Reference For Display at Dockets Management Branch (HFA- 305) upon publication of the final rule in the Federal Register

Docket Number: 02F-0181

FRDTS Number: CFSAN 200310

Food Additive Petition 2A4736

"FAP 2A4736: Safe Foods Corporation, submissions of 3/20/02, 8/23/02 and 10/4/02. Cetylpyridinium chloride (CPC) for use as an antimicrobial treatment for use on poultry."

2002F-0181

BKG 1

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: November 19, 2002

From: Division of Petition Review (HFS-265)
Chemistry Review Group

Subject: FAP 2A4736: Safe Foods Corporation, submissions of 3/20/02, 8/23/02 and 10/4/02. Cetylpyridinium chloride (CPC) for use as an antimicrobial treatment for use on poultry.

To: Division of Petition Review (HFS-265)
Regulatory Review Group II
Attn: A. Laumbach, Ph.D.

Introduction

Safe Foods Corporation (Safe Foods) is petitioning to allow for the use of cetylpyridinium chloride (CPC) as an antimicrobial treatment for use on poultry. A dilute aqueous solution typically containing 0.2% to 1.0% CPC and propylene glycol (PG) at 1.5 times the concentration of CPC will be applied to poultry (pre-chiller) at ambient temperature (73-75° F) in the form of a fine mist at a level of not more than 0.3 grams of CPC per pound of poultry.

In the 10/4/02 submission, Safe Foods amended their intended use of CPC. This amendment affected information on the use level, residue levels of CPC on poultry, and exposure to CPC that was provided in the submissions dated 3/20/02 and 8/23/02. This memorandum documents the chemistry information provided in the 3/20/02 and 8/23/02 submissions, and indicates the relevant new data provided in the 10/4/02 submission.

Identity

Name: Cetylpyridinium chloride

Other Chemical Name: 1-hexadecyl pyridinium chloride

Common Names: ceepryn chloride, cepacol chloride, cetamium, dobendan, pristacin, pyrisept

Trade Names: Cecure (as part of an aqueous solution containing PG; see Manufacturing, below)

CAS Reg. No.: 123-03-5

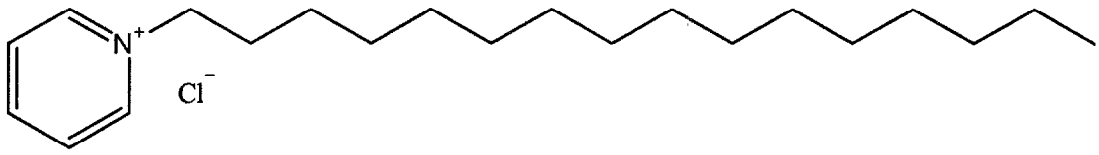
Molecular Formula: $C_{21}H_{38}NCl$

Molecular Weight: 340.05

Physical properties:

Melting point: 77-83° C
pH (1% aqueous solution): 6.0 – 7.0
Solubility: freely soluble in water, alcohol and chloroform, insoluble in ether

Structure:



CPC is presented above as the anhydrous form, however, CPC is typically present in water in the monohydrate form ($C_{21}H_{38}NCl \cdot H_2O$, CAS No. 6004-24-6).

Manufacturing

CPC

Safe Foods does not manufacture CPC. Rather, they currently purchase it from Zeeland Chemicals Inc., Zeeland, MI. Safe Foods states that CPC may be manufactured in aqueous solution (under elevated temperature and pressure) by the alkylation of pyridine with cetyl chloride. This process yields the monohydrate form of CPC.

Cecure Solution

Safe Foods produces the Cecure solution in a concentrated form. First, a known quantity of food grade PG is charged to a vessel. To this solution, a known quantity of United States Pharmacopeia (USP) grade CPC is added to yield a concentration of 40% CPC and 60% PG. The solution is then sampled and analyzed to ensure that the correct concentration of CPC and PG has been attained. If necessary, additional CPC or PG is added. The mixture is then filtered and transferred to storage containers. In the original submission, it was noted that the end-user would dilute the concentrated Cecure solution 100-fold to yield the application concentration of 0.4% CPC and 0.6% PG. In the 10/4/02 submission, however, the application level was changed to a maximum 0.3 grams CPC per pound of poultry. The new application level allows for the use of a range of diluted Cecure solutions – typically containing 0.2% to 1% CPC – that correspond to applications of 5 ounces solution per pound of poultry and 1 ounce of solution per pound of poultry, respectively. Under the new application level, the diluted Cecure solution will also contain PG at a concentration approximately 1.5 times that of CPC.

Impurities

Safe Foods states that CPC (in its powder form) may contain between 70 and 120 mg/kg of the starting material, pyridine. Safe Foods states that no other known impurities, contaminants, by-products or reaction products are present.

Specifications

CPC

Safe Foods states that the CPC used in the production of the Cecure solution will be USP grade (i.e., the CPC used will comply with the specifications set forth in USP 24/National Formulary 19, January 1, 2000). The official USP monograph for CPC is included in Appendix I. The specifications are listed below in Table 1 (as taken from the table on page 6 of Section A in the petition).

Table 1. USP 24 Specifications for CPC

Parameter	USP 24 Specification
Assay	Not less than 99.0% and not more than 102.0% of $C_{21}H_{38}ClN$, calculated on an anhydrous basis.
Identification	By infrared (IR) and ultraviolet (UV) absorption, as specified in monograph
Melting range	Between 80° C and 84° C (preliminary drying omitted)
Acidity	Not more than 2.5 mL of 0.020N NaOH to neutralize 500 mg dissolved in 50 mL of water
Water	Between 4.5% and 5.5.%
Residue on ignition	Not more than 0.2%, calculated on the anhydrous basis
Heavy metals	0.002%
Pyridine	Odor of pyridine is not immediately perceptible when 1 g is dissolved in 10 mL NaOH solution (1 in 10) without heating
Organic volatile impurities	Meets the requirements of USP Method V

Appendix I also includes the analytical methods used by Zeeland Chemicals, Inc. to verify adherence to USP specifications. The results from analysis of 49 lots of CPC are included in Appendix II.

Cecure Solution

The Cecure solution will be manufactured using USP grade CPC (as discussed above) and food grade PG complying with the specifications set in the Food Chemicals Codex, Fourth Edition (FCC IV). The FCC IV monograph for PG is included in Appendix III. Safe Foods states that the Cecure solution in its concentrated form (prior to dilution by the end-user) will contain between 39.5 and 41.5% CPC. Summary data for two lots of concentrated Cecure solution showing adherence to the CPC concentration specification are included in Appendix IV. We note that these data are also used for the stability studies, below.

We have no questions with regard to the identity and manufacture of, and specifications for the additive.

Labeling

A sample label for the Cecure solution is included in Appendix V. The current instructions state that the product must be at 65°F or above before use. Safe Food's 8/23/02 submission states that the temperature restriction should be changed to state that the product must be at 99°F or above before use. However, in the 10/4/02 submission, the temperature restriction was changed to ambient conditions (73° F to 75° F). Therefore, the high temperature restriction on the label is no longer necessary. Additionally, we note that the CAS No. for CPC on the label is 6004-24-6. This is the CAS No. for the monohydrate form.

Stability

Safe Foods provided stability data for both CPC and the Cecure solution. Appendix II presents 5-year stability data on 49 lots of CPC powder.[1] The CPC lots were examined annually to determine adherence to the specifications of odor, melt range, moisture content, CPC assay, pH in 5% aqueous solution, APHA, clarity in water and methanol, and presence of cetyl chloride and hexadecene.[2] We agree that the CPC powder samples remained stable over the 5 year testing period.

Appendix IV contains summary data from two different temperature stability tests for concentrated Cecure solution. In the first test, two lots of concentrated Cecure solution (40% CPC and 60% PG) were analyzed for % CPC and % pyridine-HCl at temperatures of 4, 20 and 48°C. The time held at each temperature was not stated. In the second test, the two lots were subjected to a freeze/thaw cycle. The time held at freezing and thawing temperatures was not stated. The test results indicate that one of the two lots was able to meet the specification limit of 39.5 to 41% CPC for all of the temperatures tested, and that the second lot did not meet the specification limit for CPC when stored at 4°C (39.4% CPC) and during the freeze/thaw test (39.3% CPC). The test results indicate that pyridine was not formed in the Cecure solutions at any of the temperatures or during the freeze/thaw cycle. It is difficult to determine any meaningful results from this stability test, as no detailed methodology or testing protocol was included.

Appendix IV also included a heat stability test for solutions of CPC. This test determined the level of free pyridine by liquid chromatography/mass spectrometry (LC/MS) in twenty-two sample solutions containing 50 mg/kg CPC. The test solution is approximately 40 to 200 times more dilute than the CPC solution that is intended to be applied to poultry (0.2% to 1%

[1]Only about 60% of the lots were analyzed for the full 5 years.

[2]The tests for pH in 5% aqueous solution, APHA, and clarity in water and methanol were eliminated in 1996. Tests for the presence of hexadecene and cetyl chloride were added in 1999.

or 2000 mg/kg to 10000 mg/kg). The test solution is heated to 95° C for up to 90 minutes. Two samples were analyzed at time zero. Ten samples (in pairs) in closed vials and ten samples (in pairs) in open vials were analyzed after 10, 20, 30, 60 and 90 minutes. The samples analyzed at time zero yielded pyridine concentrations of 98.4 mg/kg. The heated, closed vials yielded pyridine concentrations of 94.6 mg/kg after 90 minutes. This indicates that no additional pyridine is generated from the thermal decomposition of CPC. Analysis of the heated, open vials showed that no additional pyridine was generated. The open vials showed a 90% decrease in pyridine levels (to 8.5 mg/kg) after heating for 90 minutes. Safe Foods states that these results suggest that: (1) any residual pyridine present in the CPC solutions would likely be driven off upon cooking the poultry treated with CPC; and (2) CPC solution is heat stable up to 95°C. We agree with Safe Foods' assertions.

Use, Use Level, and Technical Effect

CPC will be used as an antimicrobial agent on raw poultry as a component of a dilute aqueous solution that also contains PG. The original submission indicated that the end-user would prepare an application solution containing 0.4% CPC and 0.6% PG by diluting the concentrated Cecure solution (containing 40% CPC and 60% PG) 100-fold on site. In the 10/4/02 submission, Safe Foods changed the reporting basis of the maximum use level, stating a maximum 0.3 grams of CPC would be applied per pound of poultry carcass without giblets (WOG). Safe Foods states that, by specifying the maximum application level in this way, the end-user will have more flexibility in the application of the antimicrobial agent. Typically, the dilute Cecure application solution will contain 0.2% to 1% CPC, with corresponding spray volumes of 5 ounces of solution per pound of poultry and 1 ounce of solution per pound of poultry, respectively.[3] The applied solutions will also contain PG at a concentration approximately 1.5 times that of CPC.

In the original submission, it was stated that the diluted Cecure solution would be applied at a temperature of 99° F. However, the 10/4/02 submission states that this solution will now be applied at ambient temperatures (73° F to 75° F).

The 10/4/02 submission describes a "recycle and capture technology" system that Safe Foods will supply to users of its product. The system is designed to continuously recycle CPC during the application process, and to allow for capture and disposal of 99.88% of the CPC runoff. A pictorial presentation of the Cecure recycle system is depicted in Appendix XIV. The recycle and capture system is comprised of a Cecure concentrate supply tank, a chemical

[3] 5 ounces of a 0.2% CPC solution applied to one pound of poultry would yield the following mass of CPC per pound:

$$\text{Mass CPC} = 5 \text{ ounces CPC solution} \times \frac{29.57 \text{ mL}}{1 \text{ ounce}} \times \frac{1 \text{ gram}}{1 \text{ mL}} \times \frac{0.2 \text{ g CPC}}{100 \text{ g solution}} = 0.3 \text{ g CPC}$$

Similarly, 1 ounce of a 1% CPC solution applied to one pound of poultry yields the following mass of CPC per pound:

$$\text{Mass CPC} = 1 \text{ ounce CPC solution} \times \frac{29.57 \text{ mL}}{1 \text{ ounce}} \times \frac{1 \text{ gram}}{1 \text{ mL}} \times \frac{1 \text{ g CPC}}{100 \text{ g solution}} = 0.3 \text{ g CPC}$$

feed pump, a UV spectrometer sensor and controller, a recycle tank, a system pump, a filter, a three-way valve, the CPC capture and disposal system, a drip tray extending from the spray cabinet, and a computer and appropriate software.

The application process begins by first manually filling the recycle tank to approximately 1/3 to ½ of its capacity with potable tap water (ambient temperature). After the system is turned on, the UV spectrometer sensor determines that the CPC level is below specification (by lack of absorbance at 260 nm) and signals for the addition of the Cecure concentrate to the recycle tank. Once the proper application concentration has been reached (typically from 0.2 to 1.0% CPC), the UV spectrometer sensor signals for the chemical feed pump to turn off. Next, the system pump is started, and the dilute Cecure solution is pumped to the poultry spray cabinets. The dilute Cecure solution is applied to poultry carcasses WOG as a fine mist at ambient temperatures (73-75° F) as the poultry passes through a spray cabinet (located pre-chiller) equipped with atomizing nozzles. The spray cabinet (approximately 4 feet long with a direct spray path length of 21 inches) will deliver a spray volume commensurate with the CPC concentration of the applied solution (i.e., 5 ounces per pound for a solution containing 0.2% CPC, and 1 ounce per pound for a solution containing 1% CPC) to yield an application level of no more than 0.3 g CPC per pound of poultry. This is equivalent to 0.66 mg of CPC per gram of poultry, or about 660 mg/kg.

After treatment, the poultry carcasses are allowed to drip for 2 minutes as they travel to the chiller bath. The CPC runoff from the spray cabinet and from the first 1 minute of dripping time is collected, filtered, and returned to the CPC recycle tank for reuse. The concentration of CPC in the recycle tank is continuously monitored and adjusted as necessary by the addition of fresh Cecure concentrate. Upon completion of the spraying cycle, the CPC solution in the reservoir tank is transferred to the purge tank, and then gravity fed into the CPC capture and disposal system, where the CPC is captured in disposable filters.

PG is included in the Cecure solution in order to: (1) maintain the solubility and stability of the Cecure formulation; and (2) reduce the adsorption of CPC on the treated poultry.[4] Extraction studies described in Appendix VI show that chicken skin samples treated for 10 minutes with 0.3% CPC and 0.5% PG absorbed 38% to 65% less CPC when compared to chicken skin samples treated with 0.3% CPC alone.

Safe Foods has provided the following evidence of the effectiveness of CPC as an antimicrobial on poultry:

1. Four published articles, three of which are specific to poultry, that demonstrate the antimicrobial nature of CPC in a laboratory environment (Appendix VII).

[4]Safe Foods states that PG used as a component of the Cecure solution that shall comply with 21 CFR 184.1866. The description of the use of PG as a component of the Cecure solution applied to poultry is consistent with this regulation.

2. Data from CPC studies in a poultry pilot plant conducted at the University of Arkansas (Appendix VII).[5] Sixty pre-chiller broiler carcasses were divided into six groups of 10. The treatment groups consisted of the following: (1) a prechill control employing a tap water mist; (2) postchill control; (3) 0.2% CPC, 3 second mist; (4) 0.5% CPC, 3 second prechill mist; (5) 0.2% CPC, 10 second prechill dip; and (6) 0.5% CPC 10 second prechill dip. None of the prechill treated carcasses were immersion-chilled following CPC treatment. After treatment, all control and treated carcasses were sampled for aerobic plate count (APC), *E. coli*, coliforms, and *Campylobacter*. The study states that: (1) all treatment applications significantly reduce the level of microorganisms compared to the control groups; and (2) the use of 0.5% CPC resulted in larger reductions in microorganisms compared to the use of 0.2% CPC.

3. Data from CPC studies on poultry undertaken at two USDA inspected commercial facilities (Appendix VII).[6] In both poultry plants, fecal failure cabinets (located at the exit of the immersion chiller) were modified to allow for application of CPC. In the first plant, a 20% CPC concentrate was used, while in the second plant the commercial Cecure concentrate (40% CPC and 57% PG) was used. In both cases, the solutions were diluted at the plants so as to deliver concentrations ranging from 0.2 to 0.5% CPC. Control carcasses were collected at both plants prior to the spray cabinet. All carcasses were evaluated for APC, *E. coli*, *Campylobacter, Salmonella*, and other coliforms. The study states that, in all cases, treated carcasses showed a decrease in microorganisms compared to the control groups.

4. Data from a study to determine if increasing the water temperature during CPC application would improve microbial reduction efficiency (Appendix VII). In the pilot plant and commercial plant tests listed above, room temperature water was used in the application of CPC. In the pilot study, the Cecure concentrate (40% CPC and 60% PG) solution was diluted to application levels of 0.4% CPC and 0.8% CPC, and mixed with water (70° F and 95°F). Carcasses were sprayed for 2 to 3 seconds, resulting in the application of 3.4 to 5.1 ounces per

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[5]Footnote 1 of the cover letter of the 10/4/02 submission states that the spray volumes reported in the residue and efficacy studies in the original submission were incorrect. Poultry carcasses were actually sprayed with 3 ounces of dilute Cecure application solution per pound rather than the reported 1 ounce per pound. Therefore, in this efficacy study, poultry treated with 3 ounces of dilute Cecure application solutions containing 0.2% and 0.5% CPC per pound of poultry would result in the application of 0.18 and 0.44 grams of CPC per pound of poultry, respectively. These application levels are below and are in excess of Safe Foods' maximum application level of 0.3 grams of CPC per pound of poultry, respectively.

[6] Footnote 1 of the cover letter of the 10/4/02 submission states in that the spray volumes reported in the residue and efficacy studies in the original submission were incorrect. Poultry carcasses were actually sprayed with 3 ounces of dilute Cecure application solution per pound rather than the reported 1 ounce per pound. Therefore, in this efficacy study, poultry treated with 3 ounces of dilute Cecure application solutions containing 0.2% and 0.5% CPC per pound of poultry would result in the application of 0.18 to 0.44 grams of CPC per pound of poultry. These application levels bracket Safe Foods' maximum application level of 0.3 grams of CPC per pound of poultry.

carcass.[7] All carcasses were evaluated for APC, *E. coli*, and coliform. The study states that: (1) in all cases, treated carcasses showed a decrease in microorganisms compared to the control groups; (2) there was a noticeable reduction in microorganisms in the carcasses treated with 0.4% CPC at 95° F compared to those treated at 70°F; and (3) increases in the temperature from 70°F to 95°F did not significantly change the reduction of microorganisms in the carcasses treated with 0.8% CPC.

5. Data from a study to determine if using carcass-rinse drip water instead of tap water in the Cecure reservoir tank would affect pre-chill microbial reduction efficiency (10/4/02 submission, Section C and Appendix XV) . Thirty poultry carcasses WOG were divided into three treatment groups consisting of 10 birds each: 1) control; 2) 1% CPC (1 oz/lb) in tap water; and 3) 1% CPC (1 oz/lb) in carcass-drip water obtained from a commercial facility. After treatment, all control and treated carcasses were sampled for APC, *E. coli*, coliforms, and *Campylobacter*.[8] The study states that: 1) bacterial contamination of the CPC spray solution is not likely to occur under the proposed conditions of use; 2) there was a noticeable reduction in microorganisms in the carcasses treated with either the 1% CPC solution generated with tap water or the 1% CPC solution generated with poultry drip water; and 3) reductions in microorganisms were not affected by the solution in which the CPC was prepared (poultry dip or tap water).

The information presented appears to support Safe Foods' claim that CPC is an effective antimicrobial on poultry. However, we defer to the CFSAN microbiologists to evaluate these data in detail.

Analytical Method for Determining CPC

The chemistry memorandum dated 4/30/02 (D. Folmer to A. Laumbach) noted that Safe Foods provided three separate analytical methods for the determination of CPC residues in poultry (Appendices VIII, IX and X). We requested clarification as to which method was used to determine the CPC residue levels presented in the petition. The 8/23/02 submission responded to this request. This submission stated that: (1) the method presented in Appendix VIII is the method used to determine CPC residues in poultry; (2) the method in Appendix X is essentially the same as that in Appendix VIII; and (3) that the method presented in

[7]Assuming an average carcass weight of 2.3 pounds (see Section D, p. 20 of the 3/20/02 submission), this results in the application of 1.5 to 2.2 ounces of dilute Cecure solution per pound of poultry. Therefore, use of a dilute Cecure solution containing 0.4% CPC would result in a treatment level of 0.18 to 0.26 grams of CPC per pound of poultry; and use of a dilute Cecure solution containing 0.8% CPC would result in a treatment level of 0.36 to 0.52 grams of CPC per pound of carcass. The application of the dilute Cecure solution containing 0.4% and 0.8% CPC is below and above Safe Foods' intended maximum application level of 0.3 grams of CPC per pound of poultry, respectively.

[8]The procedure outlined in Appendix XV states that after treatment and a 2 minute drip time, the carcasses were immersion-chilled for 60 minutes. We note a discrepancy in that Section C of the 10/4/02 submission does not mention that the carcasses are immersion chilled.

Appendix IX was only included for background purposes and is not relevant to the petition. The method originally described in Appendix VIII is included in Attachment 1 of the Submission dated 8/23/02. We shall summarize this method, below.

CPC residues in poultry are analyzed using a reversed phase high performance liquid chromatography (HPLC) method employing a 5 μ cyano precolumn and column, with a mobile phase consisting of 65% methanol and 35% 0.14 M acetic acid containing 8 mM tetramethylammonium hydroxide pentahydrate. Residues of CPC are extracted from poultry skin and meat as described in Appendix X. The poultry sample is extracted twice with 15 mL of 95% ethanol at 45° C. The extract solutions are combined, and then centrifuged at 2500 x g for 15 minutes. A portion of the supernatant (20 μL) is then analyzed by HPLC for the presence of CPC.

A linear calibration curve for the analytical method with a correlation coefficient > 0.999 was generated using 8 CPC standards at concentrations of 0.25, 0.5, 1.00, 2.00, 10.0, 25.0, 50.0, and 100.0 μg/mL (p. 7 of Attachment 1 in the 8/23/02 submission).

Validation

Validation data and sample chromatograms in support of the analytical method are provided in Attachment 1 of the submission dated 8/23/02. Safe Foods performed validation experiments by spiking the extracts from an untreated chicken with known amounts of CPC. An untreated broiler carcass was rinsed for 3 minutes in 1 liter of 95% ethanol. Approximately 0.4 L of the extract was then centrifuged at 5000 x g for 15 minutes. Samples of the centrifuged blank extract were then spiked with standard solutions of CPC at levels of 0.25, 0.5, 1.00, 2.00, 10.0, 25.0, 50.0, and 100.0 μg/mL.[9] Analyses of the spiked blanks were performed in triplicate. The validation studies yielded percent recoveries ranging from 92.1% to 100.8% across the range of spiking levels. By analyzing the average signal output of 10 blank extracts and using the method suggested in our Chemistry recommendations, Safe Foods determined a limit of quantitation (LOQ) and a limit of detection (LOD) for the technique of 0.37 μg/ml and 0.19 μg/ml, respectively.

We agree that the analytical method is properly validated for determining residue levels of CPC in poultry.

Residue Data

Appendix X of the original submission, and Section D and Appendix XVIII of the 10/4/02 submission describe studies performed by Safe Foods to determine the amount of residual CPC in poultry after application of the Cecure solution in a commercial setting, followed by cooking the treated poultry.

[9]These spiking levels bracket the CPC residual level of 13.2 mg/kg (10/4/02 submissison) in chicken. See Residue Data, below.

The data in Appendix X consist of two sets of tests performed in an attempt to model different commercial poultry plant settings. In the tests, commercial broiler carcasses were sprayed with dilute Cecure solutions reported to contain 0.4%, 0.6%, or 0.8% CPC and PG at a concentration 1.5 times that of CPC. However, as noted in footnote 1 of the cover letter of the 10/4/02 submission, Safe Foods actually treated the poultry in the residue tests with 3 ounces of dilute Cecure application solution per pound of poultry rather than 1 ounce per pound as was originally reported. Thus, all poultry carcasses tested for CPC residue levels were treated with more than the intended use level of 0.3 grams of CPC per pound.[10] Therefore, we will not further consider the information regarding CPC residue levels provided in the original submission (Appendix X).

In the 10/4/02 submission, Safe Foods provided new residue data that reflect the new maximum application level of 0.3 g CPC per pound of poultry. The protocol is described in Appendix XVI, and is summarized below.

The residue testing was performed using a commercial evisceration line and commercial Cecure spray cabinet. Whole prechill broiler carcasses were sprayed with ambient temperature (73.5° F to 75.5° F) dilute Cecure solutions containing 0.4% to 1% CPC (plus PG at a level 1.5 times the CPC concentration). The treatment conditions are shown in Table 2 below (taken from Table 3, Section D of the 10/4/02 submission):

Table 2. Application Parameters for CPC Residue Analysis

Concentration of CPC in Application Spray	**Ounces of Spray per Pound of Poultry**	**Grams of CPC per Pound of Treated Poultry**	**Number of Poultry Carcasses Treated**
0%	0	0	6
0.4%	1.0	0.12	6
0.8%	0.5	0.12	6
0.8%	1.0	0.24	5
1.0%	0.5	0.15	6
1.0%	1.0	0.30	6

The poultry carcasses were treated in a spray cabinet (design typical of intended use, see above). After the spray treatment, the poultry carcasses were allowed to drip for two minutes to represent the transit time in a commercial setting between spraying and passing to the immersion chiller. The carcasses were then placed in a chill water container (32 to 34° F[11]) to simulate immersion chilling for one hour with a water overflow of approximately 0.5 gallons/carcass.

[10]Poultry treated with 3 ounces of dilute Cecure application solution containing 0.4%, 0.6%, and 0.8% CPC would result in the application of 0.35, 0.53, and 0.71 grams of CPC per pound of poultry, respectively.

[11]This information was obtained from an 11/7/02 phone call between D. Folmer (HFS-265) and H. Foley (Keller and Heckman LLP).

After chilling for one hour, the entire carcass skin from the breast, sides, back and thigh area was removed. No meat samples were taken.[12] Samples were cooked in metal tins in a conventional oven at 375° F for 30 minutes, cooled to room temperature, and then emptied into plastic containers. The samples were extracted by shaking with 200 mL of 95% ethanol for 20 minutes. Next, 40 mL of the extract were transferred to a 50 mL conical tube for storage; 1 mL of this extract was centrifuged for 15 minutes at 15,000 x g. Finally, a portion of the supernatant was analyzed for CPC residue using the HPLC method described above (see Analytical Methods for Determining CPC). Raw data and all chromatograms are included in Appendix XVIII. Results are provided below in Table 3 (taken from Table 4, Section D of the 10/4/02 submission):

Table 3. Summary of CPC residue data from cooked chicken skin

CPC Treatment	Amount of CPC Applied g/lb poultry	Mean CPC Residue (mg/kg)
Control	0	Not Detected
0.4% at 1 ounce/lb	0.12	5.7
0.8% at 0.5 ounce/lb	0.12	6.6
1.0% at 0.5 ounce/lb	0.15	6.8
0.8% at 1 ounce/lb	0.24	11.6
1.0% at 1 ounce/lb	0.30	15.9

Safe Foods notes that these data confirm that the level of residual CPC on poultry is related to the total grams of CPC applied rather than either the CPC concentration of the applied solution or the volume of solution applied. We concur.

Residue testing was also performed on 17 poultry carcasses from the efficacy tests described above (Item 5 under the Use, Use Level, and Technical Effect section). In this test, poultry carcasses (independent of those analyzed for microbiological purposes) were sprayed with dilute Cecure solution containing 1.0% CPC that had been prepared using either tap water or poultry water drip from a commercial poultry processing plant. We presume that these carcasses were extracted using either the method described in Appendix X (see Analytical Method for Determining CPC), or the method described above. Samples were analyzed using the HPLC method described in Appendix VIII of the original submission. The results of this study are shown below in Table 4 (taken from Table 5 in Section D of the 10/4/02 submission).

Table 4. Summary of CPC residue data from cooked chicken skin obtained from the efficacy study (Appendix XV)

[12]This approach is consistent with the findings from residue tests in the original submission (Appendix X), in which CPC residues were not found in poultry meat in Trial 1. Consequently, Safe Foods did not collect meat samples for residue testing.

CPC Treatment	# Samples	CPC Spray Solution	Mean CPC Residue (mg/kg)
Control	6	None (tap water only)	Not Detected
1.0% at 1 ounce/lb	6	Made with tap water	11.4
1.0% at 1 ounce/lb	5	Made with carcass drip	12.1

Safe Foods states that the results indicate that the Cecure "recycle and capture" system will not lead to higher CPC residues on treated carcasses. Safe Foods also observes that the residual levels obtained in this study are consistent with those from the corresponding treatment level in the residue study described above (Table 3, 1% CPC at 1 ounce/lb).

While we concur with Safe Foods' assertions, we note that only three of the residue tests performed (Table 3 and 4) are consistent with the intended maximum application level of 0.3 g CPC/pound of poultry (i.e., application of 1 ounce of a dilute Cecure solution containing 1% CPC per pound of poultry). Therefore, taking into account only the residue tests performed at the intended maximum application level, the average CPC residue level on cooked poultry is 13.2 mg/kg.[13]

We are satisfied with the studies performed for the analysis of CPC residue levels on poultry.

Exposure

Intake of Poultry

Safe Foods provided an intake estimate of poultry consumed with and without skin (Appendix XI). They used a novel approach that combined consumption data from the USDA Continuing Survey of Food Intakes by Individuals (CSFII, 1994-1996 and 1998 Supplemental Children's Survey) with eating frequency data from the National Health and Nutrition Examination Survey III (NHANES, 1988-1994). CSFII data provide consumption data (grams of food consumed per eating occasion) obtained from a two-day (nonconsecutive) survey of approximately 21,000 people, including 5,300 children in the 1998 supplemental survey. The NHANES survey provides food eating frequency information obtained from about 40,000 people over 30 days. By combining information from the two surveys, Safe Foods intended to provide a more realistic eaters-only intake estimate for poultry.[14] A Monte Carlo sampling technique was used to multiply food consumption and frequency distributions

[13] The average CPC residue is determined in the following manner:
[(6)(15.9 mg/kg) + (6)(11.4 mg/kg) + (5)(12.1 mg/kg)] /17 = 13.2 mg/kg

[14]Safe Foods provided a paper (Appendix 3 of Appendix X; J. Lambe, et al., "The influence of survey duration on estimates of food intakes and its relevance for public health nutrition and food safety issues," *Eur. J. Cli. Nut.* (2000) **54**, 166-173) which shows that the duration of a food survey may significantly affect mean eaters-only intake estimates. In particular, the study shows that mean eaters-only intake of chicken in a 3 day survey was 53 g/d whereas the intake decreased to 24 g/d in a 14 day survey.

obtained from the two different surveys. This technique yielded a total monthly intake which was then divided by 30 to result in a daily intake.

The amount of poultry consumed with and without skin was also determined. CSFII data, which oftentimes lists whether chicken is consumed with or without skin, was used to determine that approximately 54% of poultry is consumed without skin, and 46% is consumed with skin (Appendix 2 of Appendix X).[15] Table 5, below, presents the results of Safe Foods' estimate of poultry consumption.

Table 5. Intake of Poultry with and without skin from combination of CSFII and NHANES data.[16]

		Mean (g/p/d)	90th Percentile (g/p/d)
Total Poultry Consumed	Per Capita[17]	19.4	44.2
	Eaters-only	20.9	49.2
Poultry Consumed with Skin	Per Capita	8.9	20.3
	Eaters-only	9.6	22.6
Poultry Consumed without Skin	Per Capita	10.5	23.9
	Eaters-only	11.3	26.6

Exposure to CPC

Safe Foods provided an exposure estimate for CPC in Section D of the 10/4/02 submission using the following assumptions[18]:

1. CPC will be present only in the skin of cooked poultry at an average concentration of 13.2 mg/kg.[19]

[15]In cases where no specification for "with skin" or "without skin" was given in the CSFII database, it was assumed that the same proportion of foods not specified as to skin consumption would be consumed with the skin as those foods that are specified. It was noted that this likely overestimates the amount of chicken consumed with skin, as many of these foods with no specification for "with skin" or "without skin" are typically prepared without skin.

[16]From Table 1 of Section D (original submission) and Table 6 of Section D (10/4/02 submission). The intake of poultry consumed with skin and poultry consumed without skin were obtained by multiplying the total poultry intake by 46% and 54%, respectively.

[17]The listed per capita intake values are approximately 90% of the eaters-only values. Although it is not stated in the petition, we presume that the per capita values result from multiplication of the eaters-only intake by the percent user value obtained from the NHANES data.

[18]A similar exposure estimate was included in Section D of the original submission. Safe Foods' original exposure estimate used a CPC residual level of 14.9 mg/kg to yield mean and 90th percentile exposures to CPC of 14 µg/p/d and 34 µg/p/d, respectively. The exposure estimate presented in Section D of the 10/4/02 submission supercedes the original exposure estimate.

2. The average poultry carcass is comprised of 10% skin.[20]

3. The mean and 90th percentile eaters-only intake of poultry consumed with skin are 9.6 and 22.6 g/p/d, respectively.

Thus, the mean estimated daily intake (EDI) is:

EDI_{mean} = (9.6 g poultry/p/day)(13.2 x 10^{-6} g CPC/g poultry)(0.10) = 13 µg/p/d

Likewise, the EDI for the 90th percentile consumer is 30 µg/p/d.

We concur with Safe Foods exposure estimate.

Exposure to Pyridine

We shall estimate the EDI of pyridine. Since no information on levels of residual pyridine on chicken have been provided, we shall calculate a worst-case estimate using the following assumptions:

1. CPC powder may contain a maximum 120 mg/kg pyridine (see Impurities).

2. The dilute Cecure solution will be applied at a maximum level of 0.3 g of CPC per pound of poultry carcass, which is equivalent to 0.66 mg of CPC per gram of poultry, or about 660 mg/kg.[21]

3. All of the CPC applied would remain on the chicken.

4. All of the pyridine present in CPC would remain on the chicken.[22]

Using these assumptions, the maximum concentration of pyridine applied to the poultry carcass would be 0.08 mg/kg (or 80 ppb). Therefore, the maximum eaters-only EDI for pyridine from consumption of poultry would be 1.7 µg/p/d and 3.9 µg/p/d at the mean and

[19]Based on residue studies on poultry carcasses treated with dilute Cecure solution containing 1% CPC at an application level of 1 ounce CPC per pound of poultry carcass (equivalent to the intended maximum level of 0.3 grams CPC per pound of poultry).

[20]Tan, T.K. et al., "Meat, Skin and Bone Yields of Broiler," *Singapore J. Pri. Ind.* 3(1) 12-20, 1975. See Appendix XI.

[21] $\frac{0.3\,\text{g CPC}}{\text{pound poultry}} \times \frac{1\,\text{pound}}{453.6\,\text{grams}} \times \frac{1000\,\text{g}}{1\,\text{kg}} \times \frac{1000\,\text{mg}}{1\,\text{g}} = 660\,\text{mg/kg}$

[22]The assumption that pyridine would only remain in the skin of the poultry was not used, since no experimental evidence was provided to show that pyridine would not be able to migrate to the poultry meat.

90th percentile, respectively.[23] However, since pyridine is volatile at cooking temperatures (bp 115°C), we would expect that it would be removed to an appreciable extent by cooking the poultry. Additionally, Safe Foods notes that the stability data presented in Appendix IV indicate that approximately 90% of the pyridine present in CPC concentrate solutions heated to 95°C for 90 minutes was lost. Thus, we would expect the actual mean and 90th percentile eaters-only exposure to pyridine to be negligible.

Proposed Regulation

Safe Foods has proposed wording for a regulation to appear in Part 173 (Secondary Direct Food Additives Permitted in Food for Human Consumption) for the use of CPC as an anitmicrobial agent on poultry. We suggest that the regulation appear in Subpart D (Specific Usage Additives) of Part 173. We propose the following modified wording[24]:

Cetylpyridinium chloride (CAS Reg. No. 123-03-5) may be safely used in food in accordance with the following prescribed conditions:
(a) The additive meets the specifications of the United States Pharmacopeia/National Formulary (USP 24/NF 19, January 2000), p. 370, which is incorporated by reference. Copies are available from the United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, or are available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW, Suite 700, Washington, DC 20408.

(b) The additive is used in food as an antimicrobial agent as defined at 21 CFR §170.3(o)(2) to treat the surface of raw poultry carcasses.

(c) The additive is applied as an aqueous solution that also contains propylene glycol (CAS Reg. No. 57-55-56) complying with §184.1666 of this chapter, at a concentration of approximately 1.5 times that of the cetylpyridinium chloride. The aqueous solution is applied to raw poultry carcasses as a fine mist spray at ambient temperature (73-75° F) prior to immersion in a chiller at a level not to exceed 0.3 grams per pound of raw poultry carcass.

Conclusion

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[23] CPC will be applied to poultry at a concentration of approximately 660 mg/kg, thus the maximum amount of pyridine that could be present on the treated poultry is:
$(660 \times 10^{-6}$ g CPC/g poultry) x $(120 \times 10^{-6}$ g pyridine/g CPC) = 80 ppb or 80 ng/kg.

Since it is assumed that pyridine could be found on chicken skin and meat, the total intake of poultry (20.9 g/p/d and 49.2 g/p/d 90th percentile; Table 5) is used. Thus, the mean eaters-only EDI would correspond to:
EDI_{mean} = (20.9 g poultry/p/day)$(80 \times 10^{-9}$ g pyridine/g poultry) = 1.7 µg/p/d
Similarly, the 90th percentile eaters-only EDI would be:
$EDI_{90th\%}$ = (49.2 g poultry/p/day)$(80 \times 10^{-9}$ g pyridine/g poultry) = 3.9 µg/p/d.

[24] The modified wording includes Safe Foods' revised intended use conditions (10/4/02 submission).

Safe Foods proposes to modify §173 to provide for the safe use of CPC as an antimicrobial for use on raw poultry. Safe Foods has determined the mean and 90th percentile eaters-only intakes of CPC from the petitioned use to be 13 µg/p/d and 30 µg/p/d, respectively.

We have no further questions regarding the use of CPC as an antimicrobial on raw poultry.

The petition is suitable for regulation with respect to the chemistry-related information.

Daniel E. Folmer, Ph.D.

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