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Microbial Evaluation of Cecure^{®1}-Treated (Post-Chill) Raw Poultry Carcasses and Cut-up Parts in Four Commercial Broiler Processing Facilities

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Abstract: Studies were conducted to evaluate the FDA and USDA-approved Cecure[®] antimicrobial in a post-immersion chill drench application in four broiler processing facilities. Control and Cecure[®]-treated carcasses were collected and stored on-site under typical commercial refrigerated storage conditions. On the day of treatment and on subsequent storage days, log₁₀ APC (CFU/mL) were enumerated on control and Cecure[®]-treated carcasses. In three of the four plants, treatment with Cecure[®] resulted in a significant reduction in log₁₀ APC of 1.1 to 3.4 CFU/mL on Day 0. In all plants the Cecure[®] treatment resulted in a slight extension (1 to 2 days) in carcass shelf life at 1 to 7°C. Additionally, in one plant the effect of the Cecure[®] treatment was evaluated on cut-up poultry parts from Cecure[®]-treated carcasses. Initial reductions in log₁₀ APC were statistically significant for thighs and boneless, skinless breast meat on Day 0 and slight extensions in product shelf life (1 to 2 days) were noted for wings, thighs, leg quarters, split breasts and boneless, skinless breast meat from Cecure[®]-treated carcasses. Multiple regression analysis of the slopes of the exponential growth portion (log phase) of the bacterial growth curves for the control and Cecure[®]-treated carcasses and parts showed no significant difference in the slopes of the growth curves. Thus, any extension in shelf life of Cecure[®]-treated carcasses or parts was due to initial microbial reductions at the time of treatment, demonstrating no continued technical effect during refrigerated storage.

Key words: Broilers, Cecure®, cetylpyridinium chloride, microorganisms, shelf life

INTRODUCTION

In 2004 the United States Food and Drug Administration (FDA) approved the use of cetylpyridinium chloride (CPC), as the commercial formulation Cecure[®], for use as a processing aid (as defined in 21 Code of Federal Regulations (CFR) 101.100) on raw, pre-chill poultry (21 CFR 173.375). In 2007, an amendment to the 2004 regulation was granted by FDA to allow for the treatment of raw, post-chill poultry carcasses with CPC. The United States Department of Agriculture/Food Safety Inspection Service (USDA/FSIS) also approved the use of Cecure® as an FDA-defined processing aid for pre-chill carcasses in 2004 and for post-chill raw poultry carcasses or parts in 2007 (USDA/FSIS Directive 7120.1). Since early 2004, many poultry processing plants in the United States and other poultry-producing countries including Canada, Chile, Mexico, Colombia, Panama, South Africa, Saudi Arabia and Turkey have adopted this technology for control of Salmonella, Campylobacter and other potential human pathogens and spoilage organisms on raw poultry products. In some countries Cecure® is also approved for use on other food products.

In 2006, a comprehensive three-plant study was published (Beers et al., 2006) which focused on the pre-

chill use of Cecure® for treatment of raw poultry carcasses for on-line reprocessing. In that study, the authors concluded that Cecure® could be used as a prechill spray to meet the USDA/FSIS criteria for on-line reprocessing of raw poultry carcasses. In addition, the authors found that the use of Cecure® as a pre-chill spray also provided an effective treatment for control of Salmonella, Campylobacter, aerobic plate count (APC), Escherichia coli and coliforms on pre-chill poultry carcasses. Bai et al. (2007) reported the results of two laboratory-conducted trials designed to evaluate the effects of a Cecure® spray at 0.5, 1 and 1.5% CPC (Trial 1) and 0.4, 0.5 and 1% CPC (Trial 2) on the refrigerated (2.5°C) shelf life of boneless, skinless broiler thigh meat. The authors found that a spray treatment of 0.4 or 0.5% Cecure® resulted in a 1 day extension in refrigerated shelf life while a spray treatment of 1.0 or 1.5% Cecure® resulted in a 2 day extension in the shelf life of boneless, skinless thigh meat. Since the published report by Bai et al. (2007), there have been no published results (other than an abstract by Baker et al. (2010) which highlights the preliminary results obtained from the plant labeled "Plant C" in the current study) that document the effects of using Cecure® post-immersion chill in commercial poultry facilities on immediate microbial reductions or control of microorganisms during refrigerated storage. Also, there are no additional reports that demonstrate whether extensions in shelf life as a result of commercial post-chill treatment with Cecure® is due to initial reductions in microorganisms on the day of treatment, as reported by Bai *et al.* (2007) in laboratory trials, or is due to a continued technical effect of the Cecure® treatment during refrigerated storage. This information is important due to the labeling requirements imposed by FDA for food additives.

The purpose of the present study is to determine, with a substantial amount of commercially obtained data from four USDA/FSIS-inspected broiler processing plants, that the post-chill use of Cecure[®] under USDA/FSIS and FDA-approved conditions of use does not result in a continued technical effect during refrigerated storage of raw poultry. The focus of this study is not to determine whether or not the use of Cecure[®] as a post-chill drench results in a significant extension in shelf life, but rather to focus on the explanation as to why such an extension might occur under commercial processing and refrigerated storage conditions.

MATERIALS AND METHODS

Processing plants and Cecure® application: Four USDA-inspected broiler slaughter facilities (Plants A, B, C and D), representing four different commercial poultry companies, participated in a study that was conducted over a period of seven years (November, 2007 to January, 2015). All four facilities were commercially using the Cecure® CPC antimicrobial as a post-chill drench treatment in accordance with FDA 21 CFR 173.375 which states that up to 0.8% cetylpyridinium chloride (as the commercial Cecure® formulation) can be applied to pre or post-chill raw poultry not to exceed 5 gallons (30.28 L) per carcass.

In all four plants the concentration of CPC used in the post-chill Cecure® drench cabinets was within the range of 0.2 to 0.3%. Even though up to 0.8% CPC is allowable under current government regulations, the typical usage percentage across dozens of plants is 0.2 to 0.35% (Cecure® Plant Monitor, Safe Foods Corp.) In all plants the Cecure® treatment target volume was in the range of 0.25 gallons (0.95 L) per carcass with an exposure period of <5 sec. The commercial Cecure® drench systems evaluated in these plants were comprised of stainless steel cabinets through which the carcasses were conveyed at the plant's normal operating line speed. Cabinets were equipped with spray nozzles and hertz cycle regulated booster pumps to apply the liquid treatment solution at a constant volume in liters per minute. After the Cecure® treatment, all treated carcasses were sprayed with a potable water rinse as required for post-chill usage applications.

Evaluation plan: In each of the four processing facilities, samples of whole broiler carcasses with no missing appendages were aseptically obtained immediately prior to the post-chill Cecure® treatment but after immersion chilling (control) and immediately after the post-immersion chilling Cecure® treatment and required potable water rinse (treated) on Day 0. Enough carcasses were obtained on Day 0 in each of the four plants so that refrigerated shelf life evaluations of the carcasses, or parts cut from the carcasses, could be conducted over a period of time ranging from 0 to as many as 22 days depending on the particular plant, conditions of refrigerated storage and poultry part. Days of storage, storage temperature, packaging method and specific sampling days were determined by the individual plants in accordance with their standard product handling procedures and shelf life evaluation protocols. In addition, the individual plants determined the end of shelf life for their products according to their individual standards. End of shelf life for raw poultry is typically 6.5 to 7.0 log₁₀ CFU/mL (Ayers et al., 1950; Mielnik et al., 1990). All plants agreed that at or slightly before 7 log₁₀ CFU/mL raw poultry has reached spoilage conditions.

In Plant A (November, 2007), Plant B (January, 2008), Plant C (September 2011) and Plant D (January, 2015), whole carcasses (n = 5/treatment/day for Plants A and B and n = 3/treatment/day for Plants C and D) were aseptically collected, stored under refrigeration conditions (1 to 7°C) and microbiologically evaluated. In Plant A, whole carcasses were removed from refrigerated storage and evaluated on days 0, 1, 2, 3, 4 and 7. In Plant B whole carcasses were evaluated on days 0, 2, 4, 7, 8, 9, 10 and 11. In Plant D whole carcasses were microbiologically evaluated on days 1, 2, 3, 4, 5 and 6. In Plant C, whole carcasses (n = 3/ treatment/day) and a variety of cut-up parts (n = 2/ treatment/day) were aseptically collected and evaluated. In Plant C whole carcasses were evaluated on days 0, 5, 9, 10, 11, 12, 13, 14, 15 and 16. The cut-up parts evaluated in Plant C included wings, thighs, leg quarters, split breasts (skin-on) and boneless, skinless breast meat. These parts were obtained either from carcasses that were not treated with Cecure® (control) or from post Cecure®-treated carcasses. All poultry parts were cut-up by trained plant employees. In Plant C all the various parts were sampled on days 0, 5, 10, 14, 15, 16, 17 and 18. If a group of parts had not reached spoilage levels of APC by Day 18 (6.5 to 7.0 log10 CFU/mL), the product was also sampled on some or all of the following days: 19, 20 and 21 until spoilage of the particular product occurred.

Control and Cecure®-treated whole carcasses (Plants A, B, C and D) or packages/trays of cut-up parts (Plant C) were transported within the plant, on ice, to the appropriate commercial refrigerated storage facilities.

During the refrigeration period, whole carcasses were stored individually in whole carcass rinse sampling bags whereas the various cut-up parts were stored in the plant's bulk packaging bags or on wrapped foam trays as per the plant's standard packaging method. Carcasses and cut-up parts, for all days of sampling, were stored in the individual plant's walk-in refrigeration units. Plant coolers were held at temperatures consistent with their individual operating standards, but all refrigerated storage facilities, regardless of plant, operated in the range of 1 to 7°C. Each plant's quality control personnel were responsible for maintaining and recording refrigeration temperatures during the shelf life storage periods.

Microbiological sampling procedures: Whole broiler carcasses were sampled using 400 mL Butterfield's Phosphate Diluent (BPD) in plants A, B and C and 400 mL Buffered Peptone Water (BPW) in Plant D. All carcasses were sampled in whole carcass rinse bags as outlined in USDA/FSIS Microbiology Laboratory Guidebook (USDA, 1998). The various poultry parts were sampled similarly in 100 mL of BPD. For parts that were bagged in bulk, three individual parts were obtained from the top, middle and bottom of the bulk bag, the three parts (ex. three wings) representing an individual sample. For parts that were tray-packed, two individual parts were obtained from opposite sides of the tray pack (ex. two thighs), representing a single sample. All samples were evaluated for APC using Petrifilm™ in accordance with the manufacturer's instructions.

Statistical analysis: All microbiological data were transformed to log10 CFU/mL prior to statistical analysis. The lower detection level (1 CFU/mL) was utilized for all negative samples. The purpose of this study was to determine if treating raw poultry carcasses after immersion chilling with the Cecure® antimicrobial significantly alters the slope of the exponential portion, or logarithmic growth phase, of the microbial growth curve during refrigerated storage. Thus, within each of the four plants, only APC data that demonstrated at least one doubling in log10 CFU/mL from one sampling day to the next (indicative of exponential growth in 24 h) were used in the statistical analysis. In each of the four plants, multiple linear regression analysis was utilized to statistically compare the slopes of the control and Cecure®-treated bacterial growth curves during the logarithmic phase of microbial growth (R Core Team, 2013). Additionally, within each plant, regression analysis was performed across days of refrigerated storage (for microbial data that were within the exponential growth phase of the microbial growth curve) for control and treatment groups and the slopes of the regression lines were statistically compared using the Student's t-test (Zaiontz, 2013). Statistical significance

was based on p \leq 0.05. APC means by day of storage were separated in Table 1 (whole carcasses) and Table 2 (poultry parts) using the Student's t-test at p \leq 0.05 (R Core Team, 2013).

RESULTS AND DISCUSSION

In Plant A there was a significant reduction in log10 APC (>3 logs) on the Cecure®-treated carcasses on Day 0 (Table 1). Exponential growth in log10 APC on the control and Cecure®-treated carcasses did not begin until Day 2 (Fig. 1). Therefore days 2, 3, 4 and 7 were included in the multiple linear regression analysis of the control and treated data. Analysis of the slopes of the two microbial growth curves from Plant A (Fig. 1) indicated that there was not enough evidence to report that the slopes of the two curves were significantly different (p = 0.5696). This indicates that even though there was a significant reduction in the level of APC on the Cecure®-treated carcasses on Day 0 and throughout refrigerated storage that the microorganisms did not grow at different rates on the control and Cecure®-treated samples. In Plant A, the company did not continue the shelf life study to the point where spoilage typically occurs (log10 6.5 to 7.0 CFU/mL) therefore, it is not possible to determine whether an extension in product shelf life occurred.

In Plant B there was also a significant reduction in log10 APC (2.86 logs) on the Cecure®-treated carcasses on Day 0 and on all subsequent days of sampling (Table 1). Similar reductions in APC due to the pre or post-chill application of Cecure® (0.25 to 0.8% CPC) to broiler carcasses have been reported (Waldroup et al., 2000; Beers et al., 2006). In Plant B exponential bacterial growth on control and Cecure®-treated samples was occurring on days 4, 7, 8, 9, 10 and 11; therefore, these days were included in the multiple linear regression analysis (Fig. 2). Statistical analysis of the slopes of the exponential growth portions of the two microbial growth curves in Plant B indicated that there was not enough evidence to support that the slopes of the two lines were different (p = 0.2298). Thus, the slight extension in shelf life (1 day) in Plant B was the result of an initial significant reduction in log10 APC on the day of treatment (Day 0) which in turn resulted in significantly lowered counts on subsequent sampling days. The extension in product shelf life was not due to slower growth of organisms on the Cecure®-treated carcasses during refrigerated storage indicating no continued technical effect.

In Plant C there was a significant reduction in log_{10} APC on Cecure[®]-treated carcasses on Day 0 (Table 1); however, the reduction was not as great as initial reductions in plants A or B on the day of treatment. In Plant C all days of storage, excluding Day 0, were included in the multiple linear regression analysis and the results indicated that there was not enough evidence (p = 0.1443) to report that the slopes of the two lines

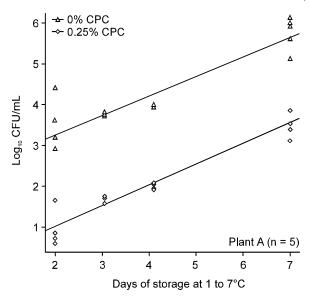


Fig. 1: Aerobic Plate Count on whole broiler carcasses treated with 0.25% Cecure[®] in a drench application after immersion chilling (Plant A)

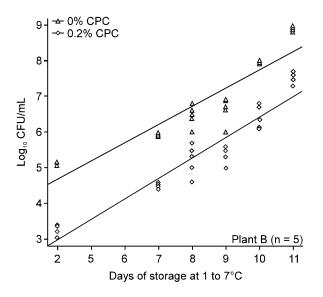


Fig. 2: Aerobic Plate Count on whole broiler carcasses treated with 0.2% Cecure[®] in a drench application after immersion chilling (Plant B)

were statistically different (Fig. 3). It is apparent from the results of this shelf life study that the storage temperature utilized in Plant C was likely in the lower range of the 1 to 7°C temperature span in comparison to plants A and B. In Plant C control product did not reach bacterial spoilage levels till between days 13 and 15 while Cecure®-treated product spoiled about 48 h later between days 15 and 16 (Table 1). Again, as noted for plants A and B, the slight extension in product shelf life (2 days) was due to initial reductions in microorganisms

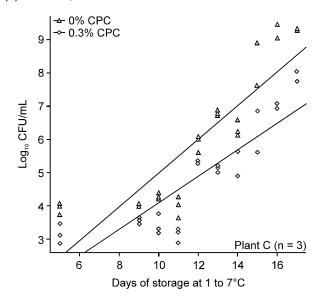


Fig. 3: Aerobic Plate Count on whole broiler carcasses treated with 0.3% Cecure[®] in a drench application after immersion chilling (Plant C)

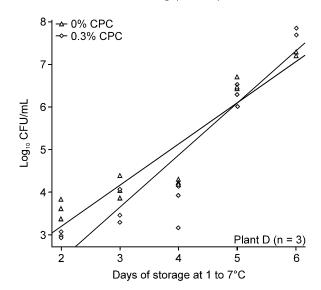


Fig. 4: Aerobic Plate Count on whole broiler carcasses treated with 0.3% Cecure[®] in a drench application after immersion chilling (Plant D)

on the day of treatment which corresponded to lower bacterial counts throughout storage. The lack in evidence to show that the slopes of the two growth curves were different indicates no continued technical effect of Cecure® during refrigerated storage.

In Plant D, there was a slight, but not statistically significant, reduction in log₁₀ APC on Day 1 on the Cecure[®]-treated carcasses (Table 1). In Plant D, no microbial sampling occurred on the day of treatment (Day 0), therefore Day 1 is the first point of data

Table 1: Microbial evaluation of a post-chill Cecure® drench application (0.2 to 0.3% cetylpyridinium chloride) on log10 Aerobic Plate Count CFU/mL (mean±SD) on raw broiler carcasses in four commercial USDA-inspected processing facilities

	Plant A			Plant B			Plant C			Plant D	
		Cecure®-			Cecure®-			Cecure®-			Cecure®-
Sampling	Control	treated	Sampling	Control	treated	Sampling	Control	treated	Sampling	Control	treated
day²	(n = 5)₃	(u = 5)	day	(9 = 0)	(u = 5)	day	(n = 3)	(n = 3)	day	(n = 3)	(n = 3)
0	3.74±0.93	0.32±0.27	0	2.90±0.19⁵	0.04±0.00°	0	4.13±0.05 ²	3.03±0.11⁵	-	2.46±0.05°	2.09±0.06
-	2.99±0.57	0.04±0.00°	7	3.47±0.15⁵	1.00±0.03⁵	52	3.91±0.17²	3.15±0.31₺	2	3.60±0.23⁵	2.97±0.11⁵
2	3.40±0.63	0.90±0.43	4	5.11±0.573	3.28±0.15⁵	တ	4.01±0.06 ²	3.56±0.11₺	က	4.09±0.27 ^a	3.60±0.41⁴
9	3.74±0.05	1.68±0.07⁵	7	5.91±0.04⁵	4.49±0.08°	10	4.26±0.09 ²	3.42±0.32°	4	4.22±0.07²	3.74±0.51
4	3.94±0.03	1.98±0.07⁵	œ	6.44±0.29₽	5.22±0.43°	7	3.95±0.33	3.13±0.20⁰	ß	6.53±0.15°	6.28±0.25
7	5.74±0.40³	3.55±0.31⁵	တ	6.61±0.36	5.34±0.23⁵	12	5.88±0.27°	5.30±0.05°	9	7.22±0.06⁵	7.75±0.09°
			10	7.95±0.05	6.41±0.33⁵	13	6.77±0.08⁵	5.13±0.12°			
			=	8.82±0.14	7.51±0.15⁵	4	6.31±0.24	5.28±0.52			
						15	8.25±0.92	6.24±0.88			
						16	9.24±0.29₽	7.00±0.11₺			

'Row means within plant (A, B, C, or D) followed by different letters are significantly different (p≤0.05). 'Samples held at 1 to 7°C during refrigerated storage. ³Raw poultry is considered spoiled at 6.5 to 7.0 (log_∞ CFU/mL)

Table 2: Microbial evaluation of a post-chill Cecure® drench application (0.3% cetylpyridinium chloride) on log., aerobic plate count¹ CFU/mL (mean±SD) on raw cut-up broiler parts in a commercial USDAinspected processing facility

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	Wings	Wings (n = 2) ³	Thig	Thighs (n = 2)	Leg quai	Leg quarters (n = 2)	Split brea	Split breasts (n = 2)	Boneless, skinl	Boneless, skinless breasts (n = 2)
Sampling day ²	Control	Cecure®-treated	Control	Cecure®-treated	Control	Cecure®-treated	Control	Cecure®-treated	Control	Cecure®-treated
0	3.43±0.50	2.08±0.90⁴	3.68±0.03	2.63±0.04⁵	4.11±0.31	3.64±0.21	4.52±0.01	3.41±0.10°	3.18±0.25	2.61±0.37
5	3.05±0.03	2.51±0.15	3.79±0.27	2.97±0.02	3.41±0.15	3.09±0.09⁵	3.81±0.42⁵	3.24±0.08	2.76±0.14 ^a	2.48±0.06 ^a
10	3.29±0.04⁵	2.88±0.01₺	3.38±0.32	2.71±0.21⁴	3.98±0.40	2.92±0.33	3.64±0.08	3.29±0.29	3.29±0.41	2.41±0.12⁵
4	5.15±0.16 ²	4.43±0.40 ^a	6.42±0.06	3.45±0.18°	3.82±0.95	2.85±0.80⁴	4.38±0.08	2.54±0.36	3.15±0.05	1.90±0.63⁴
15	6.74±0.30	4.21±0.08⁵	6.35±0.12⁵	4.30±0.56	6.91±0.46	5.22±0.91	5.79±0.21	4.87±0.31 ^a	4.68±0.32 ^a	3.34±0.26⁵
16	9	9	6.73±0.14	5.44±0.08°	6.75±0.06	5.48±0.14°	5.86±0.01	5.00±0.02°	5.57±0.04	4.38±0.32
17	7.46±0.04²	6.61±0.06⁵	Q	2	7.08±1.36	5.94±0.06	6.16±0.42°	5.12±0.16	5.41±0.15	4.45±0.88°
18			7.66±0.05	6.39±0.04°	7.84±0.99	6.97±0.10⁴	7.37±0.83	5.95±0.23	6.05±0.21 ^a	4.63±0.08°
19							7.68±0.38	6.56±0.42	6.12±0.11 ^a	5.19±0.21
20									6.66±0.17 ^a	5.81±0.07⁵
21									7.22±0.25	6.46±0.09⁵

Row means within the cut-up broiler part followed by different letters are significantly different (p<0.05)

collection. Exponential microbial bacteria growth occurred on control and Cecure®-treated samples on days 2, 3, 4, 5 and 6 in both the control and Cecure®treated groups so these days were included in the multiple linear regression analysis. The results indicated that there was not enough evidence to support that the slopes of the control and Cecure®treated microbial growth curves (Fig. 4) were different (p = 0.1284). In Plant D there was no improvement in product shelf life due to treatment with Cecure® as both groups of carcasses had reached spoilage levels of APC by Day 6 (Table 1). In fact, in Plant D the log10 APC levels were significantly higher on the Cecure®-treated carcasses at the end of the shelf life period indicating no continued technical effect due to the antimicrobial treatment

Shelf life evaluations were also conducted on various cut-up parts of control and Cecure®-treated carcasses in Plant C (Table 2). Initial reductions in log10 APC on the day of treatment (Day 0) ranged from log10 0.47 (leg quarters) to 1.35 (wings), but were significant (p≤0.05) only for the thighs and split breasts on Day 0. The lack in significance for Day 0 reductions in log10 APC for the other parts (wings, leg quarters and boneless, skinless breast meat) was likely due to the greater standard deviations associated with the day of sampling means for these parts in comparison to the standard deviations for the thighs and split breasts (Table 2). Regardless of poultry part, all Cecure®-treated parts exhibited a slight but consistent (1 to 2 days) extension in refrigerated shelf life using log10 6.5 to 7.0 CFU/mL as the criteria for determining spoilage. These findings are in agreement with the report by Bai et al. (2007) that demonstrated that a Cecure® spray could be used to extend the shelf life of boneless, skinless broiler thigh meat. But, as previously explained, the purpose of the present study was not to determine if the use of Cecure® results in an increase in product shelf life but more specifically to examine the slopes of the bacterial growth curves during the exponential growth phase in order to more fully understand the reason for any shelf life extensions. As with the whole carcasses in plants A, B, C and D, for each group of cut-up parts multiple linear regression analysis was conducted over the shelf life period comparing the slopes during the exponential growth phase. There was not enough evidence to determine any significant differences in the slopes of the control and Cecure®-treated growth curves of refrigerated wings (p = 0.2331), thighs (p = 0.3058), leg quarters (p = 0.5738), split breasts (p = 0.4259), or boneless, skinless breast meat (p = 0.5699). Thus, for all cut-up poultry parts evaluated any extension in product shelf life was likely due to slight reductions in log10 APC at the time of treatment and not to altered microbial growth during refrigerated storage.

Conclusions: Results from four plant trials, all conducted under commercial processing conditions, in USDA-inspected poultry plants confirm results from laboratory trials previously reported by Bai et al. (2007). Treatment of raw poultry carcasses or parts, with the Cecure™ antimicrobial, results in initial reductions in microorganisms on the day of treatment which may result in a slight (1 to 2 days) extension in product shelf life. Statistical analysis of the slopes of the exponential portion of the bacterial growth curves of control or Cecure®-treated broiler carcasses and cut-up parts from treated carcasses clearly indicates that there is not enough evidence to support that the slopes of the control and Cecure®-treated curves differ. Thus, the application of Cecure® as a post-chill drench treatment for raw poultry carcasses and parts cut from Cecure®-treated carcasses clearly fits the FDA and USDA/FSIS definition for a processing aid. The use of Cecure® does not result in a continued technical effect but often provides a reduction in the total microbial load at the time of treatment during poultry processing. After treatment, the remaining bacteria grow with just as much vigor and in the same manner as bacteria on untreated carcasses. Any resulting increase in shelf life that may occur appears to be due to initial reductions in organisms at the time of treatment.

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