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ORIGINAL SUBMISSION

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safe foods™ CORPORATION

January 14, 2000

The Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. S.W.
Washington, DC 20204

In accordance with the FDA's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS), we are submitting the following information regarding the use of cetylpyridinium chloride (CPC) as an antimicrobial treatment for meat and poultry products. This particular use of CPC is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is GRAS. The following documentation follows the outline for submitting a "Notice of a claim for exemption based on GRAS determination."

Sincerely,

Curtis W. Coleman
President/CEO, Safe Foods Corporation

Dr. Amy L. Waldroup
Professor

2000 JAN 19 A 11:36

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1505 Rebsamen Park Road • Little Rock, Arkansas 72202 U. S. A.
Little Rock, AR: 501-663-2383 • FAX: 501-663-8952 • E-Mail:SafeFoods@SafeFoods.net

Part I. Claim for Exemption (actual form code (c) (1))

1). Name and Address of Notifier: Mr. Curtis W. Coleman, President, Safe Foods Corporation, 1505 Rebsamen Park Road, Little Rock, AR 72202-1857.

2). Common or Usual Name of the Substance: Cetylpyridinium chloride or CPC.

3). Conditions of Use: CPC will be used to treat the outside surface of various types of raw and fully cooked food products. This may include: poultry, red meat, fish and shellfish. Regardless of food type, the concentration of CPC in the application solution will not exceed 1.0%, and will in most cases not exceed 0.5%. CPC will be utilized as an antimicrobial treatment to control bacteria, fungi, and parasites including, but not limited to, *Salmonella, Staphylococcus, Helicobacter, Campylobacter, Listeria, E. coli, Streptococcus, Yersinia, Arcobacter, Enterococcus, Shigella, Pseudomonas, Aeromonas, Bacillus, Micrococcus, Mycobacterium, Aspergillus, Penicillium*, and *Candida*.

A high percentage of the population already consumes CPC in mouthwashes, mouthrinses, and throat lozenges. Anyone who uses a daily mouthwash consumes approximately 900 mg of CPC per year. In addition, those who use any of the many types of throat lozenges that contain CPC, consume between 150 and 250 mg CPC per year in 100 lozenges. Thus, a large portion of the population currently consumes between 900 and 1300 mg CPC per year.

In this exemption claim we wish to establish that the concentration of CPC used for antimicrobial treatment of red meat, poultry, fish and shellfish would be minute at the point of application, and even less in terms of residual CPC in a given food. Currently, Americans consume 230 pounds of total meat, or 192 pounds on a boneless, trimmed-weight equivalent basis. Thus, if an aqueous CPC food treatment solution did not include a nonionic surfactant, and if all red meat, poultry, and fish produced in this country (boneless, trimmed-weight equivalent – USDA, 1998) were treated with a 1% CPC solution, then the public could be consuming as great as 1460 mg CPC per year. Obviously, this represents an extremely small increase (approximately an additional 160 mg) when compared to the total amount of CPC which is already consumed. This calculation is based on a 1% CPC solution applied at a rate of 1 ounce/pound of food product. In addition, the calculation assumes a 10% treatment absorption rate of the food. In reality, this absorption rate gives a great margin of safety because, as noted in Part IV (Scientific Procedures) below, the addition of a nonionic surfactant to the aqueous CPC solution prevents absorption by the tissue. Propylene glycol is used in the Cecure formulation as a processing aid for increasing solubility, but also has the ability to prevent the absorption of CPC into treated tissues (Kuo and Nakata, 1999; Kwak and Nakata, 1999).

4). Basis for GRAS Determination: Scientific Procedures.

5). Data and Information: The data and information that are the basis for this application of cetylpyridinium chloride for GRAS status are available for the Food and Drug Administrator's (FDA) review and copying at reasonable times, or the information will be sent to the FDA upon the Agency's request. The data and information can be obtained from Mr. Curtis W. Coleman, President, Safe Foods Corporation at 1505 Rebsamen Park Road, Little Rock, AR 72202-1857, phone: (501) 663-2383 or cwcoleman@safefoods.net.

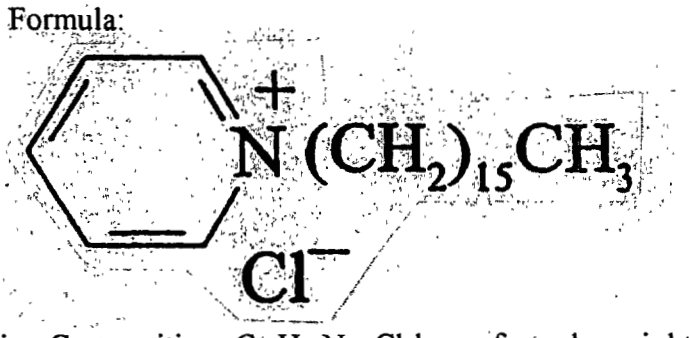
Part II. Identity of Substance (actual form code (c) (2))

1). Chemical Name: cetylpyridinium chloride.

2). Chemical Abstract Service (CAS) Registry Number: 6004-24-6.

3). Empirical Formula: $C_{21}H_{38}N$. Cl.

4). Structural Formula:



5). Quantitative Composition: $C_{21}H_{38}N$. Cl has a formula weight of 340.05; $C_{21}H_{38}N$. Cl . H_2O has a formula weight of 358.07; and $C_{21}H_{40}ClNO$ has a molecular weight of 358.01 and a mass of 339.99. Calculated elemental content is C: 70.45%; H: 11.26%; Cl: 9.90%; O: 4.47%; and, N: 3.91%.

6). Method of Manufacture: CPC can be prepared by the interaction of cetyl chloride and pyridine under pressure at an elevated temperature. In aqueous solution, CPC is synthesized by alkylation of pyridine with cetyl chloride to yield the monohydrate of the quaternary salt of pyridine and cetyl chloride.

Part III. (actual form code (c) (3))

1). Self-limiting Levels of Use: Not Applicable.

Part IV. (actual form code (c) (4))

A. Scientific Procedures (actual form code (c) (4) (i) (A))

1). Safety and Probable Consumption: CPC has been safely used in mouthwashes, rinses and throat lozenges since 1940 (Huyck, 1944). In this claim of exemption, no greater than 1.0% CPC will be used to treat various foodstuffs. This does not mean that treated foods will contain 1.0% CPC or even any measurable CPC. The reason for this is

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that the patented Cecure formulation contains an ingredient, propylene glycol, that has been shown to prevent absorption of CPC into various types of tissues. In a very recently issued patent (Kuo and Nakata, 1999) the absorption of CPC into wet tissues was prevented by adding any of a whole list of nonionic surfactants including: polyoxyethylene ethers, polyoxyethylene polyhydric alc. fatty acid esters, polyoxyethylene fatty acid esters, polyhydric alcs. fatty acid esters, polyhydric alc. alkyl ether propylene glycol fatty acid monoesters, or alkyldimethylamine oxides to the aqueous compounds. In an additional recently issued Japanese patent (Kwak and Nakata, 1999), the authors stated that "adding cationic surfactants and/or amphoteric surfactants to aqueous compounds containing the microbicide" prevents excess use of the microbicide and that any small amount of CPC that has been absorbed does not exhibit any residual antimicrobial activity. In the studies conducted to supply data for these patents, absence of a nonionic surfactant (such as propylene glycol) to the aqueous solution allowed for absorption of 16.57% of the antimicrobial solution, including the active microbicide. Even prior to these Japanese patents, researchers from the Oral-Care Research Laboratories of Lion Corporation in Tokyo reported the addition of a nonionic surfactant to oral rinse solutions containing CPC greatly reduced the adsorption of the chemical by Porphyromonas gingivalis cells (Mukasa *et al.*, 1994).

Using residual data from the literature that is specific for poultry and catfish treated with 0.1 or 0.4% CPC, residual levels of CPC in this foods is likely to be in the range of 17.8 to 33.3 mg/kg (Compadre *et al.*, 1998; Handie, 1999). However, in these studies the aqueous CPC solution used to treat the food products did not contain one of the nonionic surfactants (which is included in the Cecure treatment formulation) noted above in the two Japanese patents. Thus, the residual values noted by Compadre *et al.*, 1998 and Handie, 1999 fall in the range of values noted for the control samples (no surfactant added to solution) in the two Japanese patents previously noted (Kuo and Nakata; Kwak and Nakata, 1999).

B. (Actual form code (c) (4) (i) (B))

1). Inconsistent Reports: There are no reports of investigations of other information that appear to be inconsistent with GRAS determination.

C. (Actual form code (c) (4) (i) (C))

1). Based on the information provided in sections above (c) (1), (c) (2), (c) (3), (c) (4) (i) (A), and (c) (4) (i) (B), there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances, including CPC, added to food and there is reasonable certainty that the substance is not harmful under the intended conditions of use as specified in this claim of exemption.

Part V. CPC as an Antimicrobial Agent (not a required section of exemption)

CPC is a quaternary ammonia compound that has been safely used in some commercially available mouthwashes, throat sprays, and throat lozenges (0.045 to 1.4% CPC) for over 20 years (Barnes *et al.*, 1976; Ciancio *et al.*, 1978; Ashley *et al.*, 1984; Frost and Harris, 1994). In these types of products CPC is added to provide protection against plaque and gingivitis.

According to Huyck (1944), CPC inhibits bacterial metabolism through the formation of weakly ionized compounds from the interaction of basic cetylpyridinium ions with the acid groups of bacteria. In solution, as little as 0.002% CPC applied at room temperature resulted in close to a 90% reduction in *Salmonella typhimurium* (Breen *et al.*, 1995). There are, however, conflicting reports regarding the effects of CPC on bacterial attachment, specifically attachment of *Salmonella typhimurium*, to chicken skin (Breen *et al.*, 1995; Kim and Slavik, 1996). Breen *et al.* (1995) stated that CPC was "extremely effective at both inhibiting and reversing attachment of viable *S. typhimurium* cells to chicken skin." However, Kim and Slavik (1996) used scanning electron micrographs to show that "CPC does not detach cells from chicken skin."

Application of CPC to Poultry. In the first reported study of CPC application to poultry skin (Breen *et al.*, 1995), a 10-minute pretreatment of chicken skin with 0.1% CPC at 77° F diluted with 0.008 M buffered phosphate saline at pH 7.2 "completely inhibited the attachment of *Salmonella typhimurium*." The same treatment concentration and exposure time at 95° F, resulted in an 84% reduction in attachment. In these trials, 2.5 cm^2 (1 square inch) chicken drumstick skin samples were treated with 5 ml of various concentrations of CPC ranging from 0 to 0.1%. Considering this volume of treatment solution per skin surface area (5 ml/2.5 cm^2), and utilizing the equations for surface area of poultry carcasses (Thomas, 1978), it was determined that approximately 1.3 gallons of CPC solution would be required per processed chicken. It should be noted that in these trials the CPC was not mixed with any other chemical prior to treatment, as is the case in later studies conducted by these and other researchers.

In later studies it was reported that a 3-minute treatment with 0.4% solution of CPC plus 5% glycerin in 0.008M buffered phosphate saline at pH 7.2 to Salmonella-inoculated chicken drumstick skin resulted in a 4.8 log reduction in *Salmonella typhimurium* (Compadre *et al.*, 1996; Breen *et al.*, 1997). It was observed that when CPC was used in concentrations greater than 0.1% the CPC would quickly precipitate. The addition of 5.0% glycerin to the antimicrobial treatment serves to keep the CPC in solution. These researchers also stated that pretreatment of chicken skin with CPC could reduce carcass to carcass cross contamination during poultry slaughter because a 10-minute application of 0.8% CPC to chicken drumstick skin prevented bacterial attachment. In these studies the temperature of the CPC solution was not reported and each 2.5 cm^2 (1 square inch) skin sample was treated with 5 ml of CPC solution. Again, this would equate to using approximately 1.3 gallons of CPC solution per processed chicken carcass.

In a study conducted by Kim and Slavik (1996), CPC was evaluated for effectiveness in removing and/or killing attached *Salmonella typhimurium* on chicken skin. In this study the authors sprayed or immersed chicken skin samples in 0.1% CPC. Spray application was at either 59° F or 122° F for 1 minute. Immersion treatments were applied at room temperature for 1 minute, 1 minute plus 2 minutes "rest" time, or 3 minutes. Spraying, regardless of treatment temperature, resulted in 87 to 98% reduction in salmonellae levels. The amount of spray per unit of skin surface area was not reported. Immersion treatments, regardless of application time, resulted in salmonellae reductions similar to those noted for the spray applications. In the immersion treatments, 2.5 ml CPC solution was applied to 10 cm^2 skin samples. At this application rate, this would equate to approximately 21 ounces (0.16 gallon) per processed chicken carcass.

In trials conducted at the University of Arkansas pilot poultry processing plant prechill carcasses obtained from a local commercial facility were treated with 10 to 12 ounces (0.08 to 0.09 gallon) of 0.2% or 0.5% CPC applied at room temperature in an on-line mist cabinet (30 seconds followed by 2 minute rest period). This application would follow the inside-outside bird washer in a commercial facility, and would be applied in the same manner as an acidified sodium chlorite product (Sanova™, Alcide Corp., Redmond, WA) which is presently approved and utilized as an antimicrobial treatment in some poultry plants. These same concentrations (0.2% and 0.5%) were also evaluated as a prechill dip (immersion) for 10 seconds at room temperature. All CPC treatments, regardless of application method or concentration, significantly reduced aerobic plate count (APC), *E. coli*, other coliforms, and *Campylobacter* on postchill broilers (Brown and Waldroup, 1999, unpublished data). The 0.5% CPC dip resulted in the greatest reductions in all groups of organisms. For this treatment there was a 99.7% reduction in APC, and a > 99.9% reduction in *E. coli*, other coliforms and *Campylobacter*. In fact, *E. coli*, other coliforms, and *Campylobacter* could not be recovered from any of the chicken carcasses dipped in 0.5% room temperature CPC for 10 seconds.

In a second trial conducted in the same pilot processing facility, a 0.5% CPC 10-second room temperature prechill dip resulted in a 99.3% reduction in APC, *E. coli* and other coliforms could not be recovered from chilled carcasses (Waldroup *et al.*, 1999, unpublished data). A 10-second room temperature mist application of 0.5% CPC resulted in a 92% reduction in APC with greater than 90% reductions in *E. coli* and other coliforms. Mist applications at 0.75% and 1.0% CPC further reduced microbial levels to 99% for APC and 93% for *E. coli* and other coliforms. There was no statistical improvement in reductions in APC, *E. coli* or coliforms when the CPC concentration in the mist application was increased from 0.75% to 1.0%.

In the trial just described, all prechill carcasses were inoculated with 30,000 *Salmonella typhimurium* cells prior to the prechill dip or mist treatments. At postchill, more than 75% of control carcasses were still positive for *Salmonella*. No *Salmonella* could be recovered from carcasses treated with CPC, regardless of concentration or method of application. This finding supports the *Salmonella* inhibition studies in the literature (Breen *et al.*, 1995; 1997; Kim and Slavik, 1996). It should be noted, however, that there are some findings in the present study that needs further explanation. Our laboratory traditionally

enumerates *Salmonella typhimurium* by standard dilution and direct plating. This practice is consistent with methods used in all the previous published studies with CPC and chicken skin. When this is done, there is a lower detection level associated with the assay. In the two studies conducted by Breen *et al.*, (1995 and 1997), the lower detection level for *Salmonella typhimurium* was not published but is calculated to be 32 cfu/ml in the 1995 study and 5 cfu/ml in the 1997 study. In the 1996 study conducted by Kim and Slavik (1996), the lower detection level was not reported and can not be calculated using the information in the manuscript. In every study reported there was no preenrichment to allow for recovery of sublethally stressed cells. In fact, in the latter study the samples were direct plated on XLD agar which is highly selective for *Salmonella*, but inhibitory to sublethally stressed cells.

In our recent studies, we direct-plated for *Salmonella typhimurium*, but also allowed the entire whole carcass rinse fluid (400 ml) to preenrich for 24 hours, and then streak-swabbed the enriched sample. This allowed for a lower detection level of <1 cfu/ml for samples which are not positive by direct plating, but which are positive after overnight enrichment. Using this procedure we recovered 50 to 100% *Salmonella typhimurium* from the same samples. Thus, if the direct-plated samples are negative, but the 24-hour enriched original sample is positive for *Salmonella*, then one can conclude that at least one organism was recovered in the whole carcass rinse. This organism then had 24 hours to replicate to sufficient levels to allow for detection. One might argue that preenrichment gives the organism every chance at recovery, but this is how the USDA/FSIS *Salmonella* samples are being assayed. Current *Salmonella* performance standards are based on incidence, not level, of *Salmonella*, and one cell will result in a positive sample.

In a 4 week trial conducted in commercial broiler processing facility, the Cecure formulation (0.2 to 0.5%) was used to treat post-chill carcasses. In these studies, the final rinse cabinet or "fecal failure" cabinet that is positioned prior to grading and packaging, but after immersion chilling, was modified for application of the formulation. Cabinet modifications included changing the nozzles to allow for only small volumes (1 to 6 ounces) of the formulation per carcass, and modification of the spray pattern on the carcasses to allow for total coverage of as much surface area as possible. In addition, the length of the cabinet was extended and cabinet exhaust mechanisms were installed. The concentrated Cecure formulation was either diluted to the correct use concentration at the point of direct application to the carcass, or was diluted and held in large vessels prior to application. Regardless, the temperature of the solution was at ambient or slightly above or below depending on storage conditions.

After carcass treatment, the carcasses were allowed to drip for approximately 3 minutes prior to microbiological sampling. Carcasses were sampled using a whole carcass rinse technique in 400 mL of buffered peptone water. Samples were evaluated for incidence of *Salmonella*, and levels of *E. coli* and aerobic organisms. Control carcasses were also evaluated for these same organisms, but these carcasses were collected just prior to the modified fecal failure cabinet. During this month-long trial levels of *E. coli* and total anaerobes were significantly reduced by greater than 99%. In both trials, the incidence of *Salmonella* was significantly reduced to less than 5% positive while control carcass

Salmonella incidence rates were in some cases greater than 60%. This intervention technology is capable of providing a means for meat and poultry plants which are not presently meeting the *Salmonella* performance standard to comply with the regulation.

Application of CPC to Other Foods. Cutter and Dorsa (1998) used 1.0% CPC to treat beef samples. These authors reported complete removal of *Salmonella typhimurium* and *E. coli* O157:H7 from beef treated with a fecal slurry containing these organisms. Even after 35 days of refrigerated storage, vacuum packaged beef samples which had been initially treated with 1.0% CPC were free of these two pathogens. The authors reported no adverse organoleptic properties as determined by flavor, color, and texture of the cooked product.

Catfish skins (2.5 cm^2 or 1.0 square inch) were treated for 3 minutes with 5 ml of CPC ranging in concentration from 0.0% to 0.8% (Compadre *et al.*, 1998). Regardless of CPC concentration all treatments contained 5.0% glycerin and were diluted in 0.008 M phosphate buffer saline. Prior to antimicrobial treatment, catfish skin samples were inoculated with *Listeria monocytogenes*. At CPC concentrations of 0.2% and higher, *Listeria monocytogenes* could not be recovered from the skins, equating to a 3.67 log reduction in initial levels. However, there was no attempt in the methodology to recover any sublethally injured cells or to determine if there was a measurable amount of residual CPC in the microbial assay.

References:

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SUBMISSION END

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TELEFAX TRANSMITTAL SHEET

FOOD AND DRUG ADMINISTRATION
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Rockville, MD 20850
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PLEASE DELIVER THE FOLLOWING PAGES

TO: Linda Kahl DATE: 2-14-00

TELEPHONE #: FAX #: 202 418 3131

FROM: Tina Harper 301 827-0124

COMMENTS: FYI, here's a letter from Safe Foods to Rep. Dickey's office. Thanks for all your help.

NUMBER OF PAGES, INCLUDING COVER: 3

IF YOU DO NOT RECEIVE THE NUMBER OF PAGES INDICATED ABOVE, PLEASE CALL IMMEDIATELY

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February 8, 2000

Mr. Philip Hayes
Office of Congressman Jay Dickey
2453 Rayburn House Office Building
Washington, DC 20515

Dear Philip:

Thank you for the opportunity to visit with you on the telephone today and for your attention to this matter.

Here are the primary points:

1. Safe Foods submitted a GRAS notification to the FDA in September, 1999, and received confirmation of the FDA's receipt of the notice in late October, 1999. Our understanding from the FSIS at this point was that food processors could use Cecure™ under HACCP. Based on this information, one poultry processing plant completed modifications to their plant to use Cecure™. Other poultry, beef, fish and ready-to-eat processing facilities instituted plans for similar modifications. (It should be noted that the USDA has a policy of not issuing written letters of "approval" or "disapproval" for products that may be used as food safety interventions under HACCP, therefore requiring Safe Foods and food processors to commit large sums of money based on verbal responses from the agency.)

2. On January 7, 2000, the FDA (Drs. Robert L. Martin and Andrew Laumbach) notified me by phone that our GRAS notice cited an FDA document that contained proprietary information and therefore could not be cited. Safe Foods withdrew this GRAS notice and submitted a second notice on January 18, 2000. Safe Foods is awaiting notification of receipt from the FDA.

3. Safe Foods subsequently made several calls to the FSIS to determine the status of approval for use of Cecure™ under HACCP once the new GRAS notice was officially acknowledged. The verbal response we received was that there is no memorandum of understanding or other regulations concerning the interaction of the FDA and FSIS regarding products being reviewed for GRAS status under the new GRAS notification regulations and the new HACCP regulations.

The bottom line is this:

- The active ingredient in this antimicrobial product, (trademarked as Cecure™), has been consumed in over-the-counter products such as mouthrinses (Scope®) and throat lozenges (Cepacol®) for more than 40 years in this country.
- Cecure™ has demonstrated undeniable laboratory and commercial trial data demonstrating unparalleled efficacy against *Listeria, E. Coli, Salmonella,* and *Campylobacter*, including 6

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log reductions of *Listeria* on hot dogs and reduction of *Salmonella* contaminations on poultry from as high as 80% down to 0% (zero percent)!

- Every major food processor in the country has expressed an intention or a strong interest in using this product.
- The use of Cecure™ on food products will contribute *less than* 160 mg per year to the average person's consumption of CPC, (the product's active ingredient). We estimate the actual consumption of CPC to be closer to 50 mg/year. (The average person is estimated to consume approximately 1300 mg per year from the use of mouth rinses and throat lozenges.)
- This is a dramatically safe and effective part of the solution to the microbial food problems in our country and around the world. It deserves immediate and expeditious attention from the FDA.

Finally, let me add that we have found Dr. Robert Martin of the Office of Premarket Approval, FDA, and Dr. David Zeitz, FSIS, to be most helpful and gracious in their assistance.

To: Linda Kahl@OPA@FDA.CFSAN
Cc: [redacted]

From:
Certify: N
Priority: Normal
Subject: Letter of acknowledgement
Date: Tue Feb 15 11:24:28 2000
Attached: attach1

Dr. Kahl:

This is to confirm that we received today via fax a copy of your letter acknowledging receipt of our new GRAS notice (GRN No. 000038).

Thank You,
Curtis Coleman
Restricted Confidential Information - Intellectual Property of Safe Foods Corporation: The information contained herein is for use by authorized employees of the parties hereto and is not for general distribution within or outside their respective companies.

Happy Mail!

AM

To:
Cc:

From:
Certify: Y
Priority: Normal
Subject: RE: Letter of acknowledgement
Date: Wed Feb 16 22:18:47 2000
Attached: None

Dr. Kahl:

Thank you. I'll look forward to talking with you at 11 a.m. Eastern time.

Curtis Coleman

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-----Original Message-----
From: Linda Kahl [mailto:lkahl@bangate.fda.gov]
Sent: Wednesday, February 16, 2000 10:41 AM
To:
Cc: Kathryn Coleman (E-mail)
Subject: re: Letter of acknowledgement

Dear Mr. Coleman,

Melinda Plaisier has asked that the Center for Food Safety and Applied Nutrition return the call that you made to her office. Would Thursday at 11:00am Eastern Standard Time be a convenient time for us to call you?

Linda Kahl, Ph.D.
Division of Product Policy (HFS-206)
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
FDA
Phone: (202)418-3101
Fax: (202)418-3131
Internet: LKAHL@BANGATE.FDA.GOV

- - - - - - - - - - - - - - - Original Message - - - - - - - - - - - - - - -

Dr. Kahl:

This is to confirm that we received today via fax a copy of your letter acknowledging receipt of our new GRAS notice (GRN No. 000038).

Thank You,
Curtis Coleman

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Happy Mail!

AM

FAX TRANSMISSION

SAFE FOODS CORPORATION
1505 REBSAMEN PARK RD.
LITTLE ROCK, AR 72202
501-663-2383
FAX: 501-663-8952

To:	Dr. Linda Kahl	**Date:**	February 17, 2000
Fax #:	202-418-3131	**Pages:**	2, including this cover sheet.
From:	Curtis Coleman		
Subject:			

COMMENTS:

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February 17, 2000

Dr. Jane Henney, Commissioner
Food and Drug Administration
Parklawn Building, Room 14-71
5600 Fishers Lane
Rockville, MD 20857

Safe Foods Corporation hereby authorizes the Food and Drug Administration to discuss with the Office of Congressman Jay Dickey any and all matters pending before the FDA concerning Safe Foods Corporation. In this regard, the FDA may disclose any information requested by Congressman Dickey's Office, including information which may be designated as confidential or proprietary. This authorization shall not be deemed to waive any claim of confidentiality concerning any information disclosed pursuant to this authorization, nor shall it be deemed to authorize the disclosure of confidential information to any other person or agency by the FDA.

Curtis W. Coleman
President and CEO

c: Congressman Jay Dickey

1505 Rebsamen Park Road • Little Rock, Arkansas 72202 U. S. A.
Little Rock, AR: 501-663-2383 • FAX: 501-663-8952 • E-Mail: SafeFoods@SafeFoods.net

To: Linda Kahl@OPA@FDA.CFSAN
From:
Certify: Y
Priority: Normal
Subject: RE: RE: Letter of acknowledgement
Date: Thu Feb 17 15:33:19 2000
Attached: None

AM

Dear Dr. Kahl:

Thank you for this clarification. I did understand that I would receive the final copy of the letter and not the draft. I am, however, uncertain if it is the intention of this letter to provide a request for additional information, stipulate the inadequacies of our current notice, or to state a formal rejection of our notice.

We have, this week, discovered extensive toxicity data which will be beneficial to this notice. Also, we will have completed by this weekend extensive new residue studies which provide additional and significant information regarding the residual levels in foods treated by the CPC/propylene glycol product.

In that regard, I would like to accept your invitation to meet with you and Drs. Jackson and Pauli to gain your review of this new information and the potential benefit of its inclusion in our notice. I expect that this new information will be completed and finalized on or after February 23. This is to request a meeting with you at the first possible date after February 23.

Thank you again for your time and assistance.

Curtis Coleman

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-----Original Message-----
From: Linda Kahl [mailto:lkahl@bangate.fda.gov]
Sent: Thursday, February 17, 2000 12:18 PM
To: CWColeman@SafeFoods.net
Cc: Kathryn Coleman (E-mail)
Subject: re: RE: Letter of acknowledgement

Dear Mr Coleman:

As we discussed earlier today, a draft of our written response to your GRAS notice is under review by the Office of Premarket Approval. We will work expeditiously to complete that review and to issue the letter as described in
proposed 21 CFR 170.36. As Dr. Pauli stated, it will take at least a week to
finalize that letter, and it could take longer if the parties who review the letter recommend revisions. As soon as we finalize that letter, we will send
it to you via telefax, with a hard copy to follow by U.S. mail.

000030

At the end of our telephone conversation, we understood you to say that you would be looking for our draft letter. Given Dr. Pauli's subsequent remark

Happy Mail!

that we would work to get it finalized so that we could share it with you, we
believe that you realize that the letter that we will send you will be the agency's formal response to your GRAS notice rather than a "draft." However,
ensure that there was no miscommunication on this point, we decided that it
would be useful for us to clarify this point by written communication.

Linda Kahl
- - - - - - - - - - - - - - - Original Message - - - - - - - - - - - - - - -
Dr. Kahl:

Thank you. I'll look forward to talking with you at 11 a.m. Eastern time.

Curtis Coleman
501-663-2383

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-----Original Message-----
From: Linda Kahl [mailto:lkahl@bangate.fda.gov]
Sent: Wednesday, February 16, 2000 10:41 AM
To: CWColeman@SafeFoods.net
Cc: Kathryn Coleman (E-mail)
Subject: re: Letter of acknowledgement

ear Mr. Coleman,

Melinda Plaisier has asked that the Center for Food Safety and Applied Nutrition return the call that you made to her office. Would Thursday at 11:00am Eastern Standard Time be a convenient time for us to call you?

Linda Kahl, Ph.D.
Division of Product Policy (HFS-206)
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
FDA
Phone: (202)418-3101
Fax: (202)418-3131
Internet: LKAHL@BANGATE.FDA.GOV
- - - - - - - - - - - - - - - Original Message - - - - - - - - - - - - - - -
Dr. Kahl:

This is to confirm that we received today via fax a copy of your letter acknowledging receipt of our new GRAS notice (GRN No. 000038).

Thank You,
Curtis Coleman
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outside their respective companies.

Happy Mail!

safe foods™ CORPORATION

AM

2000 FEB 24 P 2: 25

February 22, 2000

Dr. Linda Kahl
The Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street S. W.
Washington, DC 20204

Dear Dr. Kahl:

REF: GRN000038

First, please accept my gratitude for the opportunity to talk with you and Drs. Pauli and Jackson in a telephone conversation last Thursday, February 17, 2000. I am grateful for the additional information and instruction you provided to us.

Please find enclosed the requisite documentation for our GRAS notification in accordance with the FDA's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)).

We are requesting that the enclosed documentation be treated as a revision or addendum to our GRAS notice, GRN000038. However, should that not be possible, adequate documentation is enclosed for consideration as a new GRAS notice.

The enclosed documentation includes the following:

1. Restriction of the application of cetylpyridinium chloride (CPC) to poultry products only
2. Limited and clearly defined conditions of use
3. Toxicity data
4. Residue data
5. Estimated daily intake data

We are requesting that this information be treated as a revision or addendum to the current notice (GRN000038) so that the notification and review cycle will not be required to be started over again. Regardless of how the enclosed documentation is received, (as a revision or as a new notice), we are respectfully urging the agency's expedited review of this notice. The reasons we are requesting the agency's priority attention to this notification are:

1505 Rebsamen Park Road • Little Rock, Arkansas 72202 U. S. A.
Little Rock, AR: 501-663-2383 • FAX. 501-663-8952 • E-Mail: SafeFoods@SafeFoods.net

1) Cecure™ has demonstrated - in laboratory and commercial trials - greater than 4 log reductions on the following pathogens:
   a) ***Salmonella typhimurium***
   b) ***Escherichia coli***
   c) ***Campylobacter jejuni***
   d) ***Listeria monocytogenes***
   e) ***Staphylococcus aureus***

2) USDA Secretary Dan Glickman reported that more than 9,000 Americans die every year from foodborne illnesses, and the CDC estimates that 76,000,000 million foodborne-related illnesses occur each year in the United States. The U. S. General Accounting Office estimates that the costs of treating food poisoning from *Salmonella* and other pathogens in the U. S. add up to $22 billion annually. In other words, people are getting sick and dying from foodborne illnesses, many caused by the pathogens that Cecure™ is very effective in eliminating.

3) Cecure™ is safe. The current total annual exposure to oral ingestion of Cecure™'s active ingredient, cetylpyridinium chloride (CPC), ranges from 12.9 to 16.4 mg/kg. If all of the poultry products in the U. S. were treated with Cecure™, the total annual exposure to oral ingestion of CPC would be increased by only 6.9 mg/kg.

   If all poultry products in the U. S. were treated with Cecure™, the estimated daily intake of CPC by the average poultry consumer would be 0.063 mg/kg. The acute toxic dose for humans is 50 to 500 mg/kg.

4) Cecure™ is economically feasible. The estimated cost of treating a whole broiler is 0.8¢ ($0.008). The estimated cost of treating a whole beef carcass is 26¢ per head. The estimated cost for modifying a food processing plant for the installation and use of Cecure™ is less than $10,000.00. Other treatments are expected to cost as much as 100 times more and costs for plant modifications run well into the $100,000.00's.

5) Cecure™ is desirable. There are no adverse organoleptic effects from the application of Cecure™ to foods.

6) Cecure™ is in demand. Almost every major food processor in the U. S. has either placed an order for Cecure™ or has expressed an intention to use Cecure™ upon approval. Companies in the Middle East, Europe, Africa, South America, Australia, New Zeeland, and other countries have expressed similar interests.

Please forgive me for repeating information you already know, but this product is so extraordinarily efficacious, easily applied, consumer desirable, economically feasible, and immediately available and requested by the industry, that it deserves the agency's extraordinary review.

I am available to answer the agency's questions and to work with the agency to expedite the availability of this product to the nation's food suppliers.

Thank You and Best Regards,

Curtis W. Coleman

safe foods™ CORPORATION

February 22, 2000

Dr. Linda Kahl
The Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street S. W.
Washington, DC 20204

GRAS Notification
Cetylpyridinium chloride (CPC) as an antimicrobial treatment for poultry products

Dear Dr. Kahl:

In accordance with the FDA's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)), we are submitting the following information regarding the use of cetylpyridinium chloride (CPC) as an antimicrobial treatment for poultry products. This particular use of CPC is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is GRAS. The following documentation follows the outline for submitting a "Notice of a claim for exemption on GRAS determination."

Sincerely,

Curtis W. Coleman
President/CEO
Safe Foods Corporation

2000 FEB 24 A 8:49

000038

1505 Rebsamen Park Road • Little Rock, Arkansas 72202 U. S. A.
Little Rock, AR: 501-663-2383 • FAX: 501-663-8952 • E-Mail: SafeFoods@SafeFoods.net

Part I. Claim for Exemption (actual form code (c) (1))

1). Name and Address of Notifier: Mr. Curtis W. Coleman, President, Safe Foods Corporation, 5011 Doyle Venable Drive, North Little Rock, Arkansas, 72118.

2). Common or Usual Name of the Substance: Cetylpyridinium chloride or CPC.

3). Conditions of Use: The claim of this GRAS notification is that cetylpyridinium chloride (CPC) is generally recognized as safe as used in a solution of propylene glycol (PG) and water under the following conditions:

a) This solution of CPC, PG, and water is manufactured (as a commercial product trademarked Cecure™[1]) according to good manufacturing practices (GMP) using the following ingredients:
   i) USP-grade CPC
   ii) Food grade propylene glycol
   iii) Water
b) This solution will be used to treat the outside surface of various types of raw and fully cooked poultry products.
c) The concentration of CPC in the application solution will not exceed 0.40%.
d) The method of application will be a mist or spray. The raw poultry product to be treated will be passed through a cabinet (or similar device) which is designed to safely mist or spray the prescribed application solution on to the surface of the poultry product. For raw poultry, this cabinet may be located either before or after the immersion chiller. For cooked poultry products, the solution containing CPC and PG will be sprayed or misted on to the surface of the product after cooking and before packaging.

[1] Cecure™ is a trademark of Safe Foods Corporation, Little Rock, AR. Use of the Cecure™ product as a food processing aid and/or decontamination aid is subject to certain rights pursuant to U. S. Pat. 5,366,983 and U. S. Pat. 5,855,940 and other pending applications. Safe Foods Corporation is the exclusive worldwide licensee of these patent rights. Additional patents pending. International patents and trademarks pending. All rights reserved.

e) CPC will be utilized as an antimicrobial treatment to control bacteria, fungi, and parasites including, but not limited to, *Salmonella, Staphylococcus, Helicobacter, Campylobacter, Listeria, E. coli, Streptococcus, Yersinia, Arcobacter, Enterococcus, Shigella, Pseudomonas, Aeromonas, Bacillus, Micrococcus, Mycobacterium, Aspergillus, Penicillium,* and *Candida.*

4). Basis for GRAS Determination: Scientific procedures based upon published studies and corroborated by unpublished studies and other data and information.

5). Data and Information: The data and information that are the basis for this application are available for the Food and Drug Administrator's (FDA) review and copying at reasonable times, or the information will be sent to the FDA upon the Agency's request. The data and information can be obtained from Mr. Curtis W. Coleman, President, Safe Foods Corporation at 5011 Doyle Venable Drive, North Little Rock, Arkansas, 72118, phone: (501) 663-2383, or cwcoleman@safefoods.net.

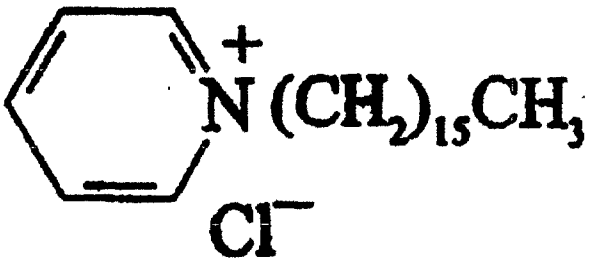
Part II. Identity of Substance (actual form code (c) (2))

1). Chemical Name: cetylpyridinium chloride.

2). Chemical Abstract Service (CAS) Registry Number: 6004-24-6.

3). Empirical Formula: $C_{21}H_{38}N$. Cl.

4). Structural Formula:



5). Quantitative Composition: $C_{21}H_{38}N$. Cl has a formula weight of 340.05; $C_{21}H_{38}N$. Cl . H_2O has a formula weight of 358.07; and $C_{21}H_{40}ClNO$ has a molecular weight of 358.01 and a mass of 339.99. Calculated elemental content is C: 70.45%; H: 11.26%; Cl: 9.90%; O: 4.47%; and, N: 3.91%.

6). Method of Manufacture: CPC can be prepared by the interaction of cetyl chloride and pyridine under pressure at an elevated temperature. In aqueous solution, CPC is synthesized by alkylation of pyridine with cetyl chloride to yield the monohydrate of the quaternary salt of pyridine and cetyl chloride.

Part III. (actual form code (c) (3))

1). Self-limiting Levels of Use: Not Applicable.

Part IV. (actual form code (c) (4))

1). Scientific Procedures (actual form code (c) (4) (i) (A))

000041

a) Toxicity: CPC has been safely used in mouthwashes, rinses, toothpaste, and throat lozenges since 1940 (Huyck, 1944; Parran, 1982). There is published toxicological data regarding oral ingestion of CPC for various animal species including rabbits, rats, mice, guinea pigs, dogs and cats (Table 1). Warren *et al.*

(1942) fed rabbits 10 or 100 mg/kg CPC daily for a duration of 4 weeks. No specific chronic exposure symptoms were noted. The LD_{50} for rabbits for single dose oral toxicity was 400 mg/kg. Lewis (1996) reported a LD_{50} for rabbits of 1000 mg/kg. Nelson and Lyster (1946) fed 30 mg/kg CPC to rats for 60 days. Temporary peripheral paralysis was noted in some rats. The LD_{50} for rats was 200 mg/kg. However, Lewis (1996) reported a LD_{50} for rats of 5080 mg/kg. Rosen *et al.* (1965) determined the LD_{50} for mice to be 108 mg/kg; however, Lewis (1996) reported a LD_{50} for mice of 1360 mg/kg. Lewis (1996) also reported an LD_{50} value for guinea pigs of 3860 mg/kg. For cats and dogs, the reported LD_{50} was 1000 mg/kg (Lewis, 1996).

Table 1. Chronic and Acute Toxicity for Cetylpyridinium Chloride

			Chronic Daily Exposure			
Author	**Year**	**Species**	**mg/kg**	**Duration**	**Symptoms**	**Acute Oral Toxicity (mg/kg) – LD_{50}**
Warren *et al.*	1942	Rabbit	10 – 100	4 weeks	None	400
Nelson and Lyster	1946	Rat	30	60 days	Temporary peripheral paralysis	200
Rosen *et al.*	1965	Mouse	No data	No data	No data	108
Lewis[1]	1996	Rabbit	No data	No data	No data	1000
		Rat	No data	No data	No data	5080
		Mouse	No data	No data	No data	1360
		Guinea pig	No data	No data	No data	3860
		Dog				
		Cat	No data	No data	No data	1000
			No data	No data	No data	1000

[1]Lewis (1996 – Appendix II) cited a study conducted by Arzndad Arzneimittel-Forschung Drug Research in 1951 in the Federal Republic of Germany. The German study was published in 1961 in the J. Pharmaceut. Sci., Amer. Pharmaceut. Assoc., 2215 Constitution Ave., NW, Washington, DC.

Cetylpyridinium chloride has a toxicity rating of 4 indicating that the estimated acute toxic dose for humans is between 50 and 500 mg/kg (Gosselin *et al.*, 1984). The acute toxic dose of CPC for humans has been estimated to be between 1 and 3 g for an average (70 kg) person (Arena and Drew, 1986)

b) Residue and Probable Consumption: Compadre *et al.* (1998) and Handie (1999) reported residual CPC values for raw poultry carcasses using radiolabelled CPC. In these studies raw, whole broiler carcasses were either immersed in 0.0125% CPC for 60 seconds or were sprayed with 0.1 or 0.4% CPC for 20 seconds. Regardless of application method, carcasses were not rinsed with water after exposure to CPC. In addition, PG was not included in the formulation. Broilers that were immersed in 0.0125% CPC had an average CPC residue of 8 to 15.7 mg/kg. For broilers which were sprayed with 0.1% CPC for 20 seconds, the CPC residue was in the range of 14.6 to 20.6 mg/kg with an average of 17.8±3.0 mg/kg. Broilers that were sprayed with 0.4% CPC for 20 seconds had an average CPC residue of 17.5 to 22.0 mg/kg with an average of 19.9±2.3 mg/kg.

Currently, the average annual per capita consumption of poultry by Americans is 47.7 kg, or 30.9 kg on a boneless, trimmed-weight equivalent basis (USDA, 1998). If one uses the average CPC residue for broilers that were sprayed with 0.4% CPC for 20 seconds (as cited in the preceding paragraph), or **19.9 mg/kg**, and an average human weight of 70 kg, then the chronic human exposure over a one year period would be as follows:

Table 2. Per Capita Poultry Consumption in 1998.

Annual poultry consumption:	**Annual chronic exposure to CPC:**
47.7 kg total	949.23 mg or 13.56 mg/kg
30.9 kg, boneless, trimmed-weight	614.91 mg or 8.78 mg/kg

A significant percentage of Americans purchase and/or consume skinless poultry, so these amounts would be considered to be "worst case" exposures. ***However,***

these annual chronic exposure estimates, to be accurate, must be reduced because of two consequential factors.

Factor One: The absorption of CPC and the corresponding resulting residue level in poultry is significantly reduced by the presence of propylene glycol in the application solution (as specified in the conditions for use in this notice). This reduction in absorption of CPC caused by the presence of various nonionic surfactants is validated in two recent Japanese patents. Kuo and Nakata (1999) reported that the absorption of CPC into wet tissues was prevented by adding any of a whole list of nonionic surfactants including: polyoxyethylene ethers, polyoxyethylene polyhydric alc. fatty acid esters, polyoxyethylene fatty acid esters, polyhydric alcs. fatty acid esters, polyhydric alc. alkyl ether propylene glycol fatty acid monoesters, or alkyldimethylamine oxides to aqueous CPC solutions. In an additional recently issued Japanese patent (Kwak and Nakata, 1999), the authors stated that "adding cationic surfactants and/or amphoteric surfactants to aqueous compounds containing the microbicide" (CPC) prevents excess use of the microbicide and that any small amount of CPC that has been absorbed does not exhibit any residual antimicrobial activity. Even prior to these Japanese patents, researchers from the Oral-Care Research Laboratories of Lion Corporation in Tokyo reported that the addition of a nonionic surfactant to oral rinse solutions containing CPC greatly reduced the adsorption of the chemical by *Porphyromonas* gingivalis cells (Mukasa *et al.*, 1994).

The reduction in absorption of CPC specifically by the addition of propylene glycol (PG) is confirmed in research conducted at the University of Arkansas by Beers *et al.* (2000) (Appendix II). These data suggest a 38% to 65% reduction in the absorption of CPC in broiler skin when PG is included in the formulation (as stipulated in the Conditions of Use in this notice). Using the more conservative value (38% reduction in absorption), the absorption of CPC in poultry is reduced from 19.9 mg/kg to **12.3 mg/kg**. Therefore, the chronic human exposure over a one-year period would be as follows:

Table 3. Annual Chronic Exposure to CPC Attributed to Proposed Use in Poultry Products

Annual per capita poultry consumption:	**Annual chronic exposure to CPC when applied in a solution including PG:**
47.7 kg total	586.71 mg or 8.38 mg/kg
30.9 kg, boneless, trimmed-weight basis	380.07 mg or 5.43 mg/kg

Factor Two: The research conducted by Beers *et al.* (2000) (Appendix II) demonstrates the effects of cooking on residual CPC in broiler skin. Cooking results in a 31.2% reduction in residual CPC. Thus, using the absorption value of 12.3 mg/kg above, absorption would be reduced to **8.5 mg/kg**. Of course some fully cooked products would not be reheated, therefore this modification will be used only when referring to raw poultry applications. For fully cooked poultry, only the effects of adding 0.5% PG to the CPC treatment will employed.

Approximately 35% of all poultry is currently sold in a fully cooked form. Thus, 16.7 kg (35% of 47.7 kg per capita annual consumption[2]) of poultry may not be subject to any heat treatment prior to consumption. Therefore, for the 16.7 kg of cooked poultry the 12.3 mg/kg CPC residue value is appropriate. For the remainder, which would be raw product (31 kg), the 8.5 mg/kg value is utilized. The annual chronic exposure to CPC using these residue factors is calculated in Table 4:

[2] As an extra margin of safety, 47.7 kg per capita annual consumption of poultry is used instead of the 30.9 kg value, which is the boneless, trimmed-weight equivalent.

Table 4. Annual Chronic Exposure to CPC as Adjusted for Cooking

Poultry Product:	**Annual per capita consumption:**	**CPC residue absorption rate:**	**Annual chronic exposure to CPC when applied in a solution including PG:**
Raw	31 kg	8.5 mg/kg	263.5 mg or 3.76 mg/kg
Fully cooked	16.7 kg	12.3 mg/kg	205.4 mg or 2.93 mg/kg
TOTALS:	**47.7 kg**	**-------**	**468.9 mg or 6.69 mg/kg**

A percentage of the population already consumes CPC (Figure 1). In fact, CPC has been consumed since 1940 as an antimicrobial in mouthwashes, mouthrinses, toothpastes, and throat lozenges (Huyck, 1944; Parran, 1982). Anyone who uses a daily mouthwash which contains CPC currently consumes approximately 900 mg of CPC per year. In addition, those who use throat lozenges that contain CPC consume between 150 and 250 mg CPC per year in 100 lozenges. Thus, a large portion of the population currently consumes between 900 and 1150 mg CPC per year. On a per kg basis, this would amount to 12.9 to 16.4 mg/kg CPC per year.

To determine annual total exposure to oral ingestion of CPC, the values noted above for mouthwashes, toothpaste, and throat lozenges (900 to 1150 mg per year) should be included. On a per kg basis as above, the exposure attributed to existing sources ranges from 12.9 to 16.4 mg/kg. ***Thus, the total chronic exposure to CPC over a given year (current uses plus proposed use on poultry) would equal 19.5 to 23.1 mg/kg*** (Figure 1).

EDI: The estimated daily intake (EDI) of CPC would be approximately 0.063 mg/kg for an average poultry consumer and 0.0756 mg/kg for a high-end poultry consumer (Figure 2). These EDI values include existing and proposed exposure. It has been previously noted in this document that the ACUTE TOXIC DOSE of CPC for humans is estimated to be 50 to 500 mg/kg (Gosselin *et al.*, 1984).

A. (Actual form code (c) (4) (i) (B))

1). Inconsistent Reports: There are no reports of investigations of other information that appear to be inconsistent with GRAS determination. However, there are some discrepancies in the LD_{50} values for some of the animal species. Nevertheless, all LD_{50} values are so much greater than the values proposed in this notice that these discrepancies are inconsequential.

B. (Actual form code (c) (4) (i) (C))

1). Based on the information provided in sections above (c) (1), (c) (2), (c) (3), (c) (4) (i) (A), and (c) (4) (i) (B), there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances, including CPC, added to food and there is reasonable certainty that the substance is not harmful under the intended conditions of use as specified in this claim of exemption.

Part V. CPC as an Antimicrobial Agent (not a required section of exemption)

CPC is a quaternary ammonia compound that has been safely used in many commercially available mouthwashes, toothpastes, throat sprays, and throat lozenges (0.045 to 1.4% CPC) for over 50 years (Barnes *et al.*, 1976; Ciancio *et al.*, 1978; Ashley *et al.*, 1984; Frost and Harris, 1994). In these types of products CPC is added to provide protection against plaque and gingivitis.

According to Huyck (1944), CPC inhibits bacterial metabolism through the formation of weakly ionized compounds from the interaction of basic cetylpyridinium ions with the acid groups of bacteria. In solution, as little as 0.002% CPC applied at room temperature resulted in close to a 90% reduction in *Salmonella typhimurium* (Breen *et al.*, 1995). There are, however, conflicting reports regarding the effects of CPC on bacterial attachment, specifically attachment of *Salmonella typhimurium*, to chicken skin (Breen *et al.*, 1995; Kim and Slavik, 1996). Breen *et al.* (1995) stated that CPC was "extremely

effective at both inhibiting and reversing attachment of viable *S. typhimurium* cells to chicken skin." However, Kim and Slavik (1996) used scanning electron micrographs to show that "CPC does not detach cells from chicken skin."

Application of CPC to Poultry. In the first reported study of CPC application to poultry skin (Breen *et al.*, 1995), a 10-minute pretreatment of chicken skin with 0.1% CPC at 77° F diluted with 0.008 M buffered phosphate saline at pH 7.2 "completely inhibited the attachment of *Salmonella typhimurium*." The same treatment concentration and exposure time at 95° F resulted in an 84% reduction in attachment. In these trials, 2.5 cm^2 (1 square inch) chicken drumstick skin samples were treated with 5 ml of various concentrations of CPC ranging from 0 to 0.1%. Considering this volume of treatment solution per skin surface area (5 ml/2.5 cm^2), and utilizing the equations for surface area of poultry carcasses (Thomas, 1978), it was determined that approximately 1.3 gallons of CPC solution would be required per processed chicken. It should be noted that in these trials the CPC was not mixed with any other chemical prior to treatment, as is the case in later studies conducted by these and other researchers.

In later studies it was reported that a 3-minute treatment with 0.4% solution of CPC plus 5% glycerin in 0.008M buffered phosphate saline at pH 7.2 to *Salmonella*-inoculated chicken drumstick skin resulted in a 4.8 log reduction in *Salmonella typhimurium* (Compadre *et al.*, 1996; Breen *et al.*, 1997). It was observed that when CPC was used in concentrations greater than 0.1% that the CPC would quickly precipitate. The addition of 0.5% glycerin to the antimicrobial treatment serves to keep the CPC in solution. These researchers also stated that pretreatment of chicken skin with CPC could reduce carcass to carcass cross contamination during poultry slaughter because a 10-minute application of 0.8% CPC to chicken drumstick skin prevented bacterial attachment. In these studies the temperature of the CPC solution was not reported and each 2.5 cm^2 (1 square inch) skin sample was treated with 5 ml of CPC solution. Again, this would equate to using approximately 1.3 gallons of CPC solution per processed chicken carcass.

In a study conducted by Kim and Slavik (1996), CPC was evaluated for effectiveness in removing and/or killing attached *Salmonella typhimurium* on chicken skin. In this study the authors sprayed or immersed chicken skin samples in 0.1% CPC.

Spray application was at either 59° F or 122° F for 1 minute. Immersion treatments were applied at room temperature for 1 minute, 1 minute plus 2 minutes "rest" time, or 3 minutes. Spraying, regardless of treatment temperature, resulted in 87 to 98% reduction in salmonellae levels. The amount of spray per unit of skin surface area was not reported. Immersion treatments, regardless of application time, resulted in salmonellae reductions similar to those noted for the spray applications. In the immersion treatments, 2.5 ml CPC solution was applied to 10 cm^2 skin samples. At this application rate, this would equate to approximately 21 ounces (0.16 gallon) per processed chicken carcass.

In trials conducted at the University of Arkansas pilot poultry processing plant, prechill carcasses obtained from a local commercial facility were treated with 10 to 12 ounces (0.08 to 0.09 gallon) of 0.2% or 0.5% CPC applied at room temperature in an on-line mist cabinet (30 seconds followed by 2 minute rest period). This application would follow the inside-outside bird washer in a commercial facility, and would be applied in the same manner as an acidified sodium chlorite product (Sanova™, Alcide Corp., Redmond, WA) which is presently approved and utilized as an antimicrobial treatment in some poultry plants. These same concentrations (0.2% and 0.5%) were also evaluated as a prechill dip (immersion) for 10 seconds at room temperature. All CPC treatments, regardless of application method or concentration, significantly reduced aerobic plate count (APC), *E. coli*, other coliforms, and *Campylobacter* on postchill broilers (Brown and Waldroup, 1999, unpublished data). The 0.5% CPC dip resulted in the greatest reductions in all groups of organisms. For this treatment there was a 99.7% reduction in APC, and a > 99.9% reduction in *E. coli*, other coliforms and *Campylobacter*. In fact, *E. coli*, other coliforms, and *Campylobacter* could not be recovered from any of the chicken carcasses dipped in 0.5% room temperature CPC for 10 seconds.

In a second trial conducted in the same pilot processing facility, a 0.5% CPC 10-second room temperature prechill dip resulted in a 99.3% reduction in APC; *E. coli* and other coliforms could not be recovered from chilled carcasses (Waldroup *et al.*, 1999, unpublished data). A 10-second room temperature mist application of 0.5% CPC resulted in a 92% reduction in APC with greater than 90% reductions in *E. coli* and other coliforms. Mist applications at 0.75% and 1.0% CPC further reduced microbial levels to 99% for APC and 93% for *E. coli* and other coliforms. There was no statistical

improvement in reductions in APC, *E. coli* or coliforms when the CPC concentration in the mist application was increased from 0.75% to 1.0%.

In the trial just described, all prechill carcasses were inoculated with 30,000 *Salmonella typhimurium* cells prior to the prechill dip or mist treatments. At postchill, more than 75% of control carcasses were still positive for *Salmonella*. No *Salmonella* could be recovered from carcasses treated with CPC, regardless of concentration or method of application. This finding supports the *Salmonella* inhibition studies in the literature (Breen *et al.*, 1995; 1997; Kim and Slavik, 1996). It should be noted, however, that there are some findings in the present study that need further explanation. Dr. Waldroup's laboratory traditionally enumerates *Salmonella typhimurium* by standard dilution and direct plating. This practice is consistent with methods used in all the previous published studies with CPC and chicken skin. When this is done, there is a lower detection level associated with the assay. In the two studies conducted by Breen *et al.* (1995 and 1997), the lower detection level for *Salmonella typhimurium* was not published but is calculated to be 32 cfu/ml in the 1995 study and 5 cfu/ml in the 1997 study. In the 1996 study conducted by Kim and Slavik (1996), the lower detection level was not reported and can not be calculated using the information in the manuscript. In every study reported there was no preenrichment to allow for recovery of sublethally stressed cells. In fact, in the latter study the samples were direct plated on XLD agar that is highly selective for *Salmonella*, but inhibitory to sublethally stressed cells.

In the recent studies conducted by Dr. Waldroup, samples were direct-plated for *Salmonella typhimurium*, but they also allowed the entire whole carcass rinse fluid (400 ml) to preenrich for 24 hours, and then streak-swabbed the enriched sample. This allowed for a lower detection level of <1 cfu/ml for samples which are not positive by direct plating, but which are positive after overnight enrichment. Using this procedure they recovered 50 to 100% *Salmonella typhimurium* from the same samples. Thus, if the direct-plated samples are negative, but the 24-hour enriched original sample is positive for *Salmonella*, then one can conclude that at least one organism was recovered in the whole carcass rinse. This organism then had 24 hours to replicate to sufficient levels to allow for detection. One might argue that preenrichment gives the organism every chance at recovery, but this is how the USDA/FSIS *Salmonella* samples are being assayed.

Current *Salmonella* performance standards are based on incidence, not level, of *Salmonella*, and one cell will result in a positive sample.

In a 4 week trial conducted in commercial broiler processing facility, the Cecure formulation (0.2 to 0.5%) was used to treat post-chill carcasses. In these studies, the final rinse cabinet or "fecal failure" cabinet that is typically positioned prior to grading and packaging but after immersion chilling, was modified for application of the Cecure™ formulation. Cabinet modifications included changing the nozzles to allow for only small volumes (1 to 6 ounces) of the formulation per carcass, and modification of the spray pattern on the carcasses to allow for total coverage of as much surface area as possible. In addition, the length of the cabinet was extended and cabinet exhaust mechanisms were installed. The concentrated Cecure formulation was either diluted to the correct use concentration at the point of direct application to the carcass, or was diluted and held in large vessels prior to application. Regardless, the temperature of the solution was at ambient or slightly above or below depending on storage conditions.

After carcass treatment, the carcasses were allowed to drip for approximately 3 minutes prior to microbiological sampling. Carcasses were sampled using a whole carcass rinse technique in 400 mL of buffered peptone water. Samples were evaluated for incidence of *Salmonella*, and levels of *E. coli* and aerobic organisms. Control carcasses were also evaluated for these same organisms, but these carcasses were collected just prior to the modified fecal failure cabinet. During this month-long trial, levels of *E. coli* and total anaerobes were significantly reduced by greater than 99%. In both trials, the incidence of *Salmonella* was significantly reduced to less than 5% positive while control carcass *Salmonella* incidence rates were in some cases greater than 60%. This intervention technology is capable of providing a means for meat and poultry plants which are not presently meeting the *Salmonella* performance standard to comply with the regulation.

Application of CPC to Other Foods. Cutter and Dorsa (1998) used 1.0% CPC to treat beef samples. These authors reported complete removal of *Salmonella typhimurium* and *E. coli* O157:H7 from beef treated with a fecal slurry containing these organisms. Even after 35 days of refrigerated storage, vacuum packaged beef samples which had been initially treated with 1.0% CPC were free of these two pathogens. The authors reported

no adverse organoleptic properties as determined by flavor, color, and texture of the cooked product.

Catfish skins (2.5 cm^2 or 1.0 square inch) were treated for 3 minutes with 5 ml of CPC ranging in concentration from 0.0% to 0.8% (Compadre *et al.*, 1998). Regardless of CPC concentration all treatments contained 5.0% glycerin and were diluted in 0.008 M phosphate buffer saline. Prior to antimicrobial treatment, catfish skin samples were inoculated with *Listeria monocytogenes.* At CPC concentrations of 0.2% and higher, *Listeria monocytogenes* could not be recovered from the skins, equating to a 3.67 log reduction in initial levels. However, there was no attempt in the methodology to recover any sublethally injured cells or to determine if there was a measurable amount of residual CPC in the microbial assay.

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Appendix I.

Sax's Dangerous Properties of Industrial Materials

R.J. Lewis, Sr., 1996

Pages 000056 - 000057 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Figures 1 and 2.

Figure 1. Existing and Proposed Annual Per Capita Consumption of CPC

Figure 2. Estimated Proposed Daily CPC Intake for Average and High Consumers of Poultry

Figure 2. Estimated proposed daily CPC intake for average and high consumers of poultry

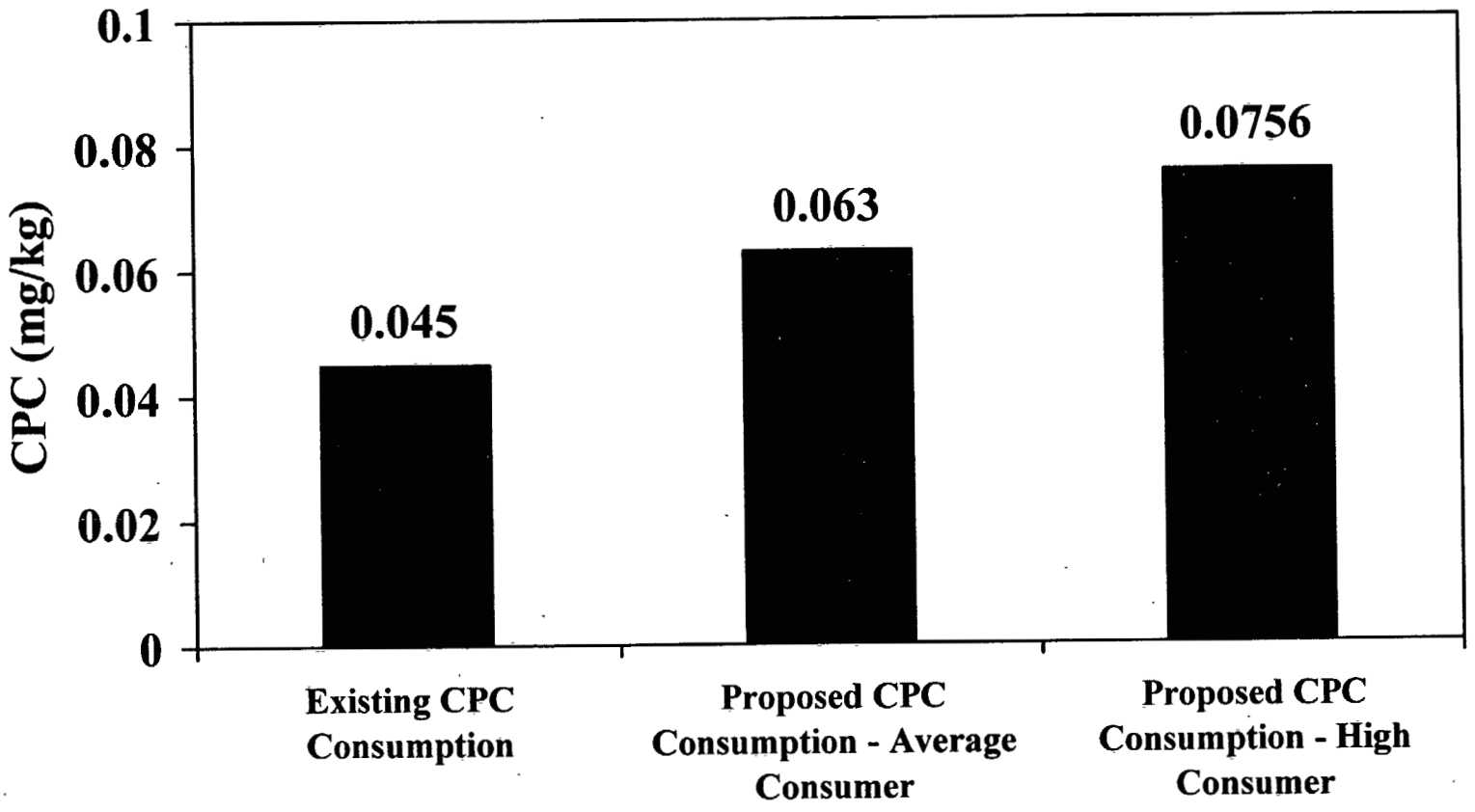

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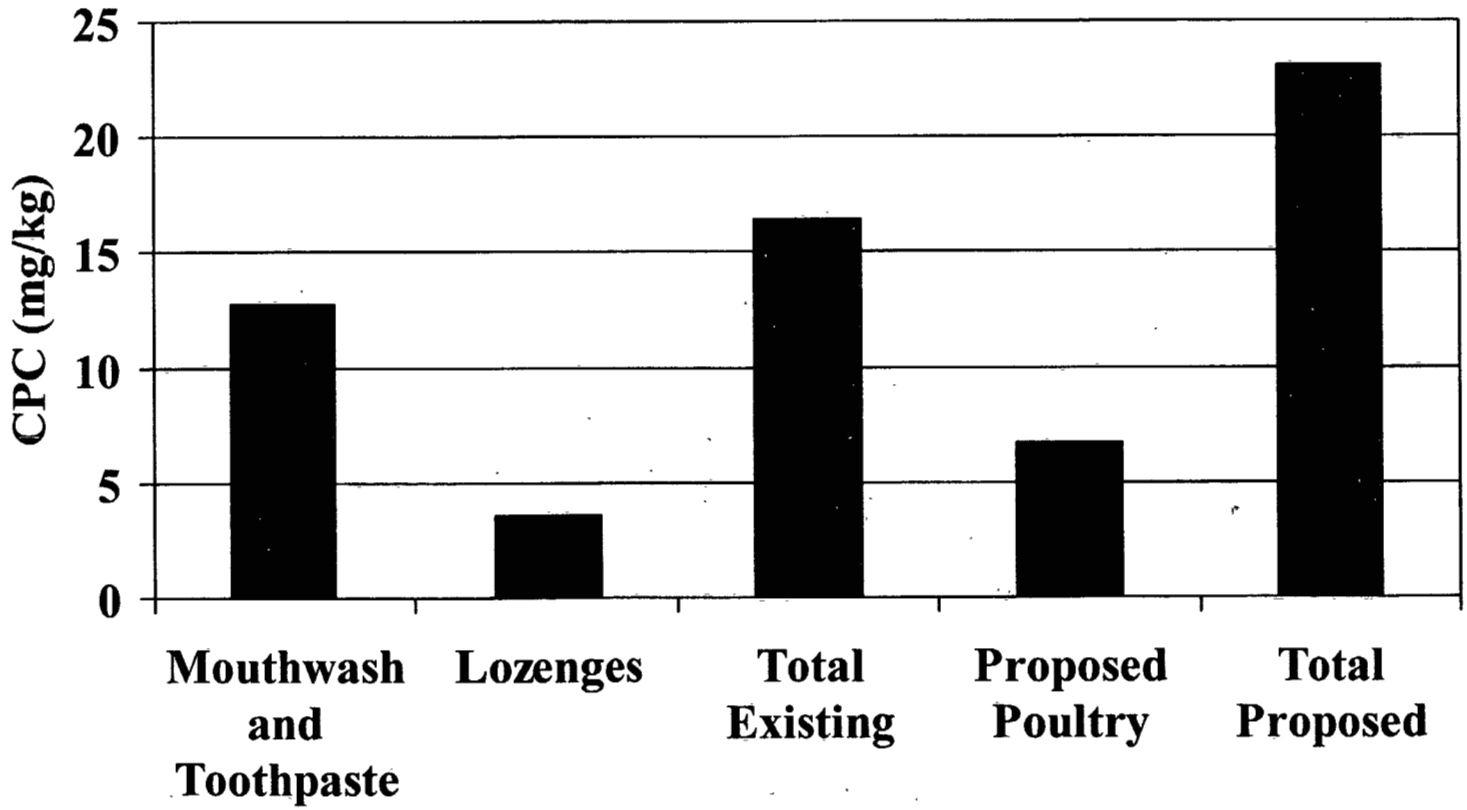
Figure 1. Existing and proposed annual per capita consumption of CPC



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Appendix II.

The Effects of Post-Treatment Water Rinsing, Cooking, and the Addition of Propylene Glycol to Aqueous Cetylpyridinium Chloride Solutions on Residual Cetylpyridinium Chloride in Broiler Skin Samples

K. Beers, E. Kroger, and A. Waldroup, 2000

The Effects of Post-Treatment Water Rinsing, Cooking, and the Addition of Propylene Glycol to Aqueous Cetylpyridinium Chloride Solutions on Residual Cetylpyridinium Chloride in Broiler Skin Samples

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General – Trials 1, 2 and 3.

Three experiments were conducted to determine the effects of the addition of propylene glycol (PG) to a 0.3% aqueous solution of cetylpyridinium chloride (CPC) on chemical absorption by poultry skin samples. In Trial 1, the effects of post-treatment water rinsing of poultry skin which had been treated with 0.3% CPC, with or without 0.5% PG, was also evaluated. In addition, the effects of cooking on poultry skin samples on retention of CPC was investigated (Trial 2). This trial was especially important because there was no published data available regarding the effects of cooking on residual CPC in any type of food product. In all experiments, fresh, chilled broiler leg quarters were purchased locally at retail. The skin was manually removed from the pieces of poultry and was cut into pieces with each piece weighing approximately 4 grams. In all trials the initial weight of each piece of skin was recorded and ranged from 3.91 to 4.09 grams.

Trial 1.

Objective. The objective of this experiment was twofold. First, the effects of water rinsing following direct exposure of chicken skin to 0.3% CPC on residual CPC in treated chicken skin samples was evaluated. In addition, the effects of including 0.5% PG in a 0.3% CPC solution on the amount of residual CPC in treated broiler skin samples was investigated.

Procedure. Each individual chicken skin sample (n=5) was placed into a 50 mL conical tube containing 40 mL 0.3% CPC or 40 mL 0.3% CPC plus 0.5% PG. Skin samples were allowed to remain in constant contact with the aqueous solutions for 10 minutes during which time the samples were gently shaken. It should be noted in a commercial application, such as treatment of raw poultry carcasses or parts, the exposure period would be considerably shorter (< 5 seconds). In addition, it should be noted that the amount of CPC solution applied to the skins in these experiments is close to 160 times the amount of solution that would typically be used in a commercial application. For example, a broiler would have to be treated with 1.25 gallons of CPC to approximate the weight to volume exposure utilized in this trial. In reality, only 3 to 4 ounces of CPC will be utilized to treat a commercial broiler. Thus, any residuals data obtained from these experiments should provide a tremendous margin of safety and represent "worst case" values.

After the 10-minute CPC exposure period, the skin samples were removed from the solutions and allowed to drain for 1 minute. Skin samples were allowed to touch the sides of their respective conical tubes in an effort to remove excess liquid. Half of the skin samples were then individually transferred to clean 50 mL conical tubes containing 40 mL of water. The samples were then rinsed in the water with gentle shaking for 10 minutes in an effort to determine if simple water rinsing post-CPC treatment would significantly reduce absorption of CPC into the skin. Again, a commercial application would only allow for a rinse exposure period of 5 seconds or less and would utilize only a fraction of the water that was utilized in these experiments.

All skin samples, regardless of treatment, were transferred to clean conical tubes each containing 30 grams 95% ethanol (EtOH). CPC was extracted for 30 minutes on a shaker table. Tubes were centrifuged for 10 minutes @ 3 K RPM. CPC residual analysis was performed by HPLC for both the water rinses and the EtOH extracts according to the method outlined by Handie, 1999.

Results. Regardless of whether the CPC solution contained PG, water washing of the skin samples immediately after CPC treatment resulted in only a very small, insignificant reduction in the residual CPC value in the skins (< 25 ppm). Basically, rinsing the skins with water only reduced CPC absorption into the skin by 5%. Again, it is important to remember that the water rinsing conducted in this study was for 10 minutes – much longer than could be accomplished during commercial poultry processing. Thus, it

appears that a post-CPC rinse would not result in a significant reduction in the amount of residual CPC in the treated skin.

The addition of PG to the 0.3% CPC treatment significantly reduced the residual CPC that was recovered from the treated broiler skins. The mean residual CPC in the skin samples which had been exposed to 0.3% CPC with no PG for 10 minutes was 749.7 ppm (ug/g). The mean residual CPC in the skin samples which had been treated with 0.3% CPC with 0.5% PG for 10 minutes was 260.1 ppm. Thus, the addition of 0.5% PG to the 0.3% CPC solution reduced absorption of CPC into the broiler skin samples by 65%.

Trial 2.

Objective. The objective of this experiment was to determine the effects of cooking on residual CPC in broiler skin. Although there is published data (Breen *et al.*, 1995) that indicates that heating CPC to 400 F (for 30 minutes) forms no mutagenic compounds, there was no data available regarding the effects of heating CPC on residual CPC content of animal tissues.

Procedures. Each individual chicken skin sample (n=10) was placed into a 50 mL conical tube containing 40 mL 0.3% CPC plus 0.5% PG. Skin samples were allowed to remain in constant contact with the aqueous solutions for 10 minutes during which time the samples were gently shaken. Skin samples were then removed from the tubes but allowed to drip into the tubes for 1 minute. Skin samples were touched against the side

of their respective tubes in an effort to remove any excess liquid. The ten skin samples were divided into two groups of five skins each. Regardless of group, each skin sample was placed individually into a foil sample boat. Five of the boats containing skins were placed on a "cookie sheet" and were held a room temperature while the other five foil boats containing skins were placed on a cookie sheet and were cooked in a conventional home oven at 300 F for 45 minutes. The cooked samples were allowed to cool to room temperature (approximately 30 minutes). Each skin sample (plus any liquid in the foil boats) was submerged into 30 g of EtOH in 50 mL conical tubes. The tubes were then placed on a shaker table for 30 minutes during the extraction process. Samples were then centrifuged for 10 minutes @ 3 K RPM. The samples were then analyzed for residual CPC using the HPLC method previously cited.

Results. The average CPC content of the raw CPC-treated skin samples was 526.7 ppm. The average CPC content of the cooked CPC-treated skin samples was 362.6 ppm. There was no difference in the standard deviation between the two groups of skins. Thus, the cooking procedure reduced the residual CPC in the skins by 31.2%. This is the only information available regarding the effects of residual CPC in any type of food product.

Trial 3.

Objective. The objective of Trial 3 was to confirm the results noted in Trial 1 and to determine why PG is capable of significantly reducing absorption of CPC by chicken skin.

Procedures. Each individual chicken skin sample (n=5) was placed into a 50 mL conical tube containing 40 mL 0.3% CPC or 40 mL 0.3% CPC plus 0.5% PG. Skin samples were allowed to remain in constant contact with the aqueous solutions for 10 minutes during which time the samples were gently shaken. After the 10-minute exposure period, the skin samples were removed from the solutions and allowed to drain for 1 minute. Skin samples were allowed to touch the sides of their respective conical tubes in an effort to remove excess liquid. In this trial, skin samples were also weighed at this point in time to determine any change in skin weight resulting from exposure of the skins to the liquid CPC solutions. Half of the skin samples were then individually transferred to clean 50 mL conical tubes containing 40 mL of water. These samples were rinsed in the water with gentle shaking for 10 minutes in an effort to determine if rinsing the skins post-CPC treatment would significantly reduce absorption of CPC into the skin.

All skin samples, regardless of treatment, were transferred to clean conical tubes each containing 30 grams 95% ethanol (EtOH). CPC was extracted for 30 minutes on a shaker table. Tubes were centrifuged for 10 minutes @ 3 K RPM. CPC residual analysis was performed by HPLC for both the water rinses and the EtOH extracts as previously cited.

Results. Regardless of whether PG was included in the 0.3% CPC treatment, water rinsing of the skins resulted in only a 1 to 4% reduction in residual CPC in the treated skins. Whereas, the addition of PG to the 0.3% CPC treatment reduced the residual CPC in the skins by 38%. It should be noted that in Trial 1 the addition of 0.5% PG to the

0.3% CPC treatment resulted in a 65% reduction in CPC residual in the skins; however, in Trial 1 the standard deviation was two times as great as in Trial 3. Because skins were weighed in this trial before and immediately after exposure to the CPC solutions, we were able to calculate the precise amount of CPC to which each skin had been exposed. For those skins which were treated with 0.3% CPC with no PG, the HPLC value (ppm) of residual CPC and the calculated value based on moisture uptake during treatment were almost identical. However, when 0.5% PG was included in the 0.3% CPC formulation the weight of the skins post-treatment was not increased. In fact, the actual weight of each skin was reduced by 0.3 to 0.5 grams. Thus, even though some CPC was absorbed by these skin samples, the PG was minimizing moisture uptake thus resulting in significantly less absorption of CPC into the skin samples.

Conslusions. Results from these studies indicate that post-treatment water rinsing of broiler skin samples which have been treated with 0.3% CPC or 0.3% CPC plus 0.5% PG does not significantly reduce residual CPC in the skins. In fact, water rinsing only reduces the residual CPC by less than 5%. Cooking broiler skin samples which have been exposed to 0.3% CPC plus 0.5% PG results in approximately a 30% reduction in residual CPC in the skin. Addition of 0.5% PG to 0.3% CPC solutions results in a significant reduction in the residual CPC in treated broiler skin. This reduction is in the range of 38 to 65% and is no doubt dependent upon a variety of factors which may include proximate composition of the skin sample, surface area to solution volume ratio, temperature of product and treatment solution, time of exposure, etc.

Figures 1, 2, and 3

Figure 1. Residual CPC Recovered from Poultry Skin by Ethanol Extraction (Experiment 1)

Figure 2. Residual CPC Recovered from Cooked Poultry Skin by Ethanol Extraction (Experiment 2)

Figure 3. Residual CPC Recovered from Poultry Skin by Ethanol Extraction (Experiment 3)

Figure 1. Residual CPC recovered from poultry skin by ethanol extraction (Experiment 1).

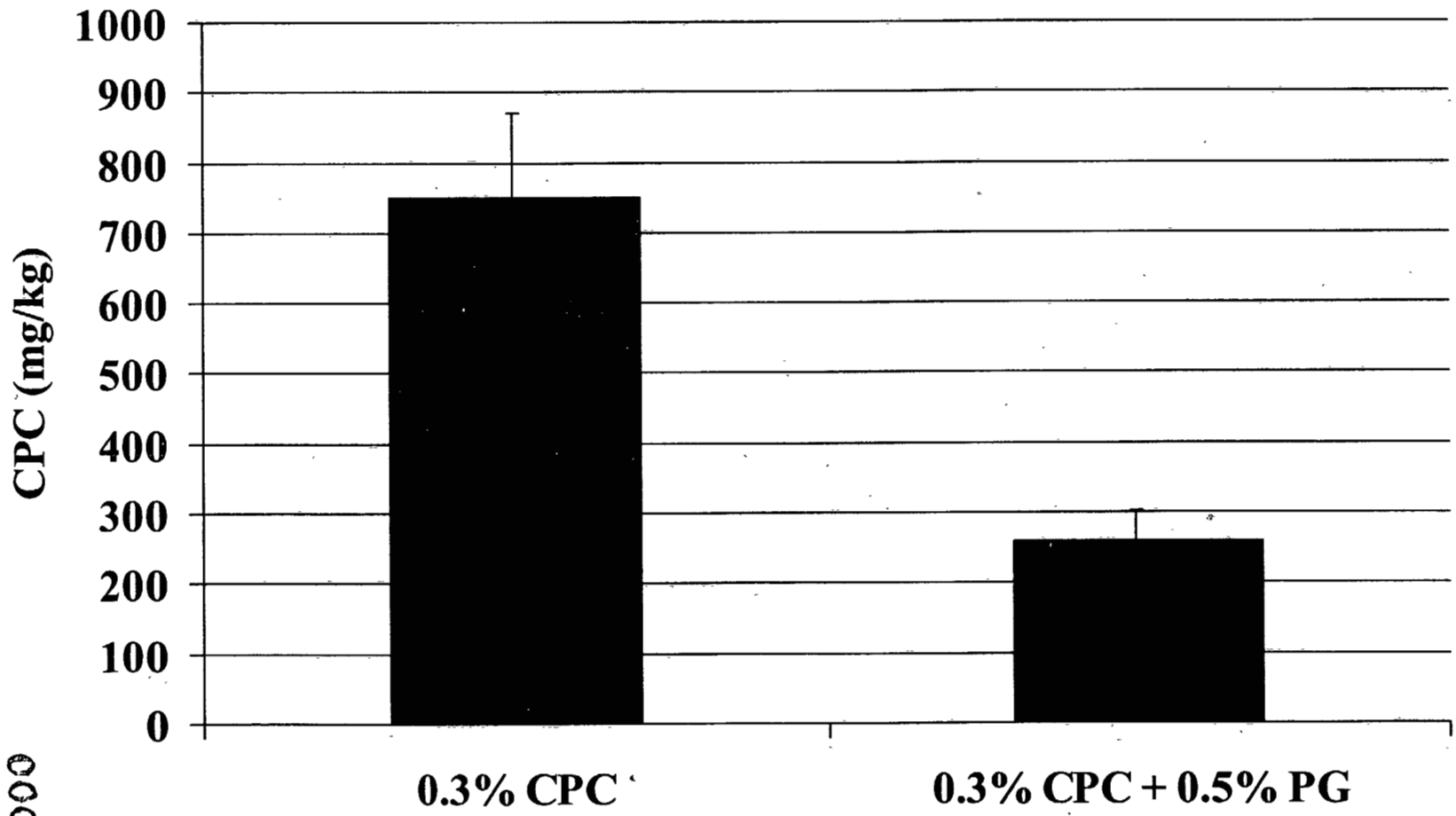


Figure 2. Residual CPC recovered from cooked poultry skin by ethanol extraction (Experiment 2).

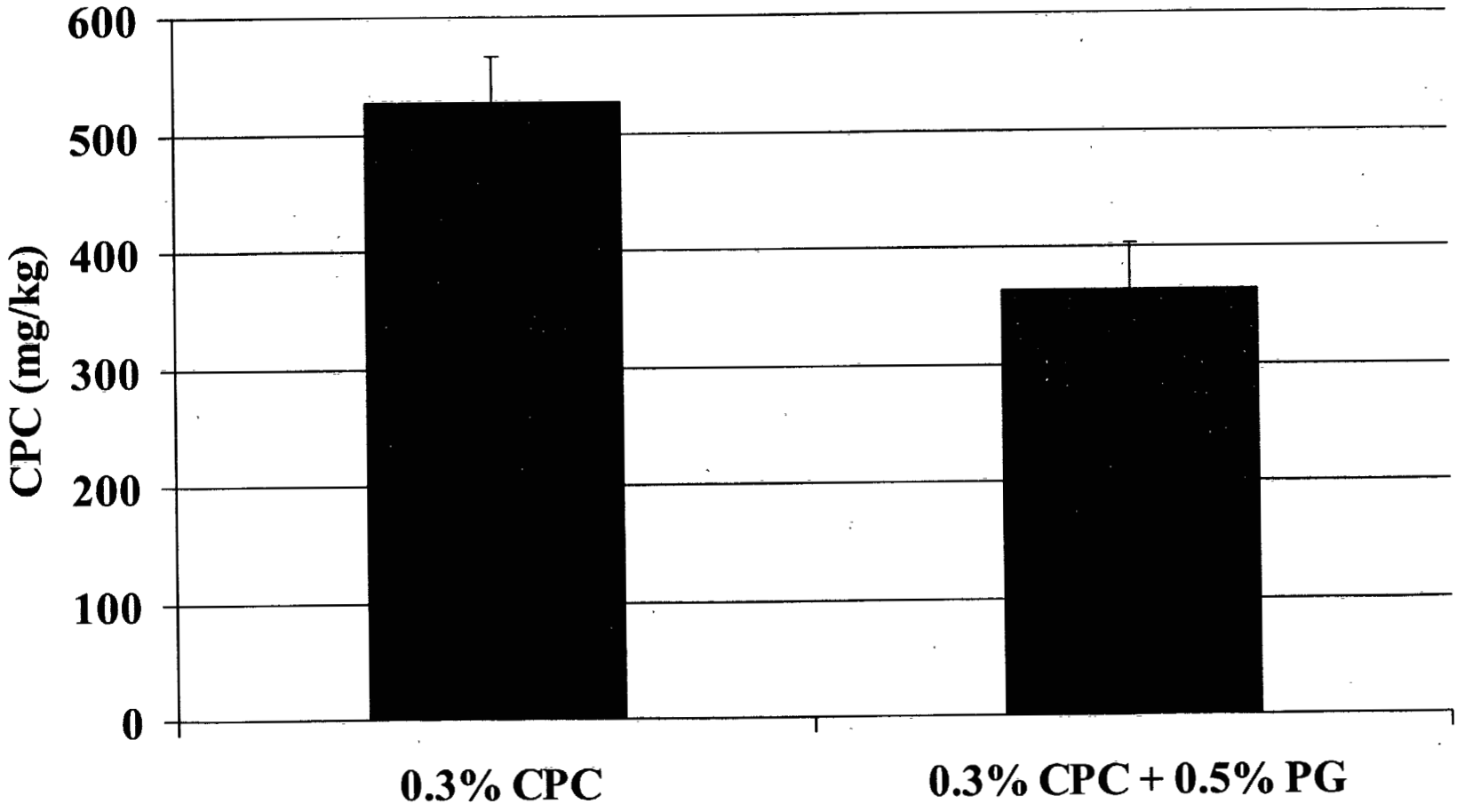
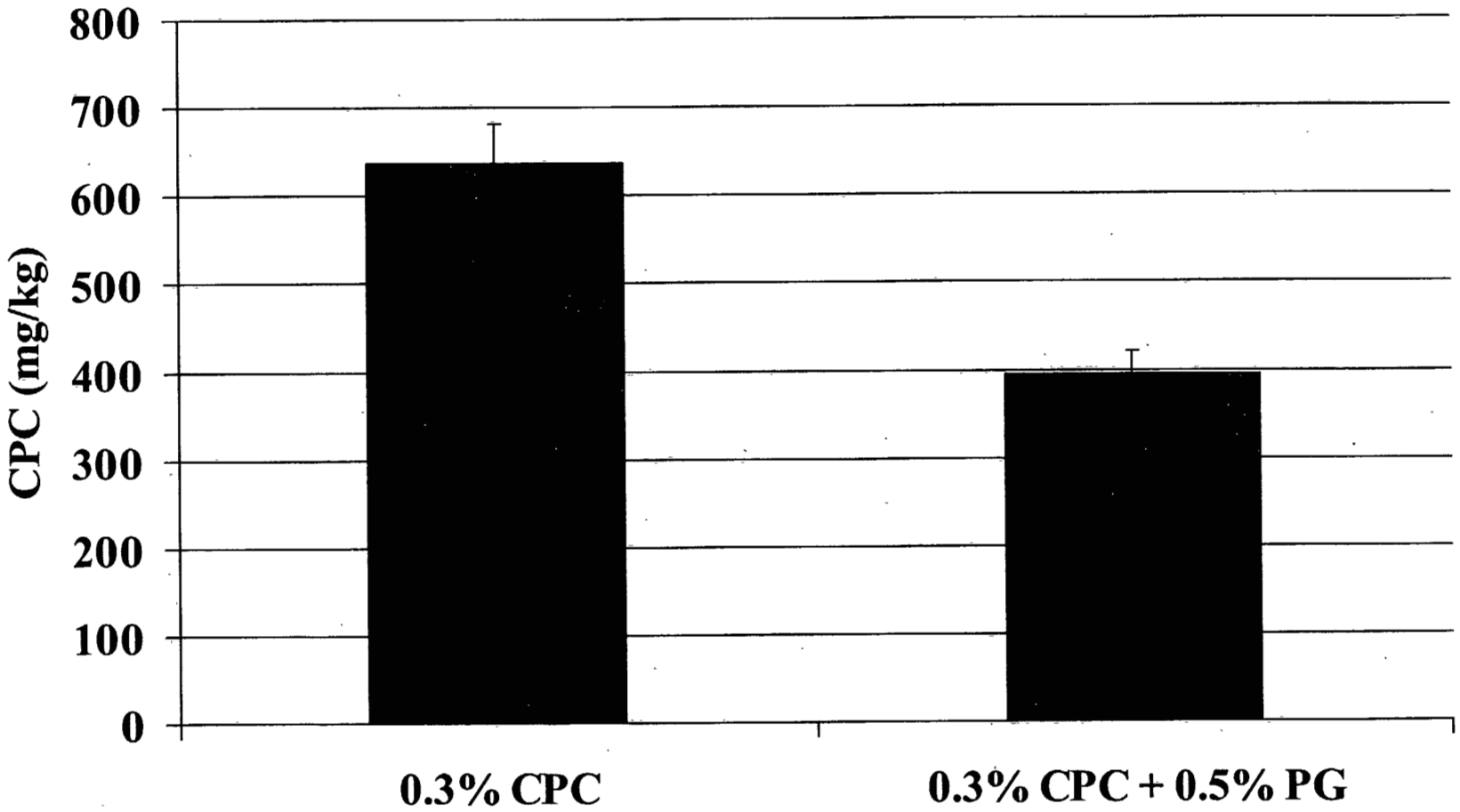


Figure 3. Residual CPC recovered from poultry skin by ethanol extraction (Experiment 3).



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000056 - 000057	Lewis, Richard J.	Cetylpyridinium Chloriide Monohydrate	1996	Saxs Dangerous Properties of Industrial Materials	Ninth Edition

NA- Not applicable