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Appendix 15

Environmental Assessment

Appendix 15

ENVIRONMENTAL ASSESSMENT

1.0	**Date**	July 31, 2006
2.0	**Name of Petitioner**	Safe Foods Corporation
3.0	**Address**	Mr. Curtis W. Coleman, President/CEO Safe Foods Corporation 4801 North Shore Drive North Little Rock, Arkansas 72118 Telephone: (501) 758-8500 Email: cwcoleman@safefoods.net
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4.0 Description of the Proposed Action

4.1 Requested Approval

The action requested in this Petition is the amendment of an existing food additive regulation, 21 C.F.R. § 173.375, to permit the use of cetylpyridinium chloride, generally abbreviated herein as CPC, as a food processing aid on raw poultry under modified treatment conditions.

CPC, in the form of an aqueous solution that also contains propylene glycol (marketed by the Petitioner under the trade name "Cecure®"), is currently permitted under Section 173.375 for use as a fine mist spray applied to the surface of raw poultry carcasses prior to immersion chilling. The maximum amount of CPC applied in that application is not to exceed 0.3 grams per pound of carcass.

This petition proposes to amend Section 173.375 to permit the use of CPC to treat raw poultry carcasses under alternative conditions. Under these conditions, CPC will be applied to raw poultry carcasses either prior to or after chilling. The concentration of CPC in the solution applied to the carcasses shall not exceed 1.0 percent by weight. Instead of a fine mist spray, the CPC will be applied by directing a flow of aqueous CPC/propylene glycol solution so as to deluge, or drench, the carcass. When treatment with CPC is not followed by immersion in a chiller, it will be followed by a potable water rinse.

The current clearance for CPC under Section 173.375 was promulgated in response to a Food Additive Petition (FAP No. 2A4736) filed by Safe Foods Corporation. The relevant information provided in the Environmental Assessment for that petition is incorporated herein by reference.

4.2 Need for Action

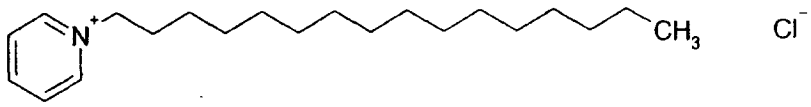
Treatment with Cecure® as described here will provide a means for poultry plants to meet more stringent food safety performance standards, and will allow poultry processors to provide consumers with raw poultry products that are significantly safer. The use of Cecure® will reduce the following microorganisms: *Salmonella, Campylobacter, E. coli* (including *O157:H7*), and other coliforms.

5.0 Identification of Chemical Substance that Is the Subject of the Proposed Action

The additive that is the subject of this Petition is Cetylpyridinium Chloride ("CPC"). Other chemical and common names for CPC include the following: 1-Hexadecyl pyridinium chloride, Ceepryn chloride, Cepacol chloride, Cetamium; Dobendan, Pristacin, and Pyrisept. The Chemical Abstract Service (CAS) Registry Number is 123-03-5.

5.1 Structure and Physical Properties

5.1.1 The structural formula for CPC is depicted below:



5.1.2 Physical Properties

The molecular formula of CPC is $C_{21}H_{38}NCl$; the molecular weight is 340. CPC is typically present in water in the monohydrate form. The monohydrate has the molecular formula $C_{21}H_{38}NClAH_2O$ and has a formula weight of 358. The calculated elemental content is C: 70.45%, H: 11.26%; Cl: 9.90%; O: 4.47%; and N: 3.91%.

CPC may be characterized in terms of the following physical properties:

Appearance/Physical form: white powder (monohydrate)

Melting Point: 77 - 83°C

pH: (1% aq soln): 6.0 - 7.0

Solubility: freely soluble in water, alcohol and chloroform but insoluble in ether.

6.0 Introduction of Substance into the Environment

6.1 Production Releases

All of the information available to Petitioner indicates that the production of the substance of interest will involve no extraordinary circumstances that will result in a significant environmental impact as a result of its manufacture. Thus, this assessment focuses on potential environmental issues relative to the use and disposal after use of CPC and Cecure®.

6.2 Use Releases

Cecure® application: The subject food processing aid (Cecure®) will be used at poultry processing plants located throughout the country in the processing of whole poultry carcasses. The point at which the treatment solution is applied may be either (a) after the live bird slaughter and evisceration and just prior to chilling of the carcasses, usually by immersion in a chill tank, or (b) just after chilling, immediately following re-hanging. The Cecure® treatment solution will be applied in a commercial, stainless steel application cabinet at commercial line speed conditions. The cabinet will be equipped with a manifold containing open ports that will apply the liquid treatment solution over the entire surface of the carcass (including the inside of the body cavity). The flow of Cecure® treatment solution through the ports (rated in gallons per minute) is controlled by regulating the hertz cycles of the booster pump. .

Section 173.375, paragraph (c), states that the additive shall be used in systems that collect and recycle solution that is not carried out of the system with the treated poultry carcasses. No change to this requirement is proposed here; thus, when CPC is applied as described herein, it will be in systems that collect and recycle the spent Cecure® solution. The use of recycle and capture technology almost entirely eliminates the introduction of CPC to the environment. A description of the Cecure® Recycle System was previously provided as Appendix XIV to FAP 2A4736, and is incorporated herein by reference.

In understanding the environmental fate of CPC and Cecure®, it is important to understand the flow of the product and waste streams in a poultry processing plant. The major steps involved in whole poultry carcass processing are described in the Environmental

Assessment for FAP 2A4736. For the Agency's ready reference, this background information is reproduced below.

> **Live Bird Slaughter:** In the U.S., virtually all poultry processing is done automatically with the aid of processing plant personnel. From the point where the live broiler enters the plant, to the point where the product leaves the plant in ready-to-eat form, many pieces of automated equipment and several gallons of water per broiler carcass are utilized. The steps involved in transforming the live broiler into a grocery-store product ready to be purchased by the consumer are addressed in the following narrative.
>
> After the live birds arrive at the processing plant, they are automatically unloaded from the catching crates onto an automated conveyor belt. The live birds move slowly on this belt as workers catch and hang them by the feet on two-point overhead stainless steel shackles. They move, still alive, upside down, to a cabinet where they receive a mild jolt of electricity. This is known as the "stunning" process. At this point in the process, the birds are moving on the line at a speed of approximately 70 birds/minute. Stunning is accomplished by wetting the bird's body, with feathers still intact, and allowing the head (primarily the comb) to come into contact with a saline solution through which an electrical current is surging. This jolt of electricity is not severe enough to permanently damage or kill the bird, but is done only to immobilize the bird and allow the body of the bird to become relaxed enough to allow for automated killing.
>
> With the birds still hanging upside down, and now with outstretched neck from the stunning operation, the bird is killed by an automated circular blade that severs the jugular vein. The bird dies within a 2-minute bleed time due to severe blood loss. After bleeding, the bird is totally submerged in a large tank of circulating hot water (136° to 140° F) for about 2 minutes to loosen the feathers. This process is called "scalding." The feathers and skin of the bird come out of the scalding process totally drenched with water. This added water aids in the picking process that is accomplished just moments after the birds exit the scalder bath. USDA requires one quart of fresh water to be added for each bird that enters the scald tank; thus, there is a continuous overflow of water from the scald tank.
>
> The picking process is accomplished automatically by a series of machines that literally "grab" the feathers off the bird using a specialized type of rubber "picking fingers." At this point, the bird has been bled and picked and is referred to as a "New York Dressed" bird. The birds are then automatically dropped off the conveyor system by cutting the feet off. As the feet are severed, the birds drop to a conveyor belt below.
>
> This conveyor belt moves the New York Dressed birds into a separate part of the plant known as the evisceration room. The feathers and blood are removed from the slaughter area using the overflow water from the scalder plus some additional fresh water. The feathers and blood are kept as two separate products and are not

typically mixed together. The blood leaves the plant in tanker trucks for rendering into blood meal. The feathers are screened to remove some of the water and leave the plant by tanker truck for rendering into feather meal. Feather meal is typically used in cattle diets and for dry pet feeds. Blood meal is typically utilized as a plant fertilizer.

Bird Evisceration: In the evisceration room, the birds are quickly re-hung upside down by the legs on the stainless steel shackles of a separate overhead conveyor system. At this point in the process, only the blood, feet and feathers have been removed. In the evisceration room the birds typically move on the line at a speed of 70 to 91 birds/minute. Some new evisceration equipment allows for even greater line speeds, in the order of 105 to 140 birds/minute.

The first process that usually occurs is removal of the preen gland. This is a small appendage on the base of the tail where an oily substance is generated that the bird uses to "preen" itself allowing some waterproofing of the feathers. The preen gland is considered to be inedible and so it is removed. The next process that typically occurs is head removal. This is accomplished by "catching" the head in a v-type bar apparatus that captures the head of the bird as the remaining part of the bird continues to be pulled down the line by the automated shackles. Thus, the head is literally pulled away from the body of the bird. Both the preen gland and the head fall into a trough that is positioned directly under the shackle line to catch this waste material. This trough is known as the "offal trough" or "offal line."

The next process to occur is dislocation and removal of the neck. An automated machine is used that applies force to the neck to disjoin it from the back of the bird. In most plants the necks also fall into the offal trough under the shackle line because there is a very limited market for poultry necks. The bird is now ready for removal of all the internal organs.

The first machine the bird encounters, still moving upside down overhead on the shackle line, is the "opening cut" machine. This machine simply cuts around the vent or anus of the bird and suctions out about the last two inches of any possible remaining fecal material. A chlorinated, water spray is utilized on this machine to keep any possible fecal material from contaminating the outside skin of the bird.

The next machine is called the "draw" machine and it simply uses a scoop-like device to pull the internal organs out of the body cavity. This machine also uses a chlorinated, water spray to keep any gut material from coming into contact with the outside surface of the bird. This machine does not totally remove the guts or "viscera" from the carcass, but gently drapes the "viscera package" onto the back of the bird where it can be viewed by the USDA inspection personnel for possible disease problems. After the USDA has viewed the entire bird, including the viscera package, the viscera are then removed from the carcass and fall into the same offal trough which previously received the preen gland, head, and neck.

In some plants the gizzard, heart, and liver are harvested from the birds for human consumption. However, the majority of processors now just let those products become part of the inedible material leaving the plant because they receive more money for those products in the animal feeds business than in the consumer market.

After the viscera are dropped into the trough or "offal line", the lungs are suctioned out of the body cavity and also enter the offal line. This fully eviscerated, or gutted, carcass hanging on the shackle line by the legs, is commonly referred to as the WOG (whole carcass without giblets).

Inside/Outside Washing: After USDA inspection and viscera removal, it is necessary to thoroughly wash the inside and outside of the carcass. While the carcasses are still moving on an overhead conveyor system, they pass through at least one, but more likely three or four, "inside/outside bird washers." These stainless steel cabinets are simple automated washing stations for the carcasses. Several gallons of water are used to clean each individual carcass – inside and out. All of the water used in these wash cabinets is directed to the offal line. Thus, the spent wash water, as well as the water that is continually used to rinse off the evisceration machinery, water from hand and knife washing stations, and fresh water as needed, is utilized to move the inedible material through the offal troughs.

Immersion Chilling: As indicated above, after the Cecure® treatment, the carcasses move via the overhead line to the chilling phase of the process. They are dropped automatically from the shackle line into a huge tank of water called the prechiller. This tank of water is typically held at 55°F and the carcasses remain in the prechiller for about 15 minutes. During this time, the carcasses absorb 4 to 5% added moisture.

The water in the prechiller is violently aerated to aid in water movement for increased chilling potential and water absorption. This aeration process, combined with the large amount of fat that is present in the prechilling water, forms a flocculent material that floats on the top of the chill water. This material, typically called "chiller skimmings," is continuously removed from the prechiller water and is diverted to the offal trough.

From the prechiller tank, the carcasses move automatically into the chiller tank. This tank is larger, colder, usually 32° to 34°F, and the carcasses stay in this tank for about 45 minutes. The carcasses pick-up an additional 3 to 4% moisture in the chiller. USDA allows poultry carcasses to gain a total of 8% added moisture. Again, constant aeration of the water, combined with the fat that is present in the chiller water, forms a large amount of chiller skimmings. As is the case in the prechiller, this material is diverted to the offal trough.

After chilling, the carcasses are rehung on another shackle line for transport to other areas of the plant. They may move to a whole carcass packaging station, may go to a separate part of the plant for cut-up or deboning, or may be shipped to a different plant for further processing and cooking.

Application of Cecure®: The current clearance for CPC permits its use on pre-chill poultry carcasses. The application process was described in the Environmental Assessment for FAP 2A4736. Because the modified use of CPC described in this petition allows for use of the product on either pre-chill or post-chill poultry carcasses, the information relevant to the use of Cecure® on pre-chill carcasses is repeated here and revised as appropriate to reflect the modified application conditions for the product. This is followed by a discussion of the use of Cecure® on post-chill carcasses.

Pre-chill use: For pre-chill application, the Cecure® application cabinet is positioned just after the last inside-outside bird washer. The birds pass through the cabinet at normal line speed for application of the Cecure® solution.

Testing described in FAP 2A4736 demonstrated that only a small amount of the CPC sprayed in the Cecure® spray cabinet ends up on the carcass. The majority of the product drains out of the cabinet to a recycle tank, from which it is returned to the spray cabinet for re-use (see Appendix XIV of FAP 2A4736). Material balance calculations set forth in that petition demonstrated that approximately 99.9% of the CPC would be captured and recycled back to the system.

The modified use of Cecure® described here also will result in the vast majority of the CPC being captured and recycled using the Cecure® recycle system. Moreover, as with the spray application system described in FAP 2A4736, the Cecure® drench-type treatment system includes the use of a "drip tray" to capture a portion of the CPC that does not drip off the carcasses during passage through the treatment cabinet. As the pre-chill birds exit the cabinet en route to the chiller tank, they pass over this drip tray, which collects any CPC-

containing fluid that drips from the wet carcasses. This tray extends for the distance covered by the carcasses in the first minute after they exit the cabinet, or typically about one-half the distance to the chiller.[1] The liquid that drips into this tray is combined with the fluid that drains from the Cecure® treatment cabinet and is recycled back to the treatment system. For the remainder of the distance to the chiller (i.e., the second minute of travel time from the treatment cabinet), any liquid that drips from the carcasses goes into the plant's existing floor offal collection system and ultimately will be collected as part of the offal.

Post-chill use: For post-chill application of Cecure®, as noted above, the carcasses will be treated after removal from the chiller tank and re-hanging on the process line. The carcasses will then pass through the Cecure® treatment cabinet in the same manner used to treat pre-chill carcasses. When the carcasses exit the treatment cabinet, there again will be a drip tray extending for the distance traveled by the carcasses in approximately one minute. The carcasses will then pass through a second cabinet where they will receive a potable water rinse, again traveling at the process line speed in place at the plant. The carcasses will then exit the rinse cabinet and continue along the line for further processing and/or packaging. It is estimated that the carcasses will drip for approximately one additional minute during this post-rinse phase.

Waste Streams for CPC: The waste streams for CPC in this poultry processing environment are explained in detail in the paragraphs that follow. As indicated previously,

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[1] As indicated in FAP 2A4736, the average line speed for the birds in a poultry processing plant is estimated to be 70 birds/minute. The shackles are 6 inches apart. Thus, the birds move down the line at 35 feet/minute (i.e., 70 birds/minute x 0.5 foot/bird). The average distance between where the Cecure® spray cabinet would be placed and the location of the chiller bath is 80 feet. Therefore, the birds drip for approximately 2 minutes (i.e., 80 feet ÷ 35 feet/minute) between the spray cabinet and the chiller bath.

the great majority of the CPC present in the treatment solution goes down the drain of the treatment cabinet, where it is sent to the recycle tank. Additional CPC concentrate is added as necessary to achieve the desired treatment concentration, and the solution is then pumped to the treatment cabinet for re-use. None of the CPC captured in this way is released to the environment. Moreover, any CPC dripping off either pre-chill or post-chill carcasses for the first minute after leaving the application cabinet also will be directed to the recycle tank.

At the end of a treatment cycle, the entire Cecure® system will be shut down and any solution remaining in the recycle tank will be sent to a purge tank. This fluid will be filtered to remove the CPC. The spent filters will be collected and disposed of by means of incineration or approved landfill. The filtered, CPC-free liquid will be combined with the plant wastewater. The frequency of this purging may vary from one plant to another, although a daily purging is believed to be likely.

Based on the foregoing considerations, the amount of CPC that may enter the environment as a result of its intended use will be limited to the following: (a) for pre-chill application, the amount of CPC that drips from the carcasses during the second half of conveyance to the chiller bath, plus the minor amount of CPC that may be lost upon immersion of the carcasses in the chiller bath[2]; or (b) for post-chill application, the amount of CPC that is washed off the treated carcasses during the potable water rinse and the amount of CPC that drips off the carcasses after rinsing.

The waste streams relevant to poultry processing, and the amount of CPC that may be present in each, are discussed below.

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[2] The latter amount was shown in FAP 2A4736 to represent approximately 5% of the CPC residue on the carcass.

Inedible Offal Materials: The inedible materials that are removed from the bird, *i.e.*, the head, preen gland, and inedible viscera are combined with other non-marketable products such as the neck and giblets (heart, gizzard, liver) and are transported via water to the materials separation facility at the processing plant. Large screen screw conveyors are used to remove as much water as possible from this material. This product is then transported via tanker truck to a poultry byproducts rendering facility.

The Petitioner has conducted testing to determine the amount of CPC that may drip or be rinsed from treated carcasses without being captured for recycling. As indicated above, this consists of the amount of CPC that drips in the second minute after exiting the treatment cabinet plus the amount that may be washed off in the chiller for pre-chill treated carcasses, or the amount that is washed off in the potable water rinse plus the amount that drips off following rinsing for post-chill treated carcasses. A report of this testing appears as Attachment A to this Environmental Assessment. As shown there, pre- and post-chill carcasses were treated with either 0.6% or 1.0% CPC; an exaggerative treatment volume of 2 gallons per carcass was investigated in all cases. (A treatment volume of 1 gallon/carcass was also investigated for pre-chill application of Cecure®.) It is expected that a volume of 2 gallons per carcass will be approximately 4 to 8 times the volume that will be applied in commercial use.

As shown in Attachment A, the maximum amount of CPC lost from post-chill treated carcasses was found with the 0.6% CPC treated samples, and consisted of **0.7 mg** lost during the potable water rinse and **5.3 mg** in the post-rinse drip interval. Pre-chill application of Cecure® resulted in a maximum CPC loss of **4.4 mg** in the second minute drip post-treatment. These residues all will be combined with the offal stream. (The CPC lost during immersion

chilling of pre-chill treated carcasses, **0.7 mg** as measured in Attachment A, will initially be present in chiller skimmings, but ultimately will be combined with the offal stream, as discussed below.)

By comparison, data set forth in FAP 2A4736 indicated that no more than 1.01 mg per carcass would be lost in the second minute of drip time. That amount was shown to represent 0.10% of the total amount of CPC applied. For the application of Cecure® described here, the maximum loss of CPC, **6.0 mg** (the sum of losses to rinsing and dripping), may be approximated as a fraction of the total quantity of CPC applied. The maximum CPC concentration in the treatment solution is 1.0%. While no maximum volume of treatment solution is proposed, in commercial practice, it is likely that the treatment volume will not exceed 2 gallons per carcass; this is also the volume that was used in the testing described in Attachment A. Application of 2 gallons of 1.0% CPC per carcass corresponds to 76 grams of CPC per carcass.[3] A loss of 6.0 mg represents 0.008% of the total amount of CPC applied. This is an order of magnitude lower than the loss of CPC determined in the previous petition, when expressed as a fraction of the total mass of CPC applied. Again, the CPC lost to non-captured drip and the potable water rinse will be directly combined with the plant's offal stream from the evisceration line.

Testing conducted by the Petitioner (see EA for FAP 2A4736) indicated that at least 99% of the CPC from a 125 ppm solution would be adsorbed by the offal within a 2-minute contact period. The offal material will ultimately become part of poultry meat and bone meal (P-MBM). P-MBM is composed of processing plant offal, processing plant condemned

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[3] 2 gal/carcass x 3.785 L/gal x 1 kg/L (density of water) x 1.0% CPC = 0.076 kg, or 76 grams, of CPC per carcass.

broilers, yellow-fat rendered from a high lipid content material known as "DAF" (dissolved air flotation material, described below), outdated grocery store products, and fryer particulate material such as excess breading (Hollingsworth, 2002). The P-MBM is routinely blended with meat and bone meal (MBM) from other animal sources and fed back to growing chickens as a source of dietary protein.

Chiller Skimmings: During immersion chilling, the carcasses are tumbled first through a pre-chiller tank (8,000 gallons, 50° to 55°F, 15 minutes), followed by a chill-water tank (25,000 gallons, 32° to 34°F, 45 minutes) for the purpose of rapidly decreasing internal body temperature of the carcasses. The chill water is continuously aerated in a manner similar to that performed in the DAF generator (for similar purposes); this process produces a surface flocculent material commonly referred to as "chiller skimmings." Chiller skimmings are of similar nature as DAF (high lipid content), but unlike DAF, chiller skimmings are of low protein content. Chiller skimmings are continuously removed (skimmed) from the chill water surface and placed into the offal trough. Since chiller skimmings are of high lipid content, it is expected that CPC rinsed from the carcasses during the chilling process will interact with this material and be removed (all but a negligible amount) from the chill water prior to entering the DAF generator. Removed chiller skimmings are combined with the offal to reduce the amount of DAF that is generated. Removal of chiller skimmings, and thus CPC, from the chill water will reduce the overall amount of CPC in the wastewater prior to delivery to the DAF generator (see below).

The testing described in Attachment A on pre-chill treated carcasses included a water rinsing step following the two-minute drip interval as a rough approximation of the effect of

immersion chilling on CPC residues. This testing resulted in **0.7 mg** of CPC lost from pre-chill treated carcasses during this rinsing.[4]

DAF generation: CPC not bound to the offal will pass with the wastewater to the DAF generator. DAF is a high-lipid content material that is produced by vigorous air turbulence of the screened processing plant wastewater (Kiepper, 2001). A typical processing plant (processing 200,000 chickens per day) will generate approximately 16,000 kg of DAF per day (Horne, 2002). Available information confirms that DAF generation is a standard practice in U.S. poultry processing plants as a method to greatly reduce the amount of particulate and lipid material that is released to domestic wastewater publicly owned treatment works ("POTWs"). Information obtained from a regional rendering operation (Smith, 2002) indicates that on a nationwide basis, approximately 50% of DAF generated at processing plants is rendered into "yellow fat" and added to P-MBM; the remaining 50% is normally soil amended.

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[4] Data set forth in FAP 2A4736 demonstrated that as much as 5% of CPC residue on pre-chill carcasses might be washed off during immersion chilling. This may be compared to the amount of CPC lost in the rinse testing by calculating the residue in the rinse water as a fraction of the CPC residue on the carcass as follows. In Section D of this FAP, the CPC residue on carcasses treated with 1.0% CPC solution (the maximum proposed CPC concentration) is calculated as 1.2 ppm, or 1.2 µg/g. Section D also notes that the current average chicken live weight, according to USDA statistics, is 5.38 pounds, which will yield an average carcass weight of 3.8 pounds. (Information from poultry processors presented in Section D indicates that the typical carcass weight is approximately 70% of the live bird weight. Thus, 5.38 lb x 70% = 3.8 lb.) A CPC residue of 1.2 µg/g on a typical chicken carcass will correspond to a total residue of 2.1 mg CPC per carcass. (3.8 lb x 453.6 g/lb x 1.2 µg/g = 2068 µg, or 2.1 mg, CPC per carcass.) If 5% of this residue were to be washed off during immersion chilling, this would amount to 0.105 mg CPC per carcass. This is lower

(footnote continued)

Due to the presence of CPC's hydrophobic tail, it is expected to bind tightly to DAF as it is formed within the generator. Experimental data presented in Appendix 1, Table 3 of the EA for FAP 2A4736 demonstrates the high binding capacity of DAF for compounds with hydrophobic moieties, such as CPC. When 2 grams of DAF were mixed with 20 ml of water containing 2 mg CPC (final CPC concentration = 1000 ppm), only 1% of the added CPC remained in the water (99% bound to DAF) after a 15 minute reaction time. The levels of CPC used in this experiment were many times higher than levels anticipated in an actual processing plant DAF generator. Therefore, at least 99% of the CPC entering the DAF generator will bind to the DAF and no more than 1% of CPC entering the DAF generator will be released with the waste water (refer to section 6.2.3 for calculated levels of CPC in DAF). Ultimately, all water associated with processing procedures must pass through the DAF generator prior to release (Horne, 2002). In actual practice, the DAF generator is positioned after the immersion chiller, thus allowing for all water used for processing prior to immersion chilling (feather removal, evisceration, carcass washing, etc.) to be in contact with the offal before screening (method of separating water and offal). The minute amount of CPC remaining in the wastewater after DAF generation will then be treated at the processing plant (if the processing plant has a pretreatment facility) or at the POTWs prior to release.

Degradates: Due to its structural nature, CPC is resistant to breakdown and subsequent generation of degradates as a result of operational steps performed routinely within poultry processing and rendering plants. Testing conducted at the University of Arkansas showed that subjecting a 0.1% (1000 ppm) solution of Cecure® to 100°C for up to

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than the 0.7 mg CPC loss measured in the rinse testing. For conservatism's sake, we will use the higher value here.

60 minutes did not alter the HPLC chromatogram (peak retention time, peak shape, or peak area) compared to an unheated control sample. In addition, no difference was observed between control and heat-treated samples when Cecure® (0.4%, 4000 ppm) was subjected to indirect steam (autoclave) as is the practice during rendering offal for preparation of P-MBM.

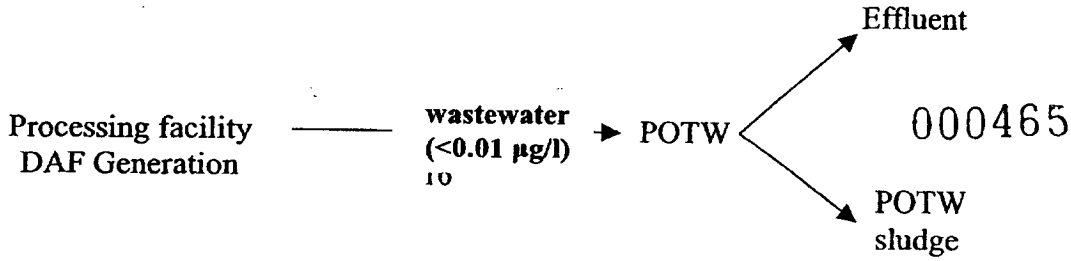
The carbon-nitrogen (C-N) bond attaching the aliphatic carbon tail to the pyridine ring is very strong (Lattin, 2002) and would require strong oxidants, not routinely used within poultry processing plants, to disrupt the bonds. In addition, the aliphatic carbon tail is fully saturated and thus contains a uniform electron distribution that would greatly hinder neucleophilic attack by chemicals typically present in poultry processing and rendering plants. Therefore, degradates of CPC, as a result of the intended use, will not present any environmental concern.

6.2.1 Air Releases

Based on its ionic nature, high molecular weight (340), and its resulting low vapor pressure, CPC is not expected to be released to the atmosphere.

6.2.2 Aquatic Environments

As noted above, approximately 99% of CPC coming into contact with poultry offal will bind to the offal and be removed from the processing plant wastewater. Of the remaining 1% of CPC, 99% will bind to the DAF during generation, further reducing the concentration of CPC in the waste water when released from the processing plant. Taken together, the



concentration of CPC in wastewater leaving the plant would be no more than 1% of 1% of the total CPC used per day that is not recycled, divided by the total amount of water (on a kilogram basis) utilized on a daily basis.

For the amount of CPC coming into contact with the poultry offal, we will use the total amount of CPC lost to drip and rinsing for either pre-chill or post-chill carcasses, based on the testing described in Attachment A. Summarizing these data again, for pre-chill carcasses, the maximum amount of CPC lost in the second-minute drip was 4.4 mg, and the amount lost in rinsing (simulating immersion chilling) was 0.7 mg; the total CPC lost was 5.1 mg. For post-chill carcasses, the maximum total CPC lost during post-treatment rinsing and dripping was 6.0 mg, consisting of 0.7 mg lost during rinsing followed by 5.3 mg lost during dripping. We will use this latter value, **6.0 mg**, in the following calculations.

The maximum concentration of CPC in the waste water therefore may be calculated as shown below. For this purpose, the Petitioner has obtained process information from a major poultry processor, Tyson Foods, Inc. The company has 60 poultry processing plants. A typical complex, or processing facility, processes 1.3 million broilers/week (200,000/day). Depending on the plant, from 5 to 11 gallons of total water may be used per bird. For calculation purposes, we will assume that 7.5 gallons of waste water are generated per bird. All of this water is discharged from the plant, after treatment or pre-treatment.

Based on the foregoing, the total amount of CPC employed per day that is not recycled to the treatment cabinet, and the resulting waste water concentration, are calculated as follows:

The total mass of CPC that is not recycled ($M_{CPC\text{-}NR}$) per day:

- Assuming a facility processing 200,000 chickens per day (whole chickens without giblets, or WOGs)
- Assuming a 1% CPC application rate
- Assuming that 6.0 mg of CPC is lost to drip and rinsing or immersion chilling
- The total mass of CPC not recycled ($M_{CPC\text{-}NR}$) would be:
  200,000 WOGs/day x 6.0 mg/WOG = **1,200,000 mg**

EC of CPC in processing plant wastewater

- Assuming 7.5 gallons of waste water generated per bird, or a total of 1,500,000 gallons of water per day (5,443,164 kg)
- Assuming $M_{CPC\text{-}NR}$ = 1,200,000 mg (calculated above)
- Assuming 1% of $M_{CPC\text{-}NR}$ passes to DAF generator (i.e., 99% binds to offal)
- Assuming 1% of CPC reaching DAF generator remains in wastewater (i.e., 99% binds to DAF)

EC of CPC in wastewater = (1,200,000 mg x 0.01 x 0.01) / 5,443,164 kg

EC of CPC in processing plant wastewater = 0.000022 mg/kg (ppm)

These calculations indicate that the maximum concentration of CPC in the waste water will be 0.022 part per billion (ppb). Furthermore, any CPC remaining in the water would then go to the POTWs, where the entire scenario would be repeated (CPC binding to sludge/DAF → large dilution of any CPC remaining in water → release of water and additional dilution).

Moreover, as pointed out by Boethling (1984; 1994), anionic surfactants (such as fabric softeners) have a neutralizing effect on quaternary ammonia compounds (QAC's). Since the concentration of anionic surfactants normally exceeds that of QAC's, especially

CPC, any potential worst-case scenario will most likely be neutralized by the greater level of anionic surfactants already present in the municipal wastewater (Boethling, 1984, 1994).

As further demonstration that CPC will be present in the waste-water only at minute concentrations, an experiment was described in FAP 2A4736 in which CPC was added to POTW sludge material containing typical microbial populations. In this testing, CPC from a 22.3 ppm solution was not detectable (minimal detectable level ~ 10 ppb) in the water in less than 1 minute reaction time (Appendix 1, Table 4, of EA for FAP 2A4736). Therefore, all available data indicates that CPC, if present at all in effluent from waste water treatment facilities, will be there in vanishingly low levels and will be of no environmental significance.

6.2.3 Terrestrial Environments

The Petitioner estimates that, five years after introduction, the use of Cecure® (CPC) for poultry processing will be in the approximate range of 35 thousand pounds per year. This figure assumes a market penetration upwards of 20% of processing facilities, and reflects the use of the recycle and capture technology. There are certain inherent uncertainties associated with this calculation, such as the quantity of CPC that would be used annually in poultry processing prior to the release of Cecure® into the marketplace. Additional areas of uncertainty relating to market volumes include the availability of other antimicrobial processing aids, their relative efficacy and ease of use, the degree to which they may already be in place in the facilities, the relative cost of Cecure®, and the cost of replacing competitive in-place installations with that of Cecure®.

The amount of CPC entering the potential terrestrial waste streams (poultry offal, DAF, and sludges from wastewater treatment) may be estimated based on the following:

(1) the projected use rate of CPC; (2) the fraction of this amount that is not captured and recycled; (3) the total mass of offal generated by the plant; 4) the percent of offal contributing to the total P-MBM, taking into account the percent at which P-MBM is blended with MBM from other animal sources; 5) the total amount of DAF generated on a daily basis; and 6) the total amount of sludge produced by the waste water processing plant on a daily basis.

Based on the above discussion, the expected concentration (EC) of CPC in poultry offal, DAF, and sludges from wastewater treatment, and subsequent incorporation of offal into P-MBM may be depicted as follows:

Estimated M_{CPC} binding to offal (CPC_{offal})

- Assuming $M_{CPC\text{-}NR}$ = 1,200,000 mg per day (calculated above)
- Assuming all but negligible amount of non-recycled CPC (carcass residue) is added to offal
- Assuming 99% of CPC in contact with offal will bind

CPC_{offal} = (1,200,000 mg x 0.99)

CPC_{offal} = 1,188,000 mg

Estimated M_{CPC} binding to DAF (CPC_{DAF})

- Assuming $M_{CPC\text{-}NR}$ = 1,200,000 mg per day
- Assuming 99% of $M_{CPC\text{-}NR}$ in contact with offal will bind to offal, so 1% goes to DAF
- Assuming 99% of CPC in contact with DAF will bind to DAF

CPC_{DAF} = (1,200,000 mg x 0.01 x 0.99)

CPC_{DAF} = 11,880 mg

Total mass of P-MBM ($M_{P\text{-}MBM}$) generated per day from processing 200,000 chickens:

- Assuming average live chicken weight is 5.38 pounds (see footnote 4 above)

- Assuming 30%, by weight, of 200,000 live chickens processed per-day ultimately becomes P-MBM (Brake *et al.*, 1993, Reonigk, 2002, Wall and Anthony, 1995)
- Assuming that P-MBM, prior to drying, is approximately 30% dry matter (DUPPS, 2002)
- Assuming that P-MBM is dried to 90% dry matter prior to usage (DUPPS, 2002)

$M_{P\text{-}MBM}$ = (200,000 chickens x 5.38 lbs x 0.30 x 0.3) / 0.90

$M_{P\text{-}MBM}$ = 107,600 lbs (48,807 kg)

EC of CPC in P-MBM

EC = CPC_{offal} / $M_{P\text{-}MBM}$ = (1,188,000 mg / 48,807 kg)

EC = 24.3 mg/kg (concentration of CPC in offal before addition of 10% non-plant material such as grocery store out-of-date items and strained fryer material)

EC in P-MBM= (24.3 x 0.9) = 21.9 mg/kg

Based on the foregoing calculations, the amount of CPC present in the P-MBM will be just 21.9 mg/kg, or 21.9 ppm. As discussed previously, the P-MBM will be used as a component of poultry feed. The level of CPC in the feed as a result of this use will be substantially less than 21.9 ppm, as shown by calculations set forth in Section 7.2 below, due to dilution with other feed components.

EC of CPC in DAF

- Assuming 16,000 kg of DAF are generated per day from 200,000 processed chickens (Horne, 2002)
- Assuming CPC_{DAF} = 11,880 mg (calculated above)

EC in DAF = (11,880 mg / 16,000 kg)

EC in DAF = 0.74 mg/kg

As previously stated, approximately 50% of DAF will be rendered into "yellow fat" and added to P-MBM. For the purpose of EA calculation, the concentration of CPC in DAF will not change with removal of 50% of the DAF. However, it should be noted that the overall mass of CPC being soil amended by this waste stream will be reduced by one-half.

The DAF calculations, as discussed above, assume that 99% of the non-recycled CPC that does not bind to offal will become bound to the DAF. This leaves 1% of 1% of the total non-recycled CPC that may be present in the wastewater. The resulting concentration in the wastewater is calculated in Section 6.2.2 above.

The maximum concentration at which CPC may be present in sludges from wastewater treatment may be calculated assuming all of the CPC in the wastewater becomes bound to the sludge. For this purpose, FDA has estimated that a poultry facility processing 200,000 chickens per day produces 1736 kg of sludge per day. (See FDA's January, 2002 guidance document for the FAP 2A4736 environmental assessment.) Thus, the maximum concentration of CPC in the sludge is as follows:

EC of CPC in Sludge

- Assuming 1736 kg of sludge are generated per day from 200,000 processed chickens
- Assuming CPC_{Sludge} = 1% of 1% of total $M_{CPC\text{-}NR}$ (i.e., 100% of CPC going to wastewater)

EC in Sludge = (1,200,000 mg x 0.01 x 0.01 / 1736 kg)

EC in Sludge = 0.069 mg/kg

As indicated by the foregoing calculations, because the vast majority of the CPC will be recycled to the treatment cabinet and virtually all of the non-recycled CPC is expected to

bind to the offal or DAF, the concentration at which CPC may become a component of sludges from wastewater treatment is very low, equivalent to 69 ppb.

6.2.4 Landfilling Sludge Environments

Due to the sorptive nature of CPC to bind to organic material such as fat and offal within the processing plant's waste stream, only extremely low, insignificant levels of CPC are expected to be present in sludge generated by POTWs. Moreover, EPA's regulations in 40 CFR part 258 governing landfills mandate new municipal solid waste landfills to be constructed with liners and collection systems to prevent leacheate from entering ground and surface water. Taken together, it can be predicted that due to the extremely low level of CPC expected to be in POTWs-generated sludges, and the fact that CPC is unlikely to leach to ground or surface water, there is no need for any concern about the presence of CPC in landfilled sludges.

7.0 Fate of Substance released into the Environment

The concentration of CPC in POTWs' sludges and water discharge is expected to be extremely low due to the sorptive interaction between poultry offal and CPC, and DAF and CPC within the poultry processing plant. Based on Petitioner's calculations, approximately 99% of the non-recycled CPC will be present in the P-MBM that subsequently will be used for poultry feed. In this regard, a series of calculations (presented below) support a sound prediction of the amount of CPC entering terrestrial environments as a result of CPC consumption by growing broilers and subsequent excretion into poultry litter.

7.1 Aquatic Environments

Because of the extremely low concentrations at which CPC is expected to be present in wastewater released from poultry processing plants, i.e., 0.022 ppb or less, no significant adverse impact on microorganisms used in biological wastewater treatment systems is expected. The lack of an adverse impact is supported by a study conducted by the Springdale, Arkansas POTW that is discussed more fully in Section 8 below.

Wastewater ($\leq$0.022 ppb) → POTW → Effluent → Receiving water

Moreover, based on the submitted experimental data and the discussions above, it is clear that CPC levels in water discharge from POTWs will be significantly less than the worst-case value of 0.022 ppb. Therefore, the presence of CPC in aquatic environments as a result of water discharge will be negligible. In addition, CPC as a leacheate from CPC in amended soil will also be negligible due to the extremely high sorptive nature of CPC onto soil (Appendix 1, Table 5 of EA for FAP 2A4736) and the low level at which CPC will be present in amended soil, as discussed more fully below.

7.2 Terrestrial Environments

The small fraction of CPC that is not captured and re-used will ultimately be amended into soil. Therefore the Petitioner conducted an experiment to determine the adsorption of CPC onto soil. The results show that CPC binds so tightly onto soil that even after 3 stringent rinses with water and one rinse with 95% ethanol, more than 99% of a 5000 ppm application is still bound to the soil. (See Appendix 1, Table 5 of EA for FAP 2A4736.) These data agree well with data presented in the report by Herrera *et al.*, 2000, that CPC binds tightly to negatively charged clay particles. Based on Petitioner's data, and the work of Herrera and co-workers (2000), it is clear that CPC amended to soil will become a constituent component and

will not leach to ground water, *i.e.*, it will not be introduced to a significant extent into aquatic environments.

Broiler Feeding Trial: Since the non-recycled CPC will combine with offal used to make P-MBM that will be subsequently fed to growing broiler chickens, a feeding trial was conducted in support of FAP 2A4736 to determine the effect of CPC consumption on broiler growth performance. Growing broiler chicks were fed a standard diet, from 2 to 5 weeks of age, containing 0, 100, or 250 ppm CPC. The levels of CPC chosen in this experiment were approximately 300 and 700 times the actual expected levels that broilers will be exposed to when they consume MBM containing P-MBM from Cecure®-treated carcasses. Growth data was collected on a weekly basis for calculation of industry standard growth parameters. In addition, several internal organs were collected and weighed to determine if CPC might be specifically affecting a particular organ system. The results from this study show quite clearly that feeding CPC did not have any effect on broiler growth performance parameters or internal organ weights. (See Appendix 2, Tables 1-5 of EA for FAP 2A4736). It should be noted that CPC used in this feeding trial was of a powder form (totally available), as opposed to CPC bound to rendered offal material as would be consumed by the chickens in actual practice. Taken together, these data present overwhelming evidence that growth performance of chickens consuming CPC, especially in the form that is bound to offal, will not be altered.

Bacterial Resistance: During their review of FAP 2A4736, FDA's Environmental Review Group expressed concerns about the development of resistant bacteria (Tattawasart *et al.*, 1999; 2000) as a result of the proposed usage of CPC. In response to this concern, experimental data addressing this issue were presented in FAP 2A4736 (Appendix 1 of the

EA, Table 6). When P-MBM containing 1125.0 ppm CPC was added to a culture of *Pseudomonas aeruginosa*, the level of bacterial growth was not different than growth observed for the P-MBM alone (not containing CPC) treatment. (Experimental methods are presented, in detail, in Appendix 1, Table 6 of EA for FAP 2A4736.) The most logical explanation for the lack of an antimicrobial effect of the offal containing CPC is that this form of CPC is so tightly bound to organic material that it is not available to function as an antimicrobial. Therefore, we assert that due to the interactive nature of CPC with other organic materials (P-MBM in particular), CPC is so tightly bound that it is not available to function as an antimicrobial when fed in this manner. In this regard, these data also firmly support the conclusion that since the CPC contained in P-MBM is tightly bound (unavailable), growth performance should not be affected and subsequent environmental effects associated with chickens consuming this form of CPC should be of no concern.

Bioconcentration of CPC: Calculations set forth in Section 6.2.3 above indicate that CPC may be present in the P-MBM at a level of 21.9 mg/kg, or 21.9 ppm. As discussed previously, the P-MBM will be used as a component of poultry feed. The concentration of CPC in the feed as a result of this use, and the total amount of CPC that may be consumed by broilers over the duration of grow-out, are calculated as follows:

7.2.1 Estimation of CPC content of broiler diets

- Assuming the EC of CPC in P-MBM = 21.9 mg/kg (calculated above)
- Assuming typical broiler diets contain 4.84% MBM (Agri-Stats, Inc., 2001), and that MBM is typically 32% P-MBM (Rudbeck, 2002). Ultimately, P-MBM would account for 1.55%, on a weight/weight basis, of a broiler diet.

EC = (21.9 mg/kg x 0.0155)

EC of CPC in broiler diet = 0.34 mg/kg

7.2.2 Estimation of Total CPC intake by broilers consuming P-MBM containing CPC

- Assuming a typical broiler will consume a total of 4.13 kg feed during grow-out[5]
- Assuming the broiler feed contains 0.34 mg/kg CPC

Total CPC consumed by broiler = 0.34 mg/kg x 4.13 kg feed

Total CPC consumed by broiler = 1.40 mg

These calculations indicate that the typical broiler will consume a total of no more than 1.4 mg of CPC over its lifetime.

Beyond the fact that very small quantities of CPC will be consumed by broilers, a number of tests and known factors discussed below demonstrate that CPC will not bioaccumulate in poultry tissues upon its consumption in the feed. For one thing, CPC is freely soluble in water, as stated in section 5.1.2 above, and has been shown in testing conducted by Safe Foods to be virtually insoluble in vegetable oil. Thus, to the extent that any free (unbound) CPC is ingested in the feed, it is expected to be readily eliminated. The fact that CPC present in the poultry feed will be bound to the P-MBM, as discussed

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[5] This value was presented in the EA for FAP 2A4736 and is based on information provided in a personal communication by Dr. Park W. Waldroup, University of Arkansas Division of Agriculture (see Attachment 4 of EA for FAP 2A4736). The most recent published value for total feed consumption, 4.8 kg, is derived from a 1994 National Academy Press publication on Nutrient Requirements of Poultry. According to Dr. Waldroup, due to geneticists selecting birds based on rapid growth and reduced food intake requirements, the current feed consumption for broiler chickens is 9.11 pounds, equivalent to 4.13 kg.

previously, makes it even less likely for the compound to be absorbed from the gastrointestinal tract.

Moreover, CPC is reported to have a log octanol/water partition coefficient (Log K_{ow}, or log P) of about 1.7.[6] This is well below the Log K_{ow} range of 5 to 8 at which bioaccumulation is most likely to occur (Hoffman, *et al.*, 1995). This is consistent with the data discussed in section 8.1 below on a closely related compound, hexadecylpyridinium bromide (HPB), which indicated very low uptake of HPB into internal organs and tissues of clams, minnows, and tadpoles (Knezovich, *et al,* 1989).

Additional information suggesting the low potential for bioaccumulation of quaternary ammonium compounds is provided by testing in which rats received orally ^{14}C-labeled hexadecyl trimethyl ammonium bromide (CTAB). About 80% of the dose of radioactivity was found in the gastrointestinal tract 8 hours after the administration, only small amounts were found in the blood plasma, and about 2% of the administrated radioactivity was excreted in the bile during the first 12 hours after treatment. The low levels of radioactivity in the plasma and bile, together with the large amount of radioactivity found in the gastrointestinal tract, indicated poor intestinal absorption of CTAB. Only small amounts of radioactivity were found in the liver, kidneys, spleen, heart, lung and skeletal muscles. Within three days of ingestion, 92% of the radioactivity was excreted via the feces and 1% via urine. (Isomaa, 1975).

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[6] Moschner, K.F., and A. Cece, 1995. *Development of a general QSAR for predicting octanol-water partition coefficients and its application to surfactants.* ASTM Special Technical Publication, Vol. STP 1218, No. Environmental Toxicology and Risk Assessment: 3rd Vol., pp. 318-31. (See Attachment 5 of EA for FAP 2A4736.) The article includes both a reported log P of 1.71 and a log P of 1.79 predicted using the model described therein.

Finally, experimental evidence of the lack of CPC bioaccumulation is provided by the results of the broiler feeding trial discussed above. In that study, following one week of continuous exposure to CPC in the feed, blood samples from birds receiving 250 ppm CPC were analyzed for CPC; no CPC was detected in the blood. (See Table 6 of Appendix 2 of EA for FAP 2A4736.) The absence of detectable CPC in the blood implies that the compound did not accumulate in edible tissues since this would require it to be transported via the circulatory system. These data strongly suggest that CPC will not be accumulated by poultry receiving the compound at the expected concentration of 0.34 ppm, more than 700 times less than the dosing level in the feeding trial.

The absence of bioaccumulation of CPC in poultry tissues ensures that the compound's presence in P-MBM that is subsequently fed to poultry will not result in any increase in the potential for human ingestion of CPC residues in food.

Material Balance: As noted in Section 6.2.3 above, the annual market for CPC is roughly estimated as 35 thousand pounds per year, based on a market penetration of 20% and taking into account the recycling of the Cecure® treatment solution. The total quantity of CPC that becomes part of poultry plant offal, and thus is incorporated into P-MBM, may be calculated based on the amount of CPC that is not recycled each time the treatment solution is applied. The calculations set forth above indicate that no more than about 6.0 mg of CPC will enter P-MBM per broiler carcass (this represents the sum of CPC directly entering the offal trough and CPC lost during immersion chilling).

The total number of broiler chickens produced annually is approximately 8.6 billion.[7] If CPC is used to treat 20% of these carcasses, and 6.0 mg of CPC enters P-MBM per carcass, the total mass of CPC entering P-MBM will be 1.0×10^{10} mg (1.0×10^{4} kg).[8]

The concentration of CPC in the P-MBM produced from the offal was calculated above as 21.9 mg/kg, and the resulting concentration of CPC in broiler diet was found to be 0.34 mg/kg.

If a total of 1.0×10^{10} mg of CPC (i.e., all non-recycled CPC) becomes incorporated into poultry feed at a concentration of 0.34 mg/kg, this represents a total quantity of poultry feed equal to 2.9×10^{10} kg.[9] By contrast, the total U.S. market for broiler feed may be calculated assuming the total quantity of feed consumed is 4.13 kg per broiler (see above) and based on the total of 8.6 billion broiler chickens produced annually. On this basis, the total broiler feed consumed annually is about 3.5×10^{10} kg.[10] This quantity is more than enough to account for all of the CPC that is not recycled.

Calculation of EEC's: CPC present in P-MBM that is incorporated into feed is expected ultimately to be excreted by the broiler chickens. The litter containing CPC is expected to be used for soil amendment.

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7 *Poultry Slaughter, 2001 Annual Summary.* United States Department of Agriculture, National Agricultural Statistics Service. April 2002.

8 (8.6×10^{9} broilers) x 20% x (6.0 mg CPC/broiler) = 1.0×10^{10} mg CPC.

9 (1.0×10^{10} mg CPC) ÷ (0.34 mg CPC/kg feed) = 2.9×10^{10} kg feed.

10 4.13 kg/broiler x 8.6×10^{9} broilers/year = 3.55×10^{10} kg/year.

In addition, CPC incorporated into DAF during DAF generation also will be soil amended. Finally, sludges from wastewater treatment may be either landfilled or soil amended. Therefore, the following calculations are presented to determine the expected environmental concentration (EEC) of CPC into soil as derived from these three waste streams. Note that the concentration of CPC in poultry litter is calculated assuming that 100% of the CPC ingested by the broilers is excreted and, thus, represents a worst-case estimate of environmental introduction by this route.

7.2.3 Estimation of CPC in poultry litter from broilers consuming CPC

- Assuming each broiler consumes 1.4 mg CPC during grow-out
- Assuming a typical broiler house containing 20,000 chickens (Tabler, 2000)
- Assuming 5 sets of broilers are grown before litter clean-out (Tabler, 2000)

M_{CPC} in poultry litter = (20,000 chickens x 1.4 mg CPC consumed x 5 grow-outs/yr)

M_{CPC} in poultry litter = 140,000 mg CPC per 200,000 chickens

7.2.4 Total mass of poultry litter (M_{litter}) for 5 grow-outs

- Assuming that 105 tons of litter are generated per 5 grow-outs (Tabler, 2000)

M_{litter} = (105 ton x 2000 lb/ton) x 0.454 kg/lb

M_{litter} = 95,340 kg

EC = TM_{CPC} in poultry litter/ M_{litter} = 140,000 mg/95,340 kg

EC of poultry litter = 1.47 mg/kg

7.2.5 Calculation of dilution rate of soil amended with poultry litter

- Assuming application rate for chicken litter is 5 tons/acre (1.1 kg/m^2)
- Assuming a soil density of 1200 kg/m^3
- Assuming litter soil amended as described by Harrass *et al.*, 1990

- Dilution rate = (1.1 kg/m^2) (100%) / (0.15 m x 1200 kg/m^3) (Harrass *et al.*, 1990)
- **Dilution rate = 0.61%**

7.2.6 Estimated EEC for CPC derived from poultry litter

- Assuming EC of 1.47 mg/kg for CPC in poultry litter
- Assuming a soil dilution rate of 0.61%

EEC = 1.47 mg/kg x 0.0061

EEC = 0.0090 mg/kg, or 9.0 ppb (*The expected environmental concentration of CPC in the soil as a result of soil amendment of poultry litter containing CPC)*

These calculations demonstrate that CPC will be applied in soil amendment of poultry litter only at negligible levels, i.e., at no more than 0.009 ppm (9 ppb) in the soil. Moreover, as discussed above, the CPC is not expected to leach from the amended soil to an appreciable extent due to its extremely strong sorptive properties.

The concentration of CPC that may be applied in soil amendment of DAF and sludges from wastewater treatment are also low as shown by the following calculations.

7.2.7 Estimated EEC for CPC derived from DAF

- Assuming EC of 0.74 mg/kg for DAF (calculated above)
- Assuming a soil dilution rate of 2.5%

EEC = 0.74 mg/kg x 0.025

EEC = 0.0185 mg/kg, or 18.5 ppb (*The expected environmental concentration of CPC in the soil as a result of soil amendment of DAF containing CPC)*

7.2.8 Estimated EEC for CPC derived from Sludge

- Assuming EC of 0.069 mg/kg for Sludge
- Assuming a soil dilution rate of 2.5%

EEC = 0.069 mg/kg x 0.025

EEC = 0.0017 mg/kg, or 1.7 ppb (*The expected environmental concentration of CPC in the soil as a result of soil amendment of sludge containing CPC)*

The maximum total level at which CPC may be present in soil as a result of the proposed use of the substance may be calculated as the sum of the maximum levels resulting from soil-amendment of poultry litter (9.0 ppb), DAF (18.5 ppb), and sludge (1.7 ppb). The result is a maximum CPC level entering terrestrial environments of approximately **29 ppb**.

8.0 Environmental Effects of Released Substance

As discussed previously, only negligible concentrations of CPC are estimated to be present in the effluent from poultry plant wastewater treatment facilities or from POTWs due to the extremely low concentration of CPC reaching these facilities. In this regard, previously provided data from the Springdale, Arkansas POTW demonstrates that CPC, even at many times the anticipated level, did not alter the bacterial nitrification process. (See Attachment 1 to EA for FAP 2A4736.) This POTW was used as a model system due, in part, to its reception of wastewater from 4 poultry processing plants and the possibility of CPC replacing a commonly used trisodium phosphate (TSP) processing aid. The Springdale POTW is facing an increasing need, from an environmental standpoint, to reduce phosphate levels in released wastewater. In fact, the Springdale POTWs initiated discussions to conduct testing in hopes that CPC may reduce the need for TSP in poultry processing plants.

8.1 Aquatic Environments

With regard to quaternary ammonium compounds in general, of which CPC is one member, acute toxicity values (LC_{50}) to aquatic organisms of approximately 1 mg/L are reportedly typical, although some species are considered to be more sensitive than others (Cooper, 1988). CPC is reported to result in mortality to just 1% of *Australorbis* sp. (snails) upon exposure at a concentration of 1 mg/L for 48 hours; 100% mortality is found at concentrations of 5 mg/L and higher. These values are well above the anticipated level of $\leq$ 0.022 ppb ($\leq$ 0.000022 mg/L) that may be released from POTWs.

Moreover, CPC is not expected to bioaccumulate to a significant extent, based on data indicating low permeability of gills to CPC (Tolls, *et al.*, 1994). This expectation is consistent with testing conducted on a related compound, hexadecylpyridinium bromide (HPB), in which clams, minnows, and tadpoles were exposed to an HPB aqueous solution (10 mg/L) for 24 hours, followed by whole body and selected tissue analysis for the compound (Knezovich, *et al.*, 1989). This testing demonstrated very low accumulation levels compared to many neutral organic compounds. HPB was detected primarily in the gills, consistent with observed acute toxicity effects. While some HPB was also detectable in the stomach and intestine due to water infiltration through the GI tract, the distribution of HPB to tissues of particular toxicological concern, e.g., liver and kidneys, was very low.

With further regard to the toxicity of CPC to aquatic organisms, a table listing pertinent LC_{50} values, as well as doses that have been associated with either 0% or 100% mortality in various species, was provided as Attachment 3 to the EA for FAP 2A4736. As indicated therein, the lowest LC_{50} reported for CPC, that for giant river prawn, is 100 μg/L (or 100 ppb). The lowest no-effect level for mortality, in the same species, is 50 μg/L (or 50

ppb). By contrast, the worst-case level of CPC in effluent from POTWs or poultry plant wastewater treatment facilities is 0.022 ppb (0.022 µg/L). This is also well below the lowest effect concentrations reported for a structural analog, alkyl (C_{12}) pyridinium chloride, for larval growth and development in clams (10 ppb) and oysters (50 ppb). (See Attachment 3 of EA for FAP 2A4736.) Based on the available data, no toxicity to aquatic organisms is expected.

Finally, as noted previously, CPC is not expected to be toxic to beneficial microorganisms in biological treatment systems (such as the Springdale, AR POTW) due to the extremely low concentrations at which it may be present in poultry plant wastewater.

8.2 Terrestrial Environments

The data provided in Section 8.1 above with respect to the toxicity of CPC to aquatic organisms may be used as a conservative reference point for purposes of assessing CPC's toxicity to terrestrial organisms. Also of relevance is the following information summarizing the results of oral toxicity testing on CPC in mammalian species:[11]

Acute Oral Toxicity (LD_{50}) for Cetylpyridinium chloride

Species	LD_{50} (mg/kg b.w.)
Rat	200 5080 428 (M) 460 (M) 335 (F)
Mouse	195 (F) 1360
Rabbit	400

[11] *See* Section E of this petition (Safety of the Food Additive) for acute toxicity data references.

	1000
Guinea pig	3860
Dog	1000
Cat	1000

These data indicate that CPC is of relatively low acute toxicity, in relation to the concentrations at which it may be released to the environment. In short-term (28-day) testing, CPC was fed to rabbits in doses of up to 100 mg/kg b.w. No gross pathological conditions were found that could be attributed to oral administration of CPC. Safe Foods has conducted both a 14-day and 28-day rat feeding trial to confirm the results of earlier feeding trials. Recently completed subchronic feeding studies in rats and dogs have established a minimum no-observed adverse effect level (NOAEL) of 375 ppm in the feed. These data additionally indicate that CPC will not be released to the environment at levels expected to give rise to adverse toxicological effects.

As shown in Section 7.2 above, the maximum level at which CPC may be present in terrestrial environments is approximately 29 ppb. Given the compound's high sorbtive properties, the actual amount of CPC to which terrestrial organisms will be exposed is likely to be considerably less than this worst-case value. None of the available toxicity data on CPC suggest a substantive toxicological concern at such low levels. Therefore, the Petitioner respectfully submits that the possible environment introduction of CPC due to soil-amendment of the various waste streams discussed above is safe.

9.0 Use of Resources and Energy

As is the case with other antimicrobial treatments for use on poultry carcasses, the production and use of Cecure® will require the consumption of natural resources and energy.

However, given the relatively small market volume estimated for the product, the amount of resources and energy required will be minimal. Moreover, because CPC will be used in place of other existing antimicrobial treatments for poultry, no net increase in the consumption of energy and resources is expected.

10.0 Mitigation Measures

As shown above, no significant adverse environmental impacts are expected to result from the proposed use of CPC as an antimicrobial treatment for poultry carcasses. This is primarily due to the low concentration at which the compound may enter the environment as a result of its use as intended, and the absence of data suggesting a substantive toxicological concern at such low levels. Thus, the use of the compound as described herein is not reasonably expected to result in any new environmental problem requiring mitigation measures of any kind.

11.0 Alternatives to the Proposed Action

No potential adverse environmental effects are identified herein which would necessitate alternative actions to those proposed in this Petition. The alternative of not approving the action proposed herein would simply result in the continued use of the materials that the subject additive would otherwise replace; such action would have no environmental impact. In view of the excellent properties of CPC as an antimicrobial treatment for poultry, the improvements in food safety that will result from its use, and the absence of any identified significant environmental impact that would result from its use, the

clearance of the use of CPC as described herein appears to be environmentally safe in every respect.

One very important point to mention is that usage of CPC as an antimicrobial agent, instead of trisodium phosphate (TSP), will result in the overall reduction of phosphates entering aquatic environments. Specifically, in Northwest Arkansas, the level of phosphates released by POTWs is aggressively regulated due to the high number of poultry processing plants using TSP as an antimicrobial. Moreover, this situation is not unique to Northwest Arkansas. In this regard, the laboratory director of the Springdale, Arkansas POTWs has expressed great enthusiasm and interest in the possibility of CPC replacing the usage of TSP. The city is currently facing the likelihood of spending millions of dollars to reduce the phosphate level in wastewater discharge.

12.0 List of Preparers

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Dr. Kelly W. Beers, Safe Foods Corporation.

Dr. Peggy Cook, Safe Foods Corporation.

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13.0 Certification

The undersigned official certifies that the information provided herein is true, accurate, and complete to the best of his knowledge.

Date: July 31, 2006

By _____
Jerome H. Heckman
Counsel for Safe Foods Corporation

14.0 References

The following sources cited as references in the foregoing Environmental Assessment were also cited in the EA for FAP 2A4736. Where appropriate, the references were provided in that petition.

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15.0 Attachments

The following is attached to this Enviromental Assessment:

A. Report of Experiment MCA-060601, Development of Cecure® Nuevo application system – quantification of environmental exposure.

The following data were provided as attachments to the Environmental Assessment for FAP 2A4736. These data are incorporated by reference and not reproduced here.

1. Report from Jennifer Enos, Laboratory Director, Springdale, Arkansas Water Utilities
2. Material Balance Table
3. Aquatic Toxicity Data
4. Letter regarding feed consumption by broilers
5. Log K_{ow} for CPC