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## Microbial Efficacy of Commercial Application of Cecure<sup>®</sup> CPC Antimicrobial<sup>1</sup> to Ingesta-Contaminated Pre-Chill Broiler Carcasses

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**Abstract:** A series of three 12-week in-plant trials were conducted in three USDA-inspected broiler processing facilities (Plants A, B, and C) to determine the antimicrobial efficacy of Cecure (cetylpyridinium chloride or CPC) as a pre-chill whole carcass spray treatment for continuous on-line reprocessing of poultry. The trials were designed to evaluate the microbial effects of treating inspection-held, "visibly contaminated" carcasses in accordance with USDA guidelines for proposed continuous on-line reprocessing trials. Treatment of this group of carcasses was necessary to determine whether Cecure could be utilized as an integral part of a plant's on-line reprocessing procedures for carcasses accidentally contaminated with digestive tract contents. During each of the three 12-week trials, carcasses were collected on 20 sampling days. On each sampling day, within each plant, four groups of carcasses (n=10) were collected including visibly clean, inspection-passed carcasses; visibly contaminated, inspection-held carcasses; visibly contaminated carcasses after Cecure treatment; and, visibly contaminated carcasses after traditional off-line reprocessing (manual washing with 20 ppm chlorinated water). All carcasses were microbiologically evaluated for aerobic plate count (APC), *Escherichia coli* (*E. coli*), total coliform, incidence and level of *Campylobacter*, and incidence of *Salmonella*. Treatment of visibly contaminated carcasses with Cecure, in comparison to the other three groups of carcasses, significantly ( $P \leq 0.0001$ ) reduced APC by 2.5 to 3.9 logs, *E. coli* by 1.6 to 2.9 logs, total coliform by 1.2 to 2.7 logs, and *Campylobacter* by 0.8 to 2.1 logs. CPC treatment also resulted in significant reductions in the incidence of *Campylobacter* and *Salmonella* on pre-chill carcasses. Incidence of both *Salmonella* and *Campylobacter* on Cecure-treated pre-chill carcasses never exceeded 9% while incidence of these two organisms was as high as 33.5% for *Salmonella* and 98.7% for *Campylobacter* on untreated visibly contaminated control carcasses. The microbial condition of Cecure-treated visibly contaminated carcasses was also significantly improved in comparison to the microbial condition of non-treated, inspection-passed "clean" carcasses and traditional off-line reprocessed carcasses. The data suggest that the use of Cecure as a pre-chill carcass spray treatment provides a viable alternative to labor intensive off-line reprocessing as well as providing all carcasses with significantly reduced overall microbial levels and decreased incidence of at least two potential human pathogens.

**Key words:** Broilers, reprocessing, CPC, *Salmonella*, *Campylobacter*

### Introduction

Researchers have estimated that as many as 1.0% or close to 70 million U.S. broilers require reprocessing each year (Blankenship *et al.*, 1975; USDA, 1991). Fletcher *et al.* (1997) estimated that some plants may reprocess as much as 5% of total production. The 1998 Code of Federal Regulations allows for the reprocessing of poultry carcasses that are accidentally contaminated during slaughter with digestive tract contents (CFR, 1989). In order to accomplish reprocessing, processors can either remove the affected carcasses from the evisceration line after inspection and clean, trim and sanitize each carcass individually, by hand, "off-line" or they may use one of several USDA approved continuous "on-line" reprocessing methods. Unlike off-line reprocessing, on-line reprocessing does not require contaminated carcasses to be removed from the evisceration line. Subsequently, on-line reprocessing results in all carcasses being subjected to

reprocessing procedures, not just those with visible contamination. As is the case with off-line reprocessing, the on-line methods must be capable of visibly and microbiologically improving the condition of the affected carcasses to a point which they are indistinguishable from non-contaminated carcasses. Currently the list of chemical substances approved for continuous on-line reprocessing includes acidified chlorine, chlorine dioxide, bromine, hydrogen peroxide plus peracetic acid, a low pH acid blend product, trisodium phosphate (including a low pH product), and most recently, Cecure (CPC antimicrobial). Additionally, some of the approved substances are used in conjunction with physical means to remove visual contaminants including one or more inside/outside bird washers, high pressure sprays, and various types of water-sprayed brushes. In order to have a process approved for on-line reprocessing an applicant must submit a research protocol to USDA for evaluation and approval. After a

protocol is approved by USDA the applicant (typically a company) must perform the research trials requested by USDA in a specified number of commercial broiler processing facilities over a given amount of time (typically 90 days per plant). The data are then sent to USDA for review and a final decision is made as to whether the process will be allowed for continuous on-line reprocessing in plants throughout the country without any additional plant trials. The criteria used to make this decision are primarily related to the microbiological condition of the carcasses, whether the process effectively removes visible contamination from the carcasses prior to immersion chilling, and whether the inspection process is adversely affected.

CPC (cetylpyridinium chloride) is the active ingredient in the Cecure antimicrobial solution (CPC diluted in food grade propylene glycol; Cecure stock solution is 40% CPC by weight). CPC is a quaternary ammonium compound with a hydrophobic tail. Additionally, CPC is a positively charged cationic surfactant which reduces surface tension of water on hydrophobic surfaces. CPC is a common ingredient in many over-the-counter mouthwashes, toothpastes, and throat lozenges. Numerous researchers have documented the antimicrobial efficacy of CPC for treatment of poultry (Breen *et al.*, 1995; Kim and Slavik, 1995; Breen *et al.*, 1997; Waldroup *et al.*, 1999). In these studies the target organisms were *Salmonella typhimurium* and *E. coli*. In all studies both organisms were reduced by greater than 1 log to as much as 4 logs (90 to 99.99%). Reductions were dependent on CPC concentration, time of direct exposure, and the ratio of treatment volume to product surface area. The FDA approved the use of CPC (as Cecure) for the antimicrobial treatment of raw poultry carcasses on May 3, 2004. The food additive petition (FAP 2A4736) defines the parameters for use as follows: cetylpyridinium chloride for use at an application rate not to exceed 0.3 g CPC per pound of poultry in an aqueous solution as an antimicrobial treatment to treat by a fine spray mist the surface of raw poultry carcasses (FDA, 2004). During the 12 months after FDA approval (June 2004 to June 2005), Cecure was evaluated by USDA as a continuous on-line processing treatment in three commercial broiler processing plants in the United States. The results of these trials were used to obtain a letter of no objection for the use of Cecure for continuous on-line reprocessing in broiler processing facilities.

## Materials and Methods

**Processing plants and evaluation plan:** Three commercial broiler processing plants (Plants A, B, and C), representing three broiler companies, participated in this study. In each of the three commercial plants, samples of carcasses were collected over a 12-week period. In each of the three plants carcasses were sampled three days per week during the first four weeks,

and one day per week during the remaining eight weeks as requested by USDA. On each sampling day, four groups of carcasses were collected (n=10 per plant) as follows:

- 1 visibly clean, USDA inspection-passed carcasses collected after the final inside-outside bird washer;
- 2 visibly contaminated, USDA inspection-held carcasses collected after the final inside-outside bird washer;
- 3 visibly contaminated, USDA inspection-held carcasses collected after the Cecure on-line spray treatment; and,
- 4 visibly contaminated, USDA inspection-held carcasses collected after traditional manual, off-line reprocessing.

In order to identify and keep track of carcasses as they moved down the evisceration line, carcasses from each of the four sampling groups were leg-banded with a different color band. No carcasses with missing parts were used in the study to minimize differences in sample weight and carcass surface area. For plants A and B there were a total of 200 samples collected per sampling group during the 12-week evaluation period. For plant C, there were a total of 180 samples collected per sampling group during the 12-week evaluation period.

In an attempt to account for normal variability in microbial contamination of poultry throughout the processing day, each day's carcass samples were collected as two 5-carcass sub-sets (each sub-set originating from a different flock of birds). This was accomplished by collecting the two sample sets at least 2 ½ hours apart and from verification from plant management. In each plant, the first sample collection period was at least 30 minutes after plant startup.

The three plant trials were not conducted simultaneously but as follows: Plant A was evaluated during the summer of 2004, Plant B was evaluated during the Fall and early Winter of 2004, and Plant C was evaluated during the late Winter of 2004 and the Spring of 2005. This sampling schedule was followed to allow time for USDA to evaluate data from the previous plant before a trial was started in another facility. Each of the three plants were processing different sized live birds as follows: Plant A birds ranged from 5.0 to 7.0 pounds, Plant B birds ranged from 6.0 to 13.0 pounds, and Plant C birds ranged from 5.5 to 7.5 pounds. The line speed in Plants A and C was 70 birds per minute, and the line speed in Plant B ranged from 11 to 52 birds per minute depending on the weight of the birds being processed.

**Sampling group location and collection:** Carcasses designated as "visibly clean, inspection-passed" were banded after inspection and were returned to the processing line. These carcasses then continued through all evisceration steps and were removed from

the line after the last inside-outside bird washer but before the Cecure spray cabinet. After removal of these carcasses from the processing line, they were placed directly into standard whole carcass rinse bags and processed as described below under microbiological procedures.

Carcasses designated as “visibly contaminated, inspection-held” were selected by the plant’s USDA personnel, removed from the line, banded, and returned to the line. These carcasses then continued through all evisceration steps and were removed from the line for sampling after the last inside/outside bird washer but just before the Cecure spray cabinet. After removal of these carcasses from the processing line they were placed directly into whole carcass rinse bags and processed as described below under microbiological procedures.

Carcasses designated as “visibly contaminated, inspection-held + Cecure” were selected by the plant’s USDA personnel, removed from the line, banded, and returned to the line. These carcasses continued through all evisceration steps including passage through the Cecure spray cabinet. Following Cecure application, these carcasses were allowed to drip for 45 to 60 seconds and were then collected into whole carcass rinse bags and processed as described below under microbiological procedures.

Carcasses designated as “visibly contaminated, inspection-held + off-line reprocessing” were selected by the plant’s USDA personnel, removed from the line, banded and transported to the off-line reprocessing station where they were washed individually, by hand with chlorinated (20 ppm) water. Following off-line reprocessing these carcasses were allowed to drip for 45 to 60 seconds and were then collected into whole carcass rinse bags and processed as described below under microbiological procedures.

**Cecure application:** Cecure was applied as a spray using stainless-steel spray nozzles inside a 4 linear foot application cabinet. The Cecure application cabinet was positioned between the last inside/outside bird washer and the chiller. The actual concentration of CPC in the Cecure spray solution was determined by use of a flow-through ultraviolet light sensor. The UV sensor was designed specifically for commercial processing plant duty and consisted of a sanitary flow cell with quartz windows, a lamp and power supply housing, and a measurement detector assembly enclosed within 316 stainless steel. All component parts were constructed of USDA and FDA acceptable materials. The UV sensor was used to measure the concentration of CPC in the Cecure spray solution, and, via computer integration and connection to a chemical feed pump, maintain the intended level of CPC. The Cecure solution was recycled during all plant trials using two sets of filtration units and

a by-pass loop between the filtered spray solution and the system pump. Extensive laboratory testing using Cecure diluted in poultry drip water collected from a commercial processing plant, demonstrated that the background interference from the drip water itself could be extracted from the CPC concentration using a dual channel sensor (FDA, 2004). The UV absorptivity of CPC at 254 nm greatly exceeds that of background material (at equal concentrations).

The Cecure application system is designed to operate under complete redundant computer control. The only information that had to be periodically input into the system was average live weight. Based on the average live weight and the percent Cecure solution to be sprayed, the application system automatically delivered the appropriate volume as dictated by line speed. Additionally, the application of Cecure spray stopped immediately when the line was halted and began again when the line started. As specified in FAP 2A4736 (FDA, 2004), Cecure can be applied at a maximum level of 0.3 g CPC/pound of pre-chill poultry. The actual spray volume and concentration varies but in these trials was typically in the range of 2 to 3.3 ounces of Cecure per pound of carcass and the CPC concentration was typically between 0.5 to 0.7%.

**Microbiological procedures:** For each sample group, sterile whole carcass rinse bags and sterile gloves were used to aseptically collect the carcasses. All carcasses were rinsed using a widely accepted whole carcass rinse technique in 400 mL of Butterfield’s Phosphate Buffer (USDA, 1998). To ensure the microbiological analysis reflected the actual bacterial levels on the Cecure-treated carcasses, all rinse solutions (even those from non-Cecure treated carcasses) were neutralized immediately after collection by the addition of activated carbon (2 g/10 mL of rinse fluid).

All samples were held on ice no longer than 30 hours (typically no longer than 6 hours) prior to initiation of microbiological plating procedures. All samples were evaluated for total aerobic plate count (APC), *E. coli*, and total coliform using Petrifilm<sup>TM3</sup>. *Salmonella* incidence was conducted in accordance with the AOAC-approved Bax® PCR System<sup>4</sup> utilizing USDA guidelines for sample volume (30 mL) (USDA, 1998). *Campylobacter* incidence and level were determined in accordance with the USDA/FSIS Microbiology Laboratory Guidebook (USDA, 1998).

All quantitative microbiological data obtained from this study were transformed to log<sub>10</sub> CFU/mL prior to statistical analysis. The lower detection level (1 CFU/mL) was utilized for all negative samples. The General Linear Model and Duncan’s Multiple Range Test of SAS (SAS Institute, 1991) were utilized to statistically analyze all data obtained from this study.

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Table 1: Effects of reprocessing procedure on microbial condition of pre-chill carcasses in three commercial poultry processing plants (Treatment means  $\pm$  SD, n = 200 for Plants A and B; n = 180 for Plant C)

Treatments <sup>1</sup>	APC	<i>E. coli</i>	Coliform	<i>Campylobacter</i>
log <sub>10</sub> CFU/mL				
Plant A1	3.2 $\pm$ 0.3 <sup>b</sup>	1.4 $\pm$ 0.3 <sup>b</sup>	0.7 $\pm$ 0.4 <sup>b</sup>	1.7 $\pm$ 0.5 <sup>b</sup>
2	3.7 $\pm$ 0.3 <sup>a</sup>	1.8 $\pm$ 0.3 <sup>a</sup>	1.3 $\pm$ 0.4 <sup>a</sup>	2.3 $\pm$ 0.4 <sup>a</sup>
3	1.2 $\pm$ 0.7 <sup>d</sup>	0.2 $\pm$ 0.2 <sup>c</sup>	0.1 $\pm$ 0.3 <sup>c</sup>	1.1 $\pm$ 0.1 <sup>c</sup>
4	3.0 $\pm$ 0.3 <sup>c</sup>	1.3 $\pm$ 1.3 <sup>b</sup>	0.8 $\pm$ 0.4 <sup>b</sup>	1.7 $\pm$ 0.4 <sup>b</sup>
Plant B1	3.7 $\pm$ 0.3 <sup>c</sup>	2.2 $\pm$ 0.3 <sup>b</sup>	1.7 $\pm$ 0.4 <sup>c</sup>	1.5 $\pm$ 0.2 <sup>c</sup>
2	3.9 $\pm$ 0.4 <sup>b</sup>	2.3 $\pm$ 0.4 <sup>b</sup>	1.9 $\pm$ 0.5 <sup>b</sup>	1.8 $\pm$ 0.4 <sup>b</sup>
3	0.5 $\pm$ 0.4 <sup>d</sup>	0.2 $\pm$ 0.3 <sup>c</sup>	0.1 $\pm$ 0.2 <sup>d</sup>	1.0 $\pm$ 0.1 <sup>d</sup>
4	4.3 $\pm$ 0.6 <sup>a</sup>	2.8 $\pm$ 0.7 <sup>a</sup>	2.4 $\pm$ 0.7 <sup>a</sup>	2.2 $\pm$ 0.6 <sup>a</sup>
Plant C1	4.7 $\pm$ 1.0 <sup>a</sup>	3.0 $\pm$ 0.9 <sup>a</sup>	2.9 $\pm$ 0.8 <sup>a</sup>	3.0 $\pm$ 0.9 <sup>a</sup>
2	4.9 $\pm$ 0.9 <sup>a</sup>	3.1 $\pm$ 0.9 <sup>a</sup>	2.9 $\pm$ 0.8 <sup>a</sup>	3.1 $\pm$ 1.0 <sup>a</sup>
3	1.0 $\pm$ 0.9 <sup>b</sup>	0.2 $\pm$ 0.5 <sup>b</sup>	0.2 $\pm$ 0.4 <sup>b</sup>	1.0 $\pm$ 0.3 <sup>b</sup>
4	4.7 $\pm$ 0.9 <sup>a</sup>	2.9 $\pm$ 0.8 <sup>a</sup>	2.8 $\pm$ 0.7 <sup>a</sup>	3.1 $\pm$ 0.9 <sup>a</sup>

<sup>1</sup>1 = Inspection-passed, no visible contamination.

2 = Inspection-held, visible contamination.

3 = Inspection-held, visible contamination + Cecure spray treatment.

4 = Inspection-held, visible contamination + off-line manual reprocessing.

<sup>abcd</sup>Means in a column with no common superscripts differ significantly ( $P \leq 0.0001$ ).

### Results and Discussion

There was a significant plant (n=3) by treatment group (n=4) interaction in levels of APC, *E. coli*, coliform, and *Campylobacter*. Thus, the data from the study is presented and discussed by plant (Tables 1 and 2). All data are presented as log<sub>10</sub> CFU/mL. In Plant A, the APC on the inspection passed pre-chill carcasses was 3.2, which was significantly lower ( $P \leq 0.0001$ ) than the level of 3.7 for the visibly contaminated, inspection-held carcasses. This same finding was noted for *E. coli*, coliforms, and *Campylobacter* in that the levels of organisms recovered were significantly ( $P \leq 0.0001$ ) lower (by approximately 0.5 log) for carcasses that were visibly clean in comparison to those that were visibly contaminated with digestive tract contents. *Salmonella* incidence on the inspection passed carcasses was 28%, while the inspection held visibly contaminated carcasses had a *Salmonella* incidence rate of 34%. These results indicate that prior to any type of reprocessing, pre-chill carcasses that are visibly contaminated with ingesta tract contents may harbor 2.5 to 4 times as many total organisms, *E. coli*, coliform and *Campylobacter* in comparison to inspection passed visibly clean carcasses. Off-line reprocessing of visibly contaminated carcasses using the standard manual rinsing procedure with 20 ppm chlorine resulted in significant ( $P \leq 0.0001$ ) reductions in APC, *E. coli*, coliforms, and *Campylobacter*. In fact, *E. coli*, coliform, and *Campylobacter* levels on the off-line reprocessed carcasses were equal to levels on the visibly clean inspection passed carcasses. Incidence of *Salmonella* was reduced from 34% for the visibly contaminated carcasses to 20% after manual reprocessing. This incidence rate was lower than the incidence rate of 28% for the inspection-passed, visibly clean carcasses. These results are in agreement with other studies that

have evaluated the microbial efficacy of off-line reprocessing (Walsh and Thayer, 1990; Blankenship *et al.*, 1993; Waldroup *et al.*, 1993; Powell *et al.*, 1995; Bilgili *et al.*, 2002). Carcasses deemed visibly contaminated by inspection personnel but treated with Cecure had significantly ( $P \leq 0.0001$ ) lower counts for all organisms evaluated in comparison to counts on all three of the other groups of carcasses. It is important to remember that Cecure treated carcasses were actually inspection held, visibly contaminated carcasses; but, that after the Cecure spray treatment the microbial counts from these carcasses were lower than counts from off-line reprocessed carcasses or counts from inspection passed carcasses that had no visible contamination.

Cecure treatment resulted in a 2 to 2.5 log reduction in APC, a 1.2 to 1.6 reduction in *E. coli*, and a 0.6 to 1.2 log reduction in coliform and *Campylobacter*. Incidence of *Campylobacter* was decreased to 8% (in comparison to >98%) and incidence of *Salmonella* to 9% (in comparison to >33%). In a study conducted by Fletcher *et al.* (1997) it was reported that continuous on-line reprocessing of visibly contaminated carcasses using 20 ppm chlorine in an inside/outside bird washer reduced visible contamination but did not significantly improve microbial levels beyond that of visibly clean carcasses. In the present study, the Cecure treatment significantly enhanced the microbial condition of visibly contaminated carcasses even in comparison to visibly clean, inspection-passed carcasses.

In Plant B the APC, coliform, and *Campylobacter* counts on inspection held, visibly contaminated carcasses were significantly higher ( $P = 0.0001$ ) than counts on inspection passed, non-contaminated carcasses as was the case in Plant A. However, the differences did not appear to be as great (0.2 to 0.3 log) as were noted

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Table 2: Effects of reprocessing procedure on incidence of *Salmonella* and *Campylobacter* on pre-chill carcasses in three commercial poultry processing plants

Treatments <sup>1</sup>	<i>Salmonella</i>		<i>Campylobacter</i>	
	Incidence <sup>2</sup>	% Positive	Incidence	%Positive
Plant A1	56/200	28.0	158/189	83.6
2	67/200	33.5	186/189	98.4
3	18/200	9.0	15/189	7.9
4	39/200	19.5	164/189	86.8
Plant B1	12/200	6.0	172/200	86.0
2	10/200	5.0	169/200	84.5
3	5/200	2.5	5/200	2.5
4	15/200	7.5	189/200	94.5
Plant C1	49/180	27.2	135/150	90.0
2	40/180	22.2	148/150	98.7
3	2/180	1.1	14/149	9.4
4	40/180	22.2	139/150	92.7

<sup>1</sup>1 = Inspection-passed, no visible contamination.

<sup>2</sup>2 = Inspection-held, visible contamination.

3 = Inspection-held, visible contamination + Cecure spray treatment.

4 = Inspection-held, visible contamination + off-line manual reprocessing.

<sup>2</sup>Number positive/number sampled.

in Plant A. In Plant B, the off-line reprocessing procedures did not effectively reduce microbial counts. In fact, off-line reprocessing resulted in significantly ( $P \leq 0.0001$ ) higher recovery of all groups of organisms. Most likely this was due to insufficient chlorine utilization at the off-line reprocessing stations or extended lapse of time between when the contaminated carcasses were removed from the evisceration line and when they were actually reprocessed. The use of Cecure on visibly contaminated, inspection held carcasses was highly effective ( $P \leq 0.0001$ ) in reducing APC (by 3.4 to 3.8 logs), *E. coli* (by 2.0 to 2.6 logs), coliform (by 1.8 to 2.3 logs), and *Campylobacter* (by 0.5 to 1.2 logs). *Salmonella* incidence was reduced to 2.5% (from as high as 7.5%) and *Campylobacter* incidence was reduced to 2.5% (from as high as 94.5%).

In Plant C the levels of APC, *E. coli*, coliform, and *Campylobacter* on inspection passed, non-contaminated and inspection-held visibly contaminated were not statistically different ( $P > 0.5$ ). This agrees with findings reported by Fletcher *et al.* (1997) in a study also conducted in a 70 bird per minute plant. In addition, off-line manual reprocessing did not reduce the level of the above groups of organisms, or incidence of *Salmonella* or *Campylobacter*. Treatment of carcasses with Cecure resulted in significant ( $P \leq 0.0001$ ) reductions in all groups of organisms. Reductions ranged from 3.7 to 3.9 logs for APC, 2.7 to 2.9 logs for *E. coli*, 2.6 to 2.7 logs for coliform, and 2.0 to 2.1 logs for *Campylobacter*. In Plant C, incidence of *Salmonella* went from as great as 27% to 1.1 % and *Campylobacter* incidence went from as great as 98.7% to less than 10% with the use of Cecure. Results from this study suggest that there is a significant amount of individual processing plant variation in the microbial condition of pre-chill broiler carcasses. In addition, the data suggest that there is a

significant amount of individual plant variation in the effectiveness of off-line manual reprocessing. In some plants the practice is justified where in other plants the process may actually be detrimental to overall microbial condition.

Data from these trials conclusively demonstrate that the use of Cecure as a pre-chill spray is highly effective for reducing the levels of APC, *E. coli*, coliform, *Campylobacter* and the incidence of *Salmonella* and *Campylobacter* on pre-chill broiler carcasses, even those which have been inspection held due to visible contamination. Based on these findings, USDA has recently approved the use of Cecure for continuous on-line reprocessing of broiler carcasses.

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