EFFECT OF SODIUM [36Cl]CHLORATE DOSE ON TOTAL RADIOACTIVE RESIDUES AND RESIDUES OF PARENT CHLORATE IN SWINE

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Abstract Sodium chlorate effectively reduces the numbers of gram-negative pathogens in gastrointestinal tracts of live animals when administered in the 24 to 72 hour period prior to slaughter. It is believed that a chlorate-based product could be a cost-effective and efficient method to reduce the contamination of carcasses during animal processing. The purpose of this study was to determine a dose of sodium chlorate in swine that would result in chlorate residues below FDA CVM-estimated safe tissue concentrations. Three sets of swine (one barrow and one gilt each) were dosed with 20, 40, or 60 mg/kg sodium [36Cl]chlorate dissolved in drinking water during a 24-hour exposure period. Animals were slaughtered after a 24-hour withdrawal period and edible tissues were removed and analyzed for chlorate content. Total radioactive residues and chlorate residues in edible tissues and excreta of swine will be reported.

Introduction A significant problem in the swine industry is the post-harvest contamination of animal carcasses with gram-negative bacteria that may subsequently cause human illness. Although several post-harvest intervention strategies have been developed that reduce the number of these pathogens on animal carcasses, none are entirely satisfactory because of cost, consumer or packer acceptance, or lack of efficacy. A fundamental problem with post-harvest intervention strategies is that they are directed towards the remediation of carcasses already contaminated with pathogens.

Anderson et al (2000) recognized that sodium chlorate (NaClO3) has the potential to serve as an extremely effective pre-harvest food safety tool in live animals. Gram-negative pathogens such as E. coli O157:H7 and Salmonella species contain respiratory nitrate reductase that normally allows the bacteria to convert nitrate to nitrite (Stewart, 1988). Anderson et al realized that chlorate may also serve as a substrate for respiratory nitrate reductase and that it could be converted to the bacterial toxin chlorite by nitrate reductase (ClO2−; van Wijk and Hutchinson; 1995). The vast majority of bacteria present in swine, however, do not possess nitrate reductase activity, making this enzyme an attractive target for development of a pathogen specific control agent. Knowing that chlorite is cytotoxic to bacteria, Anderson et al. hypothesized that when sufficient levels of chlorate are present in the alimentary tract, 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 non-ruminants have validated this hypothesis. For example, chlorate significantly reduced E. coli O157:H7 populations in gastrointestinal tracts of cattle and sheep (Callaway et al., 2002; Callaway et al., 2003), but had little effect on bacterial counts of total culturable anaerobes in ruminal fluid (Anderson et al., 2000). Market-age broilers given access to a chlorate-containing product during the 48 hours prior to slaughter had significant reductions (40-99%) in crop and cecal Salmonella populations (Byrd et al, 2003).

In swine, treatment with chlorate is highly effective at reducing populations of both E. coli O157:H7 (Anderson et al, 2001a) and Salmonella serotype Typhimurium (Anderson et al., 2001b; 2004). Gastrointestinal levels of E. Coli O157:H7 decreased 1.03 to 2.9 log units (a 62 to 99.9% reduction, depending on tissue) when sodium chlorate was administered to experimentally infected pigs (Anderson et al, 2001a) and euthanized 8 hours after the last chlorate administration. In weaned pigs artificially infected with Salmonella Typhimurium (Anderson et al, 2001b), a huge difference in pathogen numbers existed between control animals and chlorate treated animals. For example, pigs treated with chlorate contained only about 3 colony-forming units (CFU) of Salmonella Typhimurium per gram of cecal contents, whereas control animals contained approximately 1,400 CFUs of the pathogen. Commensurate with these results are those of Anderson et al, (2004) who demonstrated that chlorate eliminates Salmonella Typhimurium in finishing hogs. Both numbers of CFUs in cecal contents and the incidence of animals testing positive for Salmonella were decreased after treatment with chlorate. Independently, Burkey et al have shown...
that sodium chlorate feeding reduces fecal shedding of *Salmonella enterica* in swine (Burkey et al., 2004) and that sodium chlorate did not adversely affect animal performance (Burkey et al., 2003).

Studies investigating sodium chlorate in live hogs have progressed to the degree that knowledge of chlorate residues in swine is critical to further development of a chlorate-based product. Because sodium chlorate is not naturally occurring and will ultimately be added to swine feed or water, the US FDA CVM must approve its use. To date, no studies have been conducted investigating the magnitude of residues or degree of chlorate metabolism in hogs. Because the US FDA has provided the authors with provisional safe tissue concentrations of chlorate and chlorite, a benchmark exists with which to compare chlorate residues in treated swine. Therefore the objective of this study was to investigate the fate of radiolabeled chlorate (Na\(^{36}\)ClO\(_3\)) in swine with particular emphasis on determining a dose of chlorate that will result in safe residue concentrations in edible tissues. These data will either support or refute the safe use of sodium chlorate in hogs.

**Materials and Methods** Radiolabeled sodium chlorate (Na\(^{36}\)ClO\(_3\)) was synthesized by Ricerca Biosciences (Concord, OH) and purified in-house to a radiochemical purity of greater than 99% using Sephadex G-10 as described by (Ruiz-Cristin et al., 1989). Radiochemical purity of the purified sodium \([^{36}\text{Cl}]\)chlorate was assessed using ion and paper chromatographic techniques. Radiolabeled sodium chlorate was diluted with unlabelled sodium chlorate to a specific activity of approximately 0.18µCi/mg (~400 dpm/µg). There was no control article used in this study.

Seven cross-bred, weaned barrows (4) and gilts (3) (approximately 10 kg) were purchased, ear tagged, and weighed. Animals were provided *ad libitum* access to feed during the adaptation and training period. Six animals served as test animal and one barrow served as a source of control tissue. Hogs were adapted for 1 week prior to the initiation of training; swine were trained to metabolism crates and trained to drink from water bottles. Animals were provided water containing sodium \([^{36}\text{Cl}]\)chlorate (7.5, 15, and 22.5 mM; approximately 20, 40, and 60 mg/kg bw) and 2.5 mM sodium nitrate (Anderson et al., 2001b) for a 24 hour period. Sodium nitrate has been shown to induce respiratory nitrate reductase and renders target pathogens more susceptible to chlorate (Jung et al., 2003). After the 24-h exposure period, water was removed from each hog and replaced with untreated water for a 24 hour period. All urine and feces were collected (0-12, 12-24, 24-36, 26-48 h) during the dosing and withdrawal periods. Twenty-four hours after the final exposure to Na\(^{36}\)ClO\(_3\) animals were slaughtered by captive bolt followed by exsanguination; blood was collected into a heparinized basin, weighed and sampled. Adipose tissue, brain, diaphragm, gastrointestinal tract, kidney, liver, lung, skeletal muscle, skin, spleen, thyroid, and stomach was removed and weighed.

Radioactivity in tissues, urine, and fecal samples will be determined as described by Smith et al (2005). The identity of metabolites in tissues (after extraction with water and SPE cleanup) and urine will be determined using ion chromatography (HPLC and/or TLC) and co-chromatography with chlorate, chlorite, chloride, and/or perchlorate standards. Quantitative analysis of metabolites will be accomplished using radiochromatography, liquid scintillation of metabolites trapped as they elute from the HPLC column, and/or with the use of a Bioscan TLC plate reader.

**Results** At this writing, the analytical phase of the experiment is incomplete. Tissue residue data will either support or refute the concept that chlorate may be safely used in commercial swine operations.

**Discussion** Several facets of sodium chlorate make its development as a pre-harvest food safety tool appealing. First, oral chlorate clearly works in swine to reduce pathogen loads. Second, chlorate is inexpensive and is easily formulated into water or premixes. Third, chlorate salts are palatable and oral delivery is feasible. Fourth, the US FDA Center for Veterinary Medicine (FDA-CVM) has provided provisional safe tissue concentrations (pSTC) for chlorate and chlorite residues in edible tissues of food animals. The availability of pSTCs provides “benchmark” residue concentrations that are extremely beneficial during the development of a practical product. Fifth, the environmental impact of chlorate feeding will likely be negligible because chlorate-metabolizing bacteria are surprisingly ubiquitous in the environment (Coats et al., 1999). And sixth, parent chlorate excreted from dosed animals could potentially reduce burdens of pathogens present in animal facilities and decrease the rates of pathogen transmission.

In order for the continued development of chlorate as a pre-harvest food-safety tool, chlorate residues in edible tissues of food animals must be shown to be inconsequential to human health.
To this end, Smith et al. (2005) used a Na\textsuperscript{36}ClO\textsubscript{3} tracer to demonstrate that chlorate (ClO\textsubscript{3}) is rapidly metabolized to chloride (Cl\textsuperscript{-}) by cattle and demonstrated that chloride is the major chlorate-derived residue present in edible tissues. Because chloride is a nutrient, its presence in edible tissues of food animals is inconsequential from a food safety point of view. Chlorite, a metabolite of chlorate also of toxicological concern, was absent from beef tissues. In cattle, residues of parent chlorate fell well below the estimated safe tissue concentration provided by the FDA Center for Veterinary medicine, even when animals were dosed at 1.5 times the anticipated chlorate use level (Smith et al., unpublished). Similar residue profiles in swine should lead to further opportunities to investigate the efficacy of chlorate in larger sets of animals.

Conclusions Data generated in this study will support or refute the hypothesis that sodium chlorate may be safely used as a pre-harvest food safety tool in swine.

References


