Docket No
02F-0181

FAP 2A4736

SAFE FOODS CORP.

Appendix XIII

Environmental Assessment

02F-0181

EA-1

Environmental Assessment

1.0 Date March 20, 2002

2.0 Name of Petitioner Safe Foods Corporation

3.0 Address

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4.0 Description of the Proposed Action

4.1 Requested Approval

The action requested in this Petition is the establishment of a new food additive regulation, 21 C.F.R. § 173.---, to permit the use of cetylpyridinium chloride, generally abbreviated herein as CPC, as a food processing aid for use on raw poultry. More specifically, the substance is proposed for use at a concentration not to exceed 0.4% in an aqueous solution that also contains not to exceed 0.6% propylene glycol. The solution (marketed under the trade name "Cecure®") will be applied as a fine spray mist to treat the surface of raw poultry carcasses prior to immersion chilling.

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.

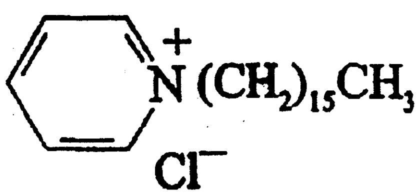
The use of Cecure will control the following microorganisms: *Salmonella, Listeria, Campylobacter, E. coli* (including *O157:H7*), and other coliforms. In addition, application of Cecure at the proposed level will reduce the total aerobic plate count, thereby extending refrigerated shelf-life.

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 Property

5.1.1 The structural formula for CPC is depicted below:



5.1.2 Physical Properties

The molecular formula of CPC is C21H38NCl; the formula weight is 340.05. The calculated elemental content is C: 70.45%, H: 11.26%; Cl: 9.90%; O: 4.47%; and N: 3.91%.

CPC is typically present in water in the monohydrate form. The monohydrate has the molecular formula C21H38NCl·H2O and has a formula weight of 358.07. 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 substances 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 spray is applied (≤1 oz/pound of carcass, ≤0.4% CPC solution) will be after the live bird slaughter and evisceration and just prior to carcass immersion into chill water. The spray will be applied in a commercial, stainless steel spray cabinet at commercial line speed conditions. The cabinet will be equipped with nozzles that will be rated in gallons per minute at a specified pressure to apply and control the appropriate amount of spray per carcass.

In understanding the environmental fate of CPC and Cecure, it is important to understand the flow of the product and waste process in a poultry processing plant.

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.

<u>Bird Evisceration</u>: 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 110 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).

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. As the carcasses leave the last inside-outside bird washer, they are totally drenched and saturated with water and are dripping considerably. It is at this point that the spray/mist cabinet for Cecure would be positioned.

A point to mention is that a dripping wet, fully saturated carcass, is going to pass through a fine mist of Cecure (≤1 ounce/pound, ≤0.4% solution) for an average of 0.6 seconds. Thus, very little of the actual product, CPC, will end up on the carcass. The majority of the product simply drains out of the cabinet and is rinsed with water, down into the offal trough positioned directly under the cabinet.

If one were to look into the offal trough under the cabinet, one would see chicken heads, intestines, chicken fat, and sometimes a little blood and feathers mixed with a large quantity of water. Again, water and gravity are used to move all of this material out of the plant into a separate material separation facility for screening. The facility used for screening the offal is typically located within the processing complex, but in a different building.

Immersion Chilling: After the Cecure treatment, the carcasses, which are still dripping wet from the several gallons of water that have been used to clean them, drip from the overhead line into the offal trough from 2 to 5 minutes until they enter 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.

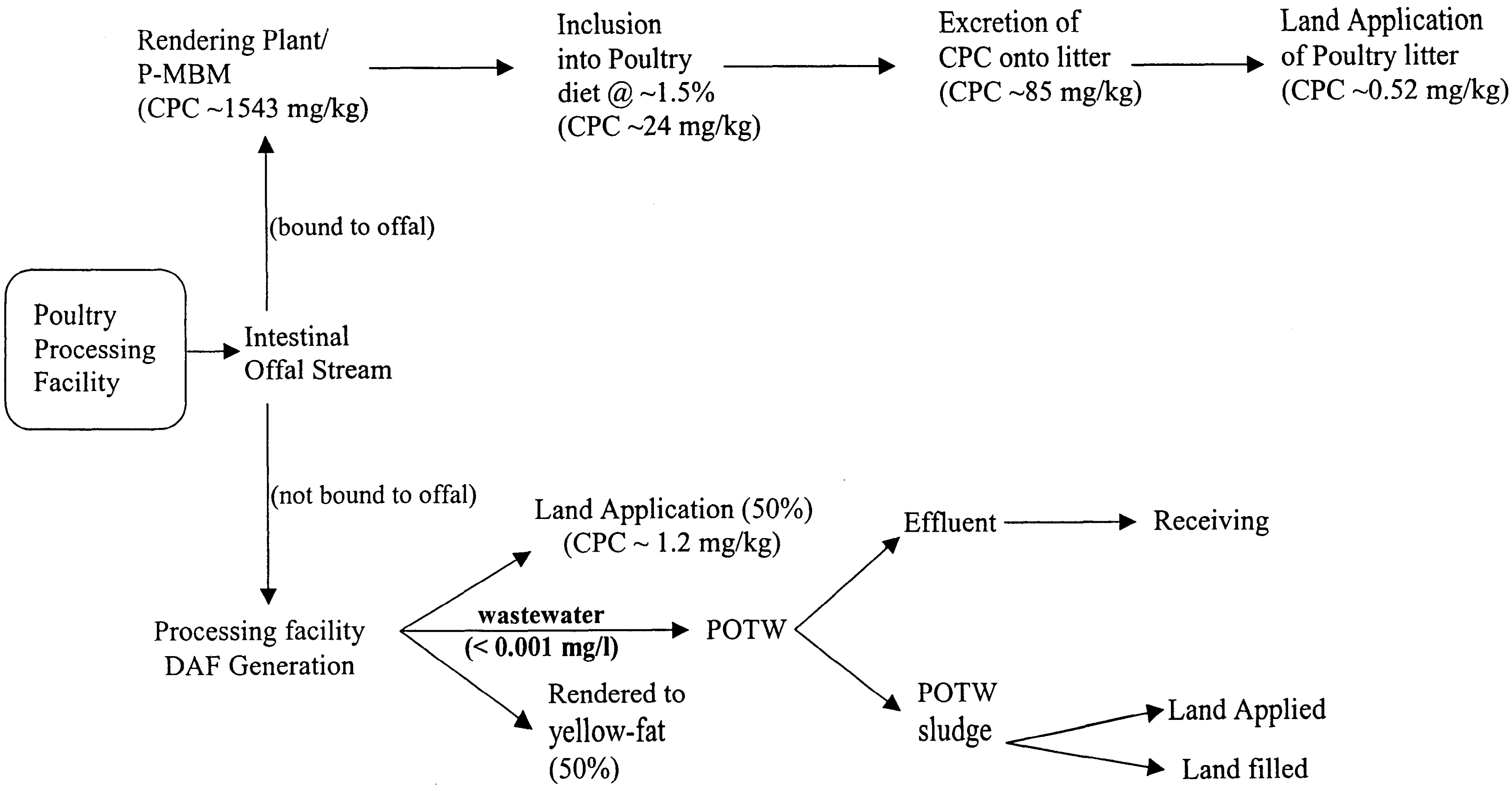
From the prechiller tank, the carcasses move automatically into the chiller tank. This tank is larger, colder, usually 32° to 34°F, and the carcasses stay in this tank for about 45 minutes. The carcasses pick-up an additional 3 to 4% moisture in the chiller. USDA allows poultry carcasses to gain a total 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.

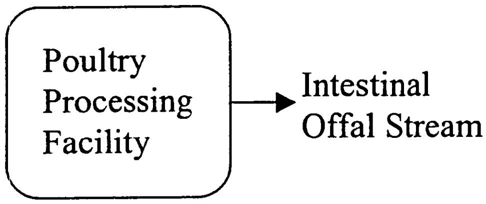
<u>Inedible Offal Materials</u>: The inedible materials that are removed from the bird, *i.e.*, the head, preen gland, and inedible viscera are combined with other non-marketable products such as the neck and giblets (heart, gizzard, liver) and are transported via water to the materials separation facility at the processing plant. Large screen screw conveyors are used to remove as much water as possible from this material. Then this product is transported via tanker truck to a poultry byproducts rendering facility. This material is combined with dead birds picked up from the farms, USDA condemned birds from the plant, and expired product from grocery stores to make a high protein feed ingredient that is used in poultry diets. In addition, bone and skin from further processing poultry plants as well as used batter and breading, and fryer skimmings are also incorporated into this rendered poultry by-product meal. This product is commonly known as poultry meal and bone meal ("P-MBM").

The proposed waste stream for CPC in this poultry processing environment is depicted on the following page and is more fully explained in detail in the paragraphs that follow.

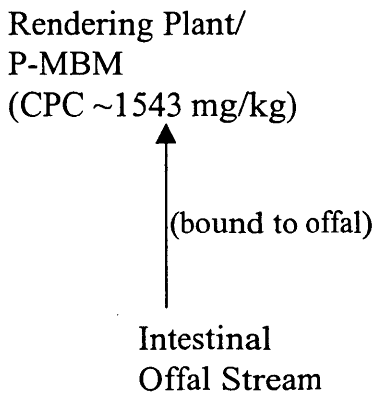
Proposed CPC Waste Stream



The Intestinal Offal Stream:



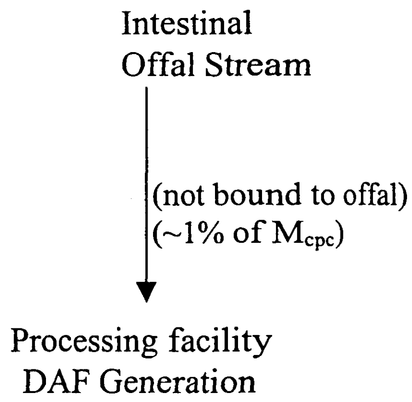
A water mist will be used inside the Cecure spray cabinet to rinse excess Cecure from the cabinet to the drain. However, the water mist will not directly contact the carcasses (i.e., there will be no direct carcass rinse following treatment with Cecure). Following drainage from the spray cabinet, the excess Cecure will be combined with the offal (head, neck, intestinal tract, other digestive organs, preen gland and fat pad) from the evisceration line, and will ultimately become part of poultry meat and bone meal (P-MBM). P-MBM is composed of processing plant offal, processing plant condemned 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.



In the course of the standard application of Cecure in poultry processing operations, not more than 3 gallons/minute of ≤0.4% (4000 ppm) spray will be diluted with at least 100 gallons/minute of water from the evisceration line, resulting in a CPC concentration in the evisceration waste stream of not more than 125 ppm.

Appropriate testing (Appendix 1, Table 1) indicates that when mixed together, at least 99% of CPC (from a 125 ppm solution) will be adsorbed by the offal within a 2-minute contact period (actual period of contact will vary from 2 to 15 minutes, depending on individual processing plant setup). In actual practice, the poultry carcass shackle line, and thus the Cecure spray cabinet, will be positioned directly above the offal trough (channel in which processing water and offal are transported through the processing plant). Little or no additional plumbing will be required to drain excess Cecure from the application cabinet to the offal trough.

Chiller Skimmings: A small percentage of sprayed CPC is expected to be present on the carcass prior to immersion chilling (Appendix 1, Table 2), and a negligible amount remains on the carcass after immersion chilling (refer to FAP, section D). During the immersion chilling process, 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. During the chilling process, the combination of ice-cold water and constant agitation act to effectively remove all but a negligible amount of CPC from the carcass (FAP, section D). In addition, 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).



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 (*Proposed CPC Waste Stream*); the remaining 50% is normally soil amended.

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 demonstrates the high binding capacity of DAF for compounds with hydrophobic moieties, such as CPC. When 2 grams of DAF were mixed with 20 mls 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 significantly 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 actual calculated levels). 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 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.

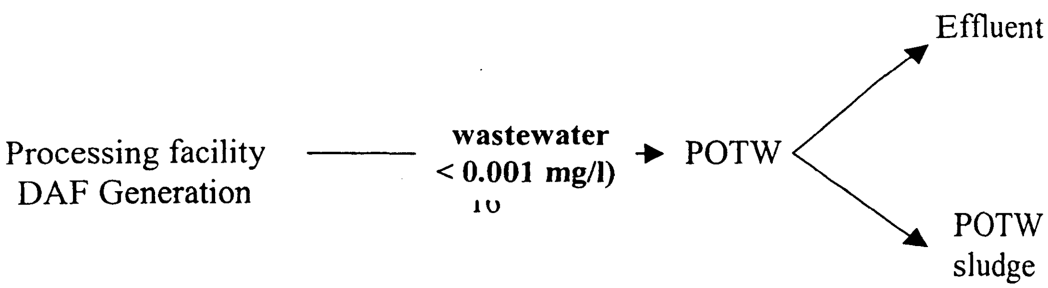
6.2.1 Air Releases

Based on its ionic nature, high molecular weight (340.05), and its resulting low vapor pressure, CPC is not expected to be released to the atmosphere.

6.2.2 Aquatic Environments

As pointed out above, approximately 99% of CPC (from a 125 ppm solution) coming into contact with poultry offal for at least 2 minutes 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, thereby dramatically decreasing 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 divided by the total amount of water (on a kilogram basis) utilized on a daily basis.

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.

For the year 2001, the average live broiler weight, as provided by the USDA's economic research service, was 5.03 pounds (personal communication, David Harvey, USDA Economic Research Services, 2002). The carcass weight (weight of the broiler at the point of application of Cecure), without giblets (WOG), for these same broilers was 3.3 pounds (Harvey, 2002). For purposes of this assessment, a WOG weight of 3.3 pounds is assumed. This Petition proposes to clear the use of CPC at a concentration of up to 0.4% (4000 ppm) in a spray that will be applied to carcasses at a maximum level of 1 ounce per pound. Thus, not more than 3.3 ounces of $\leq$0.4% CPC will be applied per carcass (on an average weight basis).

Based on the foregoing, the total amount of CPC employed per day, and the resulting waste water concentration, are calculated as follows:

The total mass of CPC (M_{CPC}) sprayed per day:

- Assuming a facility processing 200,000 chickens per day
- Assuming the average chicken carcass weight is 3.3 pounds (Harvey, 2002)
- Assuming a Cecure application rate of 1 oz. per pound of chicken (WOG basis)
- Assuming the total volume of Cecure (0.4% solution) used would be 660,000 ounces per day

The total mass of CPC would be:

M_{CPC} = (660,000 oz. x 0.004) x (1 lb./16 oz.) x 453,600 mg/lb

M_{CPC} = 74,844,000 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} = 74,844,000 mg (calculated above)
- Assuming 1% M_{CPC} passes to DAF generator
- Assuming 1% of CPC reaching DAF generator remains in wastewater

EC in wastewater = (74,844,000 mg x 0.01 x 0.01) / 5,443,164 kg

EC in processing plant wastewater = 0.0014 mg/kg

These calculations indicate that the maximum concentration of CPC in the waste water will be no more than approximately 1 part per billion (ppb). Furthermore, any CPC remaining in the water would then go to the POTWs where the whole 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 has been conducted 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). Therefore, all available data indicates that CPC, if present at all in effluent from waste water treatment facilities, will be there in such extremely low levels as to be of no environmental significance.

6.2.3 Terrestrial Environments

Petitioner estimates that, five years after introduction, the use of Cecure (CPC) for poultry processing will be in the approximate range of 2.5 million pounds per year. This figure assumes a market penetration upwards of 30% of processing facilities, an obviously high estimate. 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, 1) the projected use rate of CPC, 2) the total mass of offal generated by the plant, 3) 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, 4) the total amount of DAF generated on a daily basis, and 5) 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} = 74,844,000 mg per day (calculated above)
- Assuming all but negligible amount of CPC from carcass (from chiller skimmings) is added to offal
- Assuming 99% of sprayed CPC will bind to offal (Appendix 1, Table 3)

CPC_{offal} = (74,844,000 x 0.99)

CPC_{offal} = 74,095,560 mg

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

- Assuming M_{CPC} = 74,844,000 mg per day
- Assuming that all of M_{CPC} (100%) drains from spray cabinet (this assumes that 1.3% of M_{CPC} adheres to carcass, but is later removed by chiller skimmings and added to offal and, therefore will not become a component of DAF)
- Assuming 99% of M_{CPC} in contact with offal will bind to offal, so that 1% goes to DAF
- Assuming 99% of M_{CPC} in contact with DAF will bind to DAF

CPC_{DAF} = (74,844,000 x 0.01 x 0.99)

CPC_{DAF} = 740,956 mg

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

- Assuming average live chicken weight is 5.03 pounds (Harvey, 2002)
- 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 5.03 lbs x 0.30) x 0.3) / 0.90

$M_{P\text{-}MBM}$ = 100,600 lbs (45,632 kg)

EC of CPC in P-MBM

EC = M_{CPC} / $M_{P\text{-}MBM}$ = (74,095,560 mg / 45,632 kg)

EC = 1624 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= (1624 x 0.9) = 1543 mg/kg

Based on the foregoing calculations, the amount of CPC present in the P-MBM will be 1543 mg/kg, or approximately 0.15%. 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 0.15%, as shown by calculations set forth in Section 7.2.1 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} = 740,956 mg

EC in DAF = (740,956 mg / 16,000 kg)

EC in DAF = 46.3 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 CPC that does not bind to offal (1% of total CPC) will become bound to the DAF. This leaves 1% of 1% of the total CPC sprayed 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 this 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 CPC sprayed

EC in Sludge = (74,844,000 mg x 0.01 x 0.01 / 1736 kg)

EC in Sludge = 4.3 mg/kg

As indicated by the foregoing calculations, because the vast majority of the CPC is expected to become a part of the offal, or to bind to DAF, the concentration at which CPC may become a component of sludges from wastewater treatment is very low, equivalent to 4.3 ppm.

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 plants 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 mandates 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 sprayed 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., 0.001 mg/liter 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 (~0.001 ppm) → POTW → Effluent → Receiving water

Moreover, based on the submitted experimental data and the discussions above, it is clear that CPC levels in POTWs water discharge will be significantly less than 0.001 mg/liter. 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, more fully discussed below).

7.2 Terrestrial Environments

CPC draining from the spray cabinet will ultimately be amended into soil. Therefore the Petitioner conducted an experiment to determine the adsorption of CPC onto soil. The results (Appendix 1, Table 5) 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 (over 10,000-fold the actual expected level) is still bound to the soil. 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 CPC draining from the spray cabinet will combine with offal used to make P-MBM that will be subsequently fed to growing broiler chickens, a feeding trial was conducted 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 4 and 10 times the actual expected levels that broilers will be exposed to when they consume MBM containing P-MBM from Cecure-sprayed 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 (Appendix 2, Tables 1-5). 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: Regarding the expressed concerns of EPA's Environmental Review Group about the development of resistant bacteria (Tattawasart *et al.*, 1999; 2000) as a result of the proposed usage of CPC, experimental data dealing directly with this issue are presented (Appendix 1, 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). 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.

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.

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.

7.2.1 Estimation of CPC content of broiler diets

- Assuming the EC of CPC in P-MBM = 1543 mg/kg
- 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 = (1543 mg/kg x 0.0155)

  EC of broiler diet = 24 mg/kg (24 ppm)

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

- Assuming a typical broiler will consume 3.36 kg feed (Maynard, 2002) during grow-out
- Assuming the broiler feed contains 24 mg/kg CPC

  Total CPC consumed by broiler = (24 mg/kg x 3.36) kg feed

  Total CPC consumed by broiler = 81 mg

7.2.3 Estimation of CPC in poultry litter from broilers consuming CPC

- Assuming each broiler consumes 81 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 81 mg CPC consumed x 5 grow-outs/yr)

  M_{CPC} in poultry litter = 8,100,000 mg

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} = 95340 kg

EC = TM_{CPC} in poultry litter/ M_{litter} = 8,100,000 mg/95340 kg

EC of poultry litter = 85 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 85 mg/kg for CPC in poultry litter
- Assuming a soil dilution rate of 0.61%

EEC = 85 mg/kg x 0.0061

EEC = 0.52 mg/kg (*The expected environmental concentration of CPC in the soil as a result of soil amendment of poultry litter containing CPC)*

Inclusion Into Poultry Diet @ ~1.5% (CPC ~ 24 mg/kg)	→	Excretion of CPC onto litter (CPC ~ 85 mg/kg)	→	Land Application of Poultry litter (CPC ~ 0.52 mg/kg)

These calculations demonstrate that CPC will be applied in soil amendment of poultry litter only at negligible levels, i.e., at no more than 0.52 ppm 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 46.3 mg/kg for DAF
- Assuming a soil dilution rate of 2.5%

EEC = 46.3 mg/kg x 0.025

EEC = 1.2 mg/kg (*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 4.3 mg/kg for Sludge
- Assuming a soil dilution rate of 2.5%

EEC = 4.3 mg/kg x 0.025

EEC = 0.11 mg/kg (*The expected environmental concentration of CPC in the soil as a result of soil amendment of sludge containing CPC)*

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, experimental data (Attachment #1) from the Springdale, Arkansas POTW demonstrates that CPC, even at 8000-fold the anticipated level, did not alter the bacterial nitrification process. 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.

The available data with regard to CPC's toxicity to mammals is discussed in detail in Section E of this Petition. The available acute toxicity data are summarized below. (See Section E for toxicity data references.)

8.1 Aquatic Environments

With regard to quaternary ammonium compounds in general, of which CPC is one member, acute toxicity values (LC50) 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 <0.001 mg/L that is expected to 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.

Considering the data summarized above, CPC is not expected to be toxic to aquatic organisms at the minute concentrations at which it may be released following wastewater treatment. 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).

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

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

Species	LD50 (mg/kg b.w.)
Rat	200
	5080
	428 (M)
	460 (M)
	335 (F)
Mouse	195 (F)
	1360
Rabbit	400
	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. In a subchronic (90-day) study in rats, an apparent NOEL of 6.25 mg/kg b.w. was established. These data additionally indicate that CPC will not be released to the environment at levels expected to give rise to adverse toxicological effects. Safe Foods has conducted both a 14-day and 28-day rat feeding trial to confirm the results of earlier feeding trials.

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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12.2 Dr. Kelly W. Beers, Safe Foods Corporation.

12.3 Dr. Amy L. Waldroup, Safe Foods Corporation.

13.0 Certification

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

Date: March 20, 2002

By Curtis W. Coleman /ABB

Curtis W. Coleman
President/CEO
Safe Foods Corporation

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15.0 Attachments

#1. Report from Jennifer Enos, Laboratory Director, Springdale, Arkansas Water Utilities

Appendix 1

Table 1.

Experiment Name: CPC adsorption by Poultry Offal
Date: 1/24/2002

Offal Sample#	Treatment Level (ppm)	CPC Exposure Time (min)	Remaining in liquid (ppm)	Bound to sample (%)	Average
1	0	2	0	**0**	
2	0	2	0	**0**	
3	125	2	1.23	**99.0**	
4	125	2	1.05	**99.2**	**99.1**
5	125	30	N.D.	**100.0**	
6	125	30	N.D.	**100.0**	**100.0**

N.D. = not detected

Experimental Methods:

1. Offal (head, intestines, digestive organs, preen gland, fat pad) from one broiler was placed into a 1 gallon plastic sealable bag.
2. One liter CPC solution (125 ppm) was added to the bag and bag was sealed.
3. The contents of the bag were shaken gently for 2 minutes, then approximately 25 mls of liquid was collected into a 50 ml conical tube.
4. A portion of collected liquid was centrifuged for 15 minutes @ 35,000 x g
5. The supernate was analyzed by HPLC for CPC.

Table 2.

Experiment Name: Fate of CPC after spray on poultry carcass

Date: 3/6/02

Spray Treatment	Avg. WOG weight before spray (kg)	Total Spray volume** (ml)	WOG wt after drip (kg)	Total spray on WOG (%)	Total spray out drain (%)
Water	1.374	92	1.375	1.1	98.9
Cecure (0.4%)*	1.182	77	1.183	1.3	98.7

WOG = carcass weight without giblets (no heart, gizzard, or liver)
WOG's were allowed to drip for 2 minutes after spray
*A total of 12 carcasses were sprayed with CPC
** Total spray volume (per carcass) = 1 ounce/lb WOG

Table 4.

Experiment Name: Measurement of CPC binding to wastewater sludge
Experiment Date: 12/2001

Sample Content	Incubation Time (min)	CPC recovered from sludge (%)	CPC in water (μg/ml)
ethanol blank			N.D.
blank-1	0		22.4
blank-2	0		22.1
Control-1	0		1.0
Control-2	0		0.5
spike-1	0	86.5	N.D.
spike-2	0	84.8	N.D.
spike-3	10	84.4	N.D.
spike-4	10	82.5	N.D.
spike-5	20	89.7	N.D.
spike-6	20	83.6	N.D.
spike-7	30	76.9	N.D.
spike-8	30	80.3	N.D.
spike-9	60	80.7	N.D.
spike-10	60	80.2	N.D.
spike-11	120	78.2	N.D.
spike-12	120	73.8	N.D.

N.D. = not detected (minimum detectable limit = 10 ppb)

Experimental Conditions:

1. Sludge samples (not containing CPC) were spiked with CPC to a level of 22.3 ppm.
2. Samples containing sludge and CPC (spike-1 to spike-12) were incubated at 35^{0}C for indicated time, then centrifuged to pellet the material.
3. The liquid supernate was transferred to an HPLC autosampler vial for CPC analysis.
4. The remaining pellet was ethanol extracted for CPC assay by HPLC.
5. Samples labeled as blank-1 and blank-2 did not contain sludge material (these samples served as reference spike concentration).
6. Samples labeled as Control-1 and Control-2 contained sludge, but addition of CPC. % CPC recovered indicates the percentage of added CPC that was extracted from the pelleted material with 95% ethanol.

Table 5.

Experiment Name: CPC adsorption by soil
Date: 1/24/2002

	Applied (ppm)	Applied (mg)	CPC Total extracted (H_2O + EtOH)	Bound to sample (mg)	Bound to sample (%)
Soil	1000	2.0	N.D.	1.99	**99.8**
Soil	5000	10.0	N.D.	10.0	**100.0**

N.D. = not detected

Experimental Methods:

1. Weigh approximately 2 grams of soil into 50 ml conical tubes.
2. Add appropriate volume of di-water to each tube, cap and vortex well.
3. Transfer appropriate volume of 1% Cecure to soil solutions, to attain indicated CPC level, cap and vortex well.
4. Place tubes on platform shaker and shake for 15 minutes.
5. Centrifuge tubes at ~2500 x g for 15 minutes.
6. Decant supernate to a new 50 ml conical tube.
7. Re-extract tubes with 25 ml di-water 2 more times (decant into different tube each time) for a total of 3 water extractions.
8. After final water extraction, add 15 ml 95% EtOH to sample tubes containing pelleted soil and extracted by vortexing well, followed by 15 minutes on the shaker bath.
9. Spin EtOH extracts at ~2500 x g for 15 minutes.
10. Transfer water and EtOH extracts to HPLC autosampler vials and assay for CPC content.

Table 6.

Experiment: Antimicrobial effect of Poultry Offal	
Date: 2/16/02	
Sample	**cfu/ml**
Inoculum	4.6×10^8
Control Offal	4.2×10^7
CPC Offal (~1125 ppm)	4.9×10^7
CPC (1000 ppm)	9.9×10^5

Experimental Methods:

Sample Preparation -

1. Offal (head, intestines, digestive organs, preen gland, fat pad) from one broiler was placed into a 1 gallon plastic sealable bag and weighed.
2. One liter CPC solution (1125 ppm) was added to the bag and sealed.
3. The contents of the bag were shaken gently for 2 minutes, then approximately 20 mls of liquid was collected into a 50 ml conical tube.
4. The entire liquid portion was drained and the offal was transferred to a 1 liter glass beaker.
5. The beaker containing the CPC-treated offal was then autoclaved to ensure no bacteria were present.
6. The autoclaved material was then placed into a blender and blended into a thick slurry.
7. The material was then re-autoclaved.

Microbiology-

To compare the antimicrobial activity of Control-offal to offal containing a calculated level of 1125 ppm CPC, an aliquot of each offal (and a reference solution of 1000 ppm CPC) was added to an overnight culture of *Pseudomonas aeruginosa.* Samples were allowed to react @ 20^0C for five minutes, then neutralized by addition of Bacto neutralizing buffer for quaternary ammonia compounds. Serial dilutions were made in Butterfield's phosphate diluent, and all dilutions were plated on 3M's APC Petrifilm in accordance to supplier's instructions. The culture was then allowed to incubate for 48 hours @ 35^0C and total bacterial counts were assessed.

Appendix 2

The Effects of Feeding Cetylpyridinium Chloride to Rapidly Growing Broilers

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Overview.

A study was conducted to determine the overall effects of feeding CPC on broiler growth and performance. Feeding up to 250 ppm CPC (from 2 to 5 weeks of age) did not adversely affect any measured parameters including feed conversion, growth rate, muscle mass, or internal organ weights. It does not appear that the inclusion of CPC in the diet of rapidly growing broilers will alter any production or processed broiler parameters.

Methodology.

An experiment was conducted to evaluate the effects of feeding crystalline cetylpyridinium chloride (CPC) at 0, 100, or 250 ppm to rapidly growing commercial broiler chicks (Peterson x Arbor Acre Classic). One hundred and forty four 2-week old broiler chicks were randomly assigned to 36 grow-out battery cages; thus, a stocking density of 4 birds per battery cage. Twelve battery cages were assigned to each of the three treatments.

Over the course of the study, standard broiler grower diet and fresh water were provided *ad libitum*. Body weight data was collected on a per cage basis at the initiation of the study and at weekly intervals thereafter. In addition, all feed consumed over the course of the trial was measured weekly.

After one week exposure to the diets, blood samples (n=2 per treatment: 0 and 250 ppm CPC treatments) were collected into heparinized vaccutainer tubes. Blood samples were analyzed for CPC by HPLC analysis.

Upon completion of the study, the following variables were calculated (weekly body weight, body weight gain, feed intake, feed conversion).

After the 3-week feeding period, all birds were sacrificed by cervical dislocation. All birds from six cages per treatment (half of the total cages per treatment) were dissected (n=24 per treatment). The weights of total breast muscle (*pectoralis major* and *pectoralis minor*), abdominal and leaf fat (combined), liver and spleen were recorded. The remaining half of the birds (n=24 per treatment) also were sacrificed by cervical dislocation and the carcasses were frozen.

Results.

Statistical analysis of the data generated from this trial revealed that feeding CPC at the level of 100 or 250 ppm had no impact on any growth and feed consumption data gathered in this study (Tables 1-4). Total mortality for the study was one bird for the control treatment (0 ppm CPC), 0 for the 100 ppm CPC treatment, and 3 for the 250 ppm CPC treatment. No CPC was detected in any of the blood samples analyzed (Table 6).

The addition of 100 or 250 ppm of CPC to the diet of broilers fed from 2 to 5 weeks of age had no impact on live weight, breast yield, or fat deposition. Liver and spleen weights were not influenced by the treatments applied.

Experimental Data:

Table 1.

Effect of dietary CPC on Broiler body weights
Date: 2/14/2002

	Total Body Weight (g/bird)			
	week			
Treatment	2 (Starting)	3	4	5 (Final)
Control	374	732	1190	1608
100 ppm	379	744	1204	1609
250 ppm	379	756	1188	1600

No significant differences were found between treatments.

Table 2.

Effect of dietary CPC on weight gain/bird
Date: 2/14/2002

	Gain (g/bird)			
	week			
Treatment	2-3	3-4	4-5	Total Gain
Control	357	458	418	1234
100 ppm	365	460	405	1231
250 ppm	377	431	412	1221

No significant differences were found between treatments.

Table 3.

Effect of dietary CPC on broiler feed intake
Date: 2/14/2002

Treatment	Feed Intake (g/bird) week 2-3	3-4	4-5	Total Intake
Control	538	834	781	2166
100 ppm	522	775	766	2070
250 ppm	499	736	769	2061

No significant differences were found between treatments.

Table 4.

Effect of dietary CPC on feed/gain ratio
Date: 2/14/2002

Treatment	feed/gain ratio week 2-3	3-4	4-5	Overall
Control	1.50	1.83	1.87	1.76
100 ppm	1.44	1.69	1.91	1.68
250 ppm	1.35	1.83	1.89	1.69

No significant differences were found between treatments.

Table 5.

Effect of dietary CPC on specific organ weights
Date: 2/14/2002

Treatment	organ weight (g) @ 35 days: total body wt	liver	spleen	breast	fat pad
Control	1635	39.3	1.8	236	25.6
100 ppm	1621	39.5	1.9	234	24.6
250 ppm	1608	41.2	1.8	227	21.5

No significant differences were found between treatments.

Table 6.

CPC analysis of plasma from birds consuming CPC
Date: 2/14/2002

Treatment	Plasma CPC (μg/ml)
Control	N.D.
250 ppm	N.D.

N.D. = not detected

Experimental Methods:

1. Whole blood from wing-vein puncture was collected into heparinized vaccutainer tubes and centrifuged to separate plasma from cellular material.
2. One ml plasma was added to an equal portion of 95% ethanol and vortexed for at least 1 minute.
3. The samples were then centrifuged (12,000 x g) for 15 minutes and the clear supernate was analyzed for CPC by HPLC.

SPRINGDALE
WATER UTILITIES

525 Oak Avenue, P.O. Box 769, Springdale, Arkansas 72765-0769 (501) 751-5751

Dr. Kelly Beers
Safe Foods, Inc.
Rogers, AR

Re: **Toxicity Testing on Cecure**

February 14, 2002

Dear Dr. Beers:

Enclosed is a copy of the bench testing done on the material you certified and provided to Springdale Water Utilities for toxicity testing. As you can see from the results, no loss of nitrification was observed with a "slug" dose of 8 mg/L Cecure.

I understand from conversations with you on February 12, 2002 that you have found some literature showing 50% mortality of nitrifiers caused by 24 mg/L Cecure. In light of your discovery, it is clear that the use of your product would have to be strictly controlled to prevent high concentrations being discharged from overdosing or accidental discharge at facilities where it might be used.

Another area of concern is this product's possible effect on Springdale's wastewater treatment facility's sludge. You indicated that this material binds readily with solids, and all of it would likely end up in the solids portion of the treatment system. Springdale's sludge is treated by anaerobic digestion, then thickened and land applied in a solid or liquid form. It is unknown whether this material would be toxic to the anaerobic digesters, or whether it would have an adverse effect on Springdale's land application process. Springdale Water Utilities does not have the resources available to answer these questions. It appears that your company will have to determine the possible effects of its product on rendered solids and/or sludge. Routing this material through rendering would likely reduce its effect on wastewater treatment systems, but would likely bring up questions about its effect on poultry feed and land application of waste.

Springdale Water Utilities is very excited about your product as a possible alternative to trisodium phosphate currently being used in the poultry processing industry. We are happy to work with your company to determine whether your product has an adverse effect on our treatment system. Please keep us informed of any planned introduction of this product into Springdale's sanitary sewer system, and what you find out about Cecure's effect on solids and sludges.

Sincerely,

J. E. Enos

Jennifer E. Enos
Laboratory Director
Industrial Pretreatment Coord.

JEE/jee

Cc: Harold Hull, Plant Superintendent
Gene Andrews, Plant Manager
Rene Langston, Executive Director
file

Cecure Bench Testing

On Tuesday, February 12, 2002 bench testing was performed at the Springdale Wastewater Treatment Plant to determine the impact of the disinfectant "Cecure" on the nitrification process. Two aquarium tanks were filled with five gallons of mixed liquor collected from the first anoxic zone of the east Bardenpho train. One tank was labeled "control" and the other "sample". The "sample" tank was dosed with 15.2 mg/L of Cecure from a stock 1% solution. To simulate receiving an 8 mg/L "slug" load into the treatment plant. The "control" tank was not dosed. The tanks were aerated to maintain D.O. levels between 5 – 6 mg/L. An initial sample was taken at the start of aeration to determine the base level of ammonia in the mixed liquor. Additional samples were taken after intervals of two and five hours and analyzed for ammonia to track the progress of the nitrification process. The results are given in table 1.

Interval	Control NH3	Sample NH3
Start	3.92	3.92
2 hrs	1.60	1.30
5 hrs	0.02	0.02