amount of adipose tissue, and the collected adipose tissue samples contained a relatively high proportion of connective tissue. Analysis of the adipose tissue samples indicated that they contained 62.6 ± 7.6% fat, whereas adipose tissue from a market pig would contain approximately 90% fat (*17*).

Table 2. Distribution and Recoveries of Radiochlorine in Tissues and Excreta of Pigs[a]

	low dose[b]		medium dose		high dose	
	gilt 388 (%)	barrow 390 (%)	gilt 387 (%)	barrow 393 (%)	gilt 389 (%)	barrow 392 (%)
edible tissues						
adipose	0.0[c]	0.0	0.0	0.0	0.0	0.0
kidney	0.0	0.0	0.1	0.1	0.0	0.0
liver	0.1	0.1	0.1	0.1	0.1	0.1
skeletal muscle	1.1	1.0	0.8	0.7	0.9	0.7
total in category	1.2	1.1	1.0	0.9	1.0	0.8
inedible tissues						
blood	0.6	0.4	0.4	0.4	0.5	0.4
brain	0.0	0.0	0.0	0.0	0.0	0.0
diaphragm	0.0	0.0	0.0	0.0	0.0	0.0
GI tissue	0.4	0.4	0.3	0.3	0.4	0.5
GI contents	0.6	0.4	0.4	0.3	0.2	0.5
lung	0.1	0.1	0.1	0.1	0.1	0.1
skin	1.3	1.1	0.7	0.9	0.7	1.0
spleen	0.0	0.0	0.0	0.0	0.0	0.0
thyroid gland	0.0	0.0	0.0	0.0	0.0	0.0
heart	0.0	0.0	0.0	0.0	0.0	0.0
bone	2.0	1.8	1.4	1.4	1.2	1.6
bile	0.0	0.0	0.0	0.0	0.0	0.0
remainder of carcass	0.1	0.1	0.1	0.1	0.0	0.1
total in category	5.1	4.3	3.4	3.5	3.1	4.2
urine						
0–12 h	46.6	55.0	62.3	63.0	54.4	44.8
0–12 h spill					10.2	0.7
12–24 h	28.6	25.2	4.0	21.4	18.4	33.4
24–30 h	4.5	3.3	14.3	2.4	1.7	4.1
total in category	79.7	83.5	80.6	86.8	84.7	83.0
feces						
0–12 h	NF[d]	0.0	1.8	NF	0.0	0.0
12–24 h	1.3	NF	1.0	NF	NF	NF
24–36 h	0.4	0.0	1.7	0.1	0.0	0.2
total in category	1.7	0.0	4.5	0.1	0.0	0.2
cage wash	7.7	8.1	8.2	6.3	7.1	8.5
total recovery	95.4	97.0	97.7	97.6	95.9	96.7

[a] Data are expressed as percentages of the total radiochlorine administered. [b] Doses were delivered in approximately 500 mL of 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate in drinking water. [c] Items containing 0% radioactivity did not necessarily have nondetectable residues (see **Table 3**); generally, the tissues were not of sufficient mass to contain >0.1% of the dosed radiochlorine. [d] NF, no feces were excreted during the indicated time period.

Nature of Residues. *Nature of Urinary and Fecal Residues.* The composition of radiochlorine excreted in urine and feces of swine is shown in **Table 4**. In no instance was chlorite ion detected in urine or fecal samples. Urinary radiochlorine composition was greater than 97% chlorate, regardless of dose or time of radiochlorine excretion. During the initial 12 h of collection, all radiochlorine detected in the urine was parent chlorate. Radioactive residues present in feces collected in the 6 h period prior to slaughter were composed of both chlorate and chloride ions. Chlorate comprised from 39 to 77% of the total fecal residue. Barrows tended to excrete more parent chlorate in feces than gilts.

Nature of Residues in Tissues. The composition of radioactive residues in edible tissues of swine is shown in **Table 5**. In contrast to the composition of residues in excreta, tissue residues were composed primarily of chloride ion rather than chlorate ion. In general, radioactive residues were greatest in adipose tissue (0.13−0.49 ppm) and kidney (0.18−0.20 ppm), followed by skeletal muscle (0.07−0.18 ppm). Chlorate residues were always below 0.04 ppm in the liver, regardless of dose. In contrast to edible tissues, a relatively high concentration of parent chlorate was retained by the thyroid gland (**Table 5**).

Table 3. Concentrations (ppm) of Total Radioactive Residues in Tissues and Excreta of Swine Administered [^{36}Cl]Chlorate in Drinking Water[a]

	low dose[b]		medium dose		high dose	
	gilt 388 (ppm)	barrow 390 (ppm)	gilt 387 (ppm)	barrow 393 (ppm)	gilt 389 (ppm)	barrow 392 (ppm)
edible tissues						
adipose	1.5	1.5	1.6	1.3	2.8	2.8
kidney	2.4	1.7	2.9	2.6	4.5	5.0
liver	0.9	0.7	1.2	1.0	1.7	2.1
skeletal muscle	0.5	0.5	0.9	0.6	1.4	1.3
inedible tissues						
blood	3.5	3.2	4.4	3.9	7.5	7.7
brain	1.4	1.2	2.0	1.5	2.9	3.2
diaphragm	1.2	1.2	1.5	1.1	2.3	2.6
GI tissue	1.6	1.3	2.0	1.9	3.3	4.5
GI contents	1.9	1.0	2.2	1.7	2.8	4.1
lung	2.1	2.0	3.2	2.8	4.5	5.4
skin	1.6	1.4	2.1	2.0	3.2	4.0
spleen	1.4	1.4	2.2	1.7	3.4	4.5
thyroid gland	18.2	19.8	14.0	18.3	17.6	59.8
heart	1.2	1.0	1.9	1.4	2.6	2.9
bone	1.8	1.5	2.6	2.1	3.1	4.1
bile	1.9	1.5	0.0	2.2	4.4	6.1
remainder of carcass	2.0	1.6	3.5	2.2	7.9	5.2
urine						
0–12 h	830	835	2387	1413	2627	1904
12–24 h	423	314	570	412	421	1060
24–30 h	73	86	354	75	62	267
feces						
0–12 h	NF[c]	NDR[d]	524	NF[c]	36	<1
12–24 h	199	NF[c]	307	NF[c]	NF[c]	NF[c]
24–36 h	30	19	215	102	13	55

[a] Data are expressed as sodium chlorate equivalents. [b] Doses were delivered in approximately 500 mL of 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate in drinking water. [c] NF, no feces were excreted during the indicated time period. [d] NDR, no detectable residue.

Table 4. Chlorate Composition of Urinary and Fecal Radiochlorine in Pigs Administered [^{36}Cl]Chlorate in Drinking Water[a]

	low dose[b]		medium dose		high dose	
time	gilt 388	barrow 390	gilt 387	barrow 393	gilt 389	barrow 392
urine						
0–12	100.0	100.0	100.0	100.0	100.0	100.0
12–24	96.2	99.7	98.5	99.1	98.5	98.5
24–30	99.3	99.1	100.0	100.0	97.4	99.5
feces[c]						
24–30	38.8	65.1	50.9	73.1	53.3	76.6

[a] Data are expressed as the percentage of total radiochorine excreted as parent chlorate; the balance of the radiochlorine was excreted solely as chloride ion. [b] Doses were delivered in approximately 500 mL of 7.5, 15, and 22.5 mM sodium [^{36}Cl]chlorate in drinking water. [c] Residues in feces were speciated only for the 24–30 h time period.

For example, chlorate residues in the thyroid glands ranged from a low of 3.4 ppm in pig no. 387 to a high of 41.9 ppm in pig no. 392.

DISCUSSION

Data generated in this study clearly demonstrate that chlorate was rapidly absorbed and excreted in the urine of swine. In these pigs, 83.1 ± 2.6% of the dose was excreted in the urine during the 30 h study period with 56.2 ± 8.5% of the dosed radiochlorine excreted during the first 12 h of the study. Overall, 67% of the radiochlorine excreted in the urine was excreted during the first 12 h of the study. This 12 h period included the 6 h dosing period and the subsequent 6 h period after completion of dosing. The rapid absorption and elimination of chlorate clearly indicate that oral delivery of chlorate via the drinking water is an inefficient means to deliver chlorate to the lower GI tract. Presumably, a more efficient delivery of chlorate to the lower GI tract would increase the efficacy at killing pathogens. Nevertheless, even with the inefficient delivery of chlorate to the lower GI tract, numerous studies have demonstrated chlorate's efficacy against *E. coli* and *Salmonella enterica* in swine (*7*−*9*) dosed in a manner similar to the procedure used in this study.

Chlorate concentrations of 1.25 mM (equivalent to 160 ppm) in bovine ruminal fluid were sufficient to cause 3 log unit reductions of *E. coli* O157:H7 and *S. Typhimurium* (*2*). Feces excreted during the 6 h period immediately prior to slaughter contained 13−215 ppm of total radioactive residue, of which chlorate residues ranged from 7 to 110 ppm. Thus, chlorate concentrations in these swine were typically below chlorate concentrations shown to be active against relevant pathogens in vitro. Total radioactive residues in GI contents (whole tract contents) at slaughter were only 1−4 ppm. It is not known if chlorate is active against Gram-negative pathogens at levels below this, but the low GI residues at 24 h might help to explain why chlorate reduced cecal *S. Typhimurium* concentrations about 3 log units 16 h after the last exposure to chlorate but not 24 h after the last chlorate dose (*8*). Anderson et al. (*8*) suggested that the absence of a chlorate effect at 24 h was a function of the kinetics of chlorate in live swine. The current study serves to emphasize Anderson et al.'s point that there is a "need to develop practical administration procedures that optimize delivery and maintenance of effective concentrations of chlorate to the lower gut."

In contrast to previous reports (*18*, *19*) suggesting that chlorate is metabolized to chlorite (ClO_2^-) and excreted as the chlorite ion, no evidence for the existence of chlorite in urine or tissues of swine was generated in this study. In this regard, swine are similar to cattle (*12*, *16*). This finding diverges from studies conducted in the 1980s, which indicated that rats metabolize chlorate to chlorite and that chlorite is excreted as a urinary metabolite in appreciable quantities (i.e., up to 12% of the dosed chlorate). As discussed by Smith et al. (*16*), and as verified by Hakk et al. (submitted for publication) in a replication of Abdel Rahman's rat study, the analytical method used to measure chlorite in rat excreta (*20*) was not adequate, and results from the studies in rats (*18*, *19*) could not be corroborated (Hakk et al., submitted for publication). Subsequent studies in our laboratory utilizing $^{36}ClO_3^-$ in rats demonstrate that chlorite is not present in rat tissues or excreta. The absence of chlorite in tissues of food animals treated with chlorate has important food safety implications because chlorite is a strong oxidizing agent with toxicological concerns (*21*) of its own.

Residues of parent chlorate in edible tissues of these swine were generally less than 1% of the total radioactive residue. As indicated by the urinary chlorate levels, chlorate is apparently actively excreted, presumably because of extremely poor tubular resorption in the kidney. In contrast, little to no radioactive chloride was excreted into urine during the study. Under normal physiological conditions, about 99% of the chloride ion filtered through the glomerulous is resorbed in the proximal and distal tubules (*22*). Suh and Abdel-Rahman (*23*) determined that the half-lives of chloride absorption and excretion in rats are

Table 5. Total Radioactive Residues, Chloride Residues, and Chlorate Residues in Edible Tissues and Thyroid Glands of Swine

			tissue													
			liver			kidney			skeletal muscle			adipose tissue			thyroid gland		
dose (mg/kg)	animal	sex	TRR[a] (ppm)	Cl^{-b} (ppm)	ClO_3^{-c} (ppm)	TRR[a] (ppm)	Cl^{-b} (ppm)	ClO_3^{-c} (ppm)	TRR[a] (ppm)	Cl^{-b} (ppm)	ClO_3^{-c} (ppm)	TRR[a] (ppm)	Cl^{-b} (ppm)	ClO_3^{-c} (ppm)	TRR[a] (ppm)	Cl^{-b} (ppm)	ClO_3^{-c} (ppm)
20	390	barrow	0.74	0.74	0.01	1.75	1.68	0.07	0.52	0.45	0.09	1.50	1.23	0.27	19.8	12.9	6.4
	388	gilt	0.92	0.91	0.01	2.40	2.05	0.30	0.53	0.47	0.06	1.51	1.40	0.11	18.2	7.8	10.3
		average		0.82	**0.01**		1.86	**0.18**		0.46	**0.07**		1.31	**0.19**	19.0	10.3	**8.4**
40	393	barrow	1.00	0.99	0.02	2.55	2.39	0.17	0.63	0.55	0.08	1.43	1.24	0.17	18.3	6.2	11.9
	387	gilt	1.19	1.18	0.02	2.94	2.69	0.24	0.89	0.83	0.07	1.62	1.52	0.09	14.0	9.9	3.4
		average		1.09	**0.02**		2.54	**0.20**		0.69	**0.07**		1.38	**0.13**	16.2	8.1	**7.7**
60	392	barrow	2.11	2.09	0.03	5.03	4.87	0.17	1.28	0.97	0.26	2.83	2.26	0.58	59.8	17.0	41.9
	389	gilt	1.68	1.61	0.05	4.46	4.24	0.22	1.43	1.31	0.10	2.75	2.35	0.40	17.6	8.1	9.1
		average		1.85	**0.04**		4.55	**0.19**		1.14	**0.18**		2.30	**0.49**	38.7		**25.4**

[a] TRR, total radioactive residues expressed in parts per million of chlorate equivalents; the sum of chloride and chlorate fractions may not equal TRR due to rounding. [b] Chloride residue calculated by multiplying the percentage chloride in extracted sample by the ppm total radioactive residue. The concentrations of chloride do not reflect the physiological concentration of chloride in tissues, only that fraction of total residue present as radioactive chloride ion. [c] Chlorate residue calculated by multiplying the percentage chlorate in extracted sample by the ppm of total radioactive residues.

approximately 19 and 52 h, respectively. In contrast, the absorption and elimination half-lives of chlorate in cattle were approximately 0.7 and 7.7 h, respectively (*24*). If the kinetics of chlorate and chloride in swine are consistent with measurements taken from cattle and rats, then the preponderance of radioactive residues present as chloride ion in tissues of these swine is easily explained: Chlorate is rapidly eliminated whereas chloride is retained in the body.

From a chemical residue perspective, the chlorate residues remaining in edible tissues of swine, regardless of dose, were always 25% or less than chlorate concentrations provisionally estimated by the U.S. Food and Drug Administration to be safe in edible tissues (unpublished results). The relatively high concentration of chlorate in the thyroid gland would be of no concern to humans because thyroid gland is not a common food. It is also not of concern from a swine health perspective because chlorate is envisioned as a food safety tool to be used during a time just before slaughter.

The sites and mechanism(s) of chlorate conversion to chloride ion within swine are not known. It is likely that some reduction of chlorate to chloride could occur via bacterial reduction. Oliver et al. (unpublished results) have shown that approximately 50% of the [^{36}Cl]chlorate fortified into bovine ruminal fluid (100 ppm) was converted to chloride residue within about 24 h. Not all of the conversion appeared to be related to bacteria, however, as there was some reduction of chlorate in ruminal fluid that had been autoclaved prior to incubation. Thus, enzymatic and nonenzymatic processes are likely occurring. Biotransformation of chlorate after absorption also occurs. Smith et al. (*16*) reported that chlorate residues in skeletal muscle from cattle orally dosed with chlorate were converted to chloride during refrigeration (designed to mimic carcass-aging processes) but that chlorate residues in beef cattle muscle were stable when stored frozen for 6 months. Chlorate degradation during refrigeration (4−6 °C) of fortified skeletal muscle has also been observed (Smith et al., unpublished results). Whether chlorate degradation is due to enzymatic processes or due to the direct reduction by physiologic reducing agents within tissues is not known.

Total radioactive residues in thyroid tissues were clearly greater than total residue levels in other tissues. Although a substantial portion of the radiochlorine was chloride, concentrations of chlorate in the thyroid gland were substantially greater than in the liver, kidney, or skeletal muscle. Perchlorate also accumulates in thyroid tissues of rats (*25*−*27*). Radiochemical analysis of extracted radiochlorine from thyroids of these swine clearly indicated that chloride and chlorate, not perchlorate, were present. Thus, these data suggest that chlorate is similar to perchlorate in that it will accumulate in the thyroid tissues of treated animals. This accumulation may be related to the chronic effects of high dose chlorate on cellular proliferation in the thyroid (*28*).

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