Cetylpyridinium Chloride

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
Cetylpyridinium chloride
Cetyl pyridinium chloride
1-hexadecylpyridinium chloride

Other Names:
CPC
Hexadecylpyridinium chloride
Cetylpyridinium chloride monohydrate
Cepacol
Ceprim

Trade Names:
Cecure®

CAS Numbers:
123-03-5
6004-24-6 (monohydrate)

Other Codes:
EC No. 204-593-9
RTECS No. UU4900000
UNII No. 6 BR7T22E2S

Summary of Petitioned Use

The Safe Foods Corporation has petitioned the United States Department of Agriculture (USDA) National Organic Program (NOP) for the addition of cetylpyridinium chloride to the National List as a synthetic substance approved for use in organic processing and handling (USDA 2019). This petition includes the use of cetylpyridinium chloride as an antimicrobial agent in poultry processing. In response to the petition by Safe Foods Corporation, the National Organic Standards Board (NOSB) Materials Subcommittee has requested a technical report on cetylpyridinium chloride.

Characterization of Petitioned Substance

<u>Composition of the Substance:</u>
Cetylpyridinium chloride is an ionic compound made up of a quaternary ammonium cation and a chloride anion, as shown in Figure 1. The quaternary ammonium produces a cation without acidic or basic properties (PC 31239, EFSA 2012). The aromatic nature of the pyridine ring, quaternary ammonium, and saturated hydrocarbon component results in low chemical reactivity for the substance (EFSA 2012). Cetylpyridinium chloride is a white hydroscopic solid that forms a monohydrate when it absorbs water and is commercially available in >95% purity (Fisher 2007, Parchem 2015, Vertellus 2015, USDA 2019, ACS 2021).

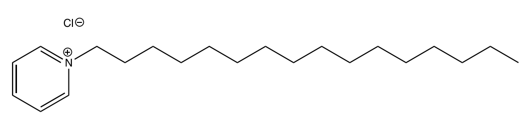
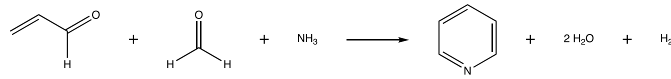


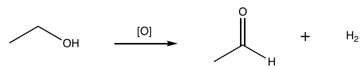
Figure 1. The chemical structure of cetylpyridinium chloride

<u>Source or Origin of the Substance:</u>
Cetylpyridinium chloride is a synthetic substance produced by reacting pyridine and cetyl chloride (1-chlorohexadecane) at an elevated temperature and pressure. The majority of commercial pyridine is produced through the Chichibabin reaction between acrolein, formaldehyde, and ammonia, as shown in Equation 1 (Frank and Seven 1949, Zhang et al. 2020).

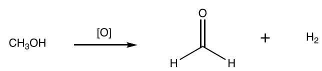


Equation 1

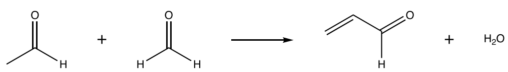
According to the petition, the pyridine for this synthesis is produced exclusively from bioethanol components (USDA 2019). Bioethanol is formed through the fermentation of biomass such as corn or sugarcane to form biologically sourced ethanol (Raynes and Taylor 2021). Once formed, ethanol and methanol can be oxidized to produce acetaldehyde and formaldehyde, respectively, as shown in Equations 2 and 3 (Brucie 2014). Acetaldehyde and formaldehyde can then be combined to produce acrolein, shown in Equation 4, which can be further reacted with formaldehyde and ammonia to form pyridine via the Chichibabin reaction described in Equation 1 (Zhang et al. 2020, Raynes and Taylor 2021).



Equation 2



Equation 3



Equation 4

Cetyl chloride is a synthetic substance that can be formed through many different reaction types, utilizing several organic functional groups. Cetyl chloride is an alkyl halide compound which can be synthesized from hexadecane and chlorine radicals from Cl_2 via a radical mechanism, the hydrohalogenation of 1-hexadecene with hydrochloric acid (HCl), or the activated nucleophilic substation of cetyl alcohol with a chloride (Cl^-) source (Brucie 2014).

<u>Properties of the Substance:</u>

General properties of cetylpyridinium chloride and cetylpyridinium chloride monohydrate are listed in Table 1. The NOP petition and literature sources do not differentiate between the anhydrous and monohydrate forms listed below and reference only cetylpyridinium chloride. Both the anhydrous and monohydrate forms are listed in Table 1 due to the hygroscopic nature of cetylpyridinium chloride, which are likely to convert anhydrous forms to the hydrate form.

Table 1. Properties of cetylpyridinium chloride

Property	Cetylpyridinium chloride	Cetylpyridinium chloride monohydrate
Molecular formula	$C_{21}H_{38}NCl$	$C_{21}H_{38}NCl \bullet H_2O$
Molecular weight	339.98 g/mol	358.01 g/mol
CAS No.	123-03-5	6004-24-6
Physical appearance	White powder	White crystals or powder
Odor	Slight pyridine odor	Slight pyridine odor
Solubility	68 g/L	Soluble in water, short chain alcohols, and chloroform. Slightly soluble in benzene and ether.
Melting point	77 °C	80–84 °C
Decomposition temperature	234 °C	234 °C
pH	5.2 (10 g/L H_2O)	4.0–6.0 (in 10% aqueous solution)

Sources: PC 31239, Fisher 2007, Parchem 2015, Vertellus 2015, SF 2019, ACS 2021, ECHA 2021

Specific Uses of the Substance:

Cetylpyridinium chloride is most commonly used as an antiseptic, an antimicrobial agent, and a disinfectant, although it is also used as a surfactant and detergent (PC 31239, Bosilevac et al. 2004, Lim and Mustapha 2004, ACS 2021, Nasila et al. 2021). Cetylpyridinium chloride is included as an antimicrobial ingredient in many oral hygiene products, including toothpaste, mouthwash, and lozenges (Cutter et al. 2000, Sreenivasan et al. 2012, Herrera et al. 2018, USDA 2019, ACS 2021, Nasila et al. 2021). Within these products, cetylpyridinium chloride has been reported to reduce plaque formation and gingivitis (Cutter et al. 2000, Sreenivasan et al. 2012, Herrera et al. 2018, Nasila et al. 2021).

Cetylpyridinium chloride is petitioned for use as an antimicrobial agent in the processing of poultry products, including raw poultry carcasses and parts. Cetylpyridinium chloride is used as an antimicrobial agent in the processing and packaging of a variety of foods, including poultry, beef, ground meats, hides, fruits, and vegetables, in conventional agricultural production (Bosilevac et al. 2004, Lim and Mustapha 2004, Moore et al. 2017, Saucedo-Alderete et al. 2017, Zhang et al. 2019, Massey et al. 2020). Within food processing, cetylpyridinium chloride is most frequently used to disinfect *Salmonella* and *Campylobacter*, although it has been shown to be effective as a broad-spectrum antimicrobial agent (Cutter et al. 2000, Bosilevac et al. 2004, Lim and Mustapha 2007, Sreenivasan et al. 2012, Thanissery et al. 2012, Thames and Sukumaran 2020, Nasila et al. 2021). In meat processing, cetylpyridinium chloride is applied as a pre- or post-chiller immersion treatment and may be applied as a dip, mist, spray, or drench (Kim and Slavik 1995, Lim and Mustapha 2004, Singh et al. 2005, Lim and Mustapha 2007, EFSA 2012, Scott et al. 2015, USDA 2019, Zhang et al. 2019, Massey et al. 2020, FSIS 2021a).

Cetylpyridinium chloride has been applied to cattle hides preslaughter in beef processing as an intervention against *Escherichia coli (E. coli)* and *Enterobacteriaceae* (Bosilevac et al. 2004). In this study, cetylpyridinium chloride was applied to cattle immediately before stunning the animals, although Bosilevac et al. state that the intervention would likely be more effective if applied post-stun to eliminate bruising associated with the additional pre-stun application and the deactivation of the spray by additional organic matter.

Approved Legal Uses of the Substance:

The USDA Food Safety and Inspection Service (FSIS) has designated cetylpyridinium chloride as a "safe and suitable ingredient used in the production of meat and poultry products." It is a "chemical intervention that can be used to potentially reduce *Salmonella* in poultry products during processing (post-chill)…without additional approval if used as detailed in the directive [FSIS guidelines]" (FSIS 2021a, FSIS 2021b).

The United States Food and Drug Administration (FDA) has approved the use of cetylpyridinium chloride "as an antimicrobial agent…to treat the surface of raw poultry carcasses" (21 CFR 173.375). The FDA requires cetylpyridinium chloride to be combined with polyethylene glycol, which must be included "at a concentration of 1.5 times that of cetylpyridinium chloride." When used as an antimicrobial additive in poultry processing, the FDA has outlined its use in §173.375:

> (1) As a fine mist spray of an ambient temperature aqueous solution applied to raw poultry carcasses prior to immersion in a chiller, at a level not to exceed 0.3 gram cetylpyridinium chloride per pound of raw poultry carcass, provided that the additive is used in systems that collect and recycle solution that is not carried out of the system with the treated poultry carcasses; or
>
> (2) As a liquid aqueous solution applied to raw poultry carcasses either prior to or after chilling at an amount not to exceed 5 gallons of solution per carcass, provided that the additive is used in systems that recapture at least 99 percent of the solution that is applied to the poultry carcasses. The concentration of cetylpyridinium chloride in the solution applied to the carcasses shall not exceed 0.8 percent by weight. When the application of the additive is not followed by immersion in a chiller, the treatment will be followed by a potable water rinse of the carcass.

The United States Environmental Protection Agency (EPA) has listed cetylpyridinium chloride as a pesticide active ingredient, which can be removed from manufacturing effluent with activated carbon (40 CFR 455.67). The EPA is currently reviewing cetylpyridinium chloride for use in pesticides and closed public comments on December 18, 2020 (EPA 2020).

<u>Action of the Substance:</u>

When used as petitioned, cetylpyridinium chloride is an antimicrobial agent. It deactivates bacteria through disruptions to the membrane structure (Lim and Mustapha 2007, Saucedo-Alderete et al. 2017, Yegin et al. 2019). The hydrocarbon tail of the substance facilitates the rearrangement of membrane lipids and results in the leakage or rupture of bacterial membranes (PC 31239, Lim and Mustapha 2007, Saucedo-Alderete et al. 2017, Yegin et al. 2019, Nasila et al. 2021). Cetylpyridinium chloride treatments have been shown to reduce bacterial populations across several food products in inoculated studies (Cutter et al. 2000, Bosilevac et al. 2004, Lim and Mustapha et al. 2004, Singh et al. 2005, Moore et al. 2017, Zhang et al. 2019). Once initial bacterial populations have been reduced, cetylpyridinium-chloride-treated meat products have been shown to maintain reduced bacterial populations when stored between 14 and 42 days (Cutter et al. 2000, Singh et al. 2005).

While cetylpyridinium chloride is recognized as a broad-spectrum antimicrobial, it has been reported to be more effective against Gram-positive bacteria, such as *Salmonella, Listeria monocytogenes* (*L. monocytogenes*), and *Staphylococcus aureus* (*S. aureus*). Gram-positive bacteria have membrane surfaces that bear a negative charge, improving the efficacy of cetylpyridinium binding (Cutter et al. 2000, Lim and Mustapha 2004, Lim and Mustapha 2007, Yegin et al. 2019, Nasila et al. 2021). The positively charged pyridinium portion of the substance binds to the negatively charged bacterial membrane through electrostatic interactions. The electrostatic attraction improves the ability of the substance to rearrange membrane lipids (Cutter et al. 2000, Lim and Mustapha 2007, Yegin et al. 2019). Additionally, the binding of the positively charged pyridinium portion of the substance disrupts membrane function and bacterial metabolism, which may deactivate bacteria without rearrangement of the membrane structure (Kim and Slavik 1995, Cutter et al. 2000, Yegin et al. 2019, Nasila et al. 2021).

Contamination of meat products (both beef and poultry) is most likely to occur before slaughter and processing (Bosilevac et al. 2004, Saucedo-Alderete et al. 2017, Thames and Sukumaran 2020). When used on cattle hides at the outset of the slaughter process, either before or after stunning, cetylpyridinium chloride has been reported to reduce bacterial populations by 20–80%. Adoption of cetylpyridinium chloride within the slaughter process may reduce or eliminate bacterial populations before processing occurs, which would further reduce cross-contamination within processing (Bosilevac et al. 2004).

When used as a surfactant or detergent, cetylpyridinium chloride enables the dissolution of insoluble compounds (Silberberg 2003, Nasila et al. 2021). In surfactant applications, the pyridinium portion of the compound interacts with water and other polar compounds, while the hydrocarbon portion interacts with non-polar compounds. Additionally, since cetylpyridinium chloride is neither an acid nor a base, it is able to maintain its function as a surfactant across a broad range of solution pH (Nasila et al. 2021).

Combinations of the Substance:

When used as petitioned in food processing applications, cetylpyridinium chloride must be combined with propylene glycol at 1.5 times the concentration of cetylpyridinium chloride (EFSA 2012, USDA 2019, Massey et al. 2020, FSIS 2021a). Propylene glycol acts as a stabilizer and solubility enhancer for cetylpyridinium chloride (USDA 2019, Massey et al. 2020). Additionally, the use of propylene glycol reduces the absorption of cetylpyridinium chloride into treated poultry products. The petition also states that all propylene glycol used in cetylpyridinium chloride formulations is produced from renewable resources, including vegetable oil and glycerin byproducts from biodiesel manufacturing (USDA 2019).

The FDA has designated propylene glycol as a direct food substance that is generally recognized as safe (GRAS) (21 CFR 184.1666) and as a GRAS general food additive (§582.1666). According to FDA regulations, the concentration of propylene glycol must be 1.5 times that of cetylpyridinium chloride in antimicrobial formulations (21 CFR 173.375). Concentrated formulations of cetylpyridinium chloride, such as Cecure®, are sold as 40% cetylpyridinium chloride and 60% propylene glycol (Vertellus 2015, SF 2019, Massey et al. 2020). The concentrated solution is diluted with water to give cetylpyridinium chloride concentrations of ≤1.0% for use in the processing and handling of food products (EFSA 2012, USDA 2019).

Status

Historic Use:

Historically, cetylpyridinium chloride has not been used in organic agriculture production. However, it has been a component of oral hygiene products since the 1950s and remains a common active ingredient in toothpaste, mouthwash, and lozenges (Cutter et al. 2000, Sreenivasan et al. 2012, Herrera et al. 2018, ACS 2021, Nasila et al. 2021). As described in the "Specific Uses of the Substance" section, cetylpyridinium chloride is an antimicrobial agent commonly used in food processing, including poultry, beef, ground meats, and raw produce, since the 1990s (Bosilevac et al. 2004, Lim and Mustapha 2004, Moore et al. 2017, Saucedo-Alderete et al. 2017, Zhang et al. 2019, Massey et al. 2020, Nasila et al. 2021). It is frequently used as a disinfectant against *Salmonella* and *Campylobacter*, although it has been reported to be a broad-spectrum antimicrobial (Cutter et al. 2000, Bosilevac et al. 2004, Lim and Mustapha 2004, Moore et al. 2017, Zhang et al. 2019, Thames and Sukumaran 2020, Nasila et al. 2021).

Organic Foods Production Act, USDA Final Rule:

Cetylpyridinium chloride is not listed in the Organic Foods Production Act (OFPA) of 1990 or the USDA organic standards (7 CFR 205).

International

Canada, Canadian General Standards Board—CAN/CGSB-32.311-2015, Organic Production Systems Permitted Substances List

Cetylpyridinium chloride is not listed in the Canadian Organic Production Systems Permitted Substances List.

CODEX Alimentarius Commission—Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)
Cetylpyridinium chloride is not listed in the CODEX.

European Economic Community (EEC) Council Regulation—EC No. 834/2007 and 889/2008
Cetylpyridinium chloride is not listed in EC No. 834/2007 or EC No. 889/2008.

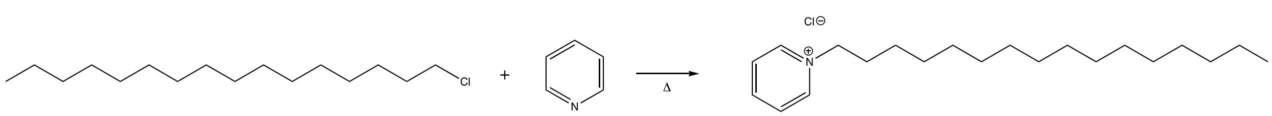
Japan Agricultural Standard (JAS) for Organic Production
Cetylpyridinium chloride is not listed in the JAS for Organic Production.

International Federation of Organic Agriculture Movements (IFOAM)
Cetylpyridinium chloride is not listed in the IFOAM NORMS for Organic Production and Processing.

Evaluation Questions for Substances to be used in Organic Handling

<u>Evaluation Question #1:</u> Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502(21)).

Cetylpyridinium chloride is synthesized by a nucleophilic substitution reaction between cetyl chloride and pyridine at elevated temperatures and pressures. As described in Equation 5, the pyridine nitrogen displaces the chlorine in cetyl chloride, which gives a chemical change through the formation of a new bond with carbon, to produce a quaternary ammonium ion and a chloride ion. Both pyridine and cetyl chloride are liquids, and the reaction is completed neat, with a slight excess of pyridine. Once the reaction reaches completion, the product is purified with activated carbon and recrystallized with a mixed solvent methyl ethyl ketone and alcohol system (PC 31239).



Equation 5

As discussed above in the "Source or Origin of the Substance" section, the petition states that all cetylpyridinium chloride for their poultry-processing formulations uses pyridine manufactured from bioethanol sources (USDA 2019). The primary method of pyridine production is the Chichibabin reaction (Equation 1). Cetyl chloride can be produced through several synthetic methods, although the petition does not describe the primary source of cetyl chloride used in cetylpyridinium chloride production.

<u>Evaluation Question #2:</u> Discuss whether the petitioned substance is formulated or manufactured by a chemical process or created by naturally occurring biological processes (7 U.S.C. § 6502(21)). Discuss whether the petitioned substance is derived from an agricultural source.

As described in Evaluation Question #1, cetylpyridinium chloride is produced through a nucleophilic substitution reaction between cetyl chloride and pyridine, which is a chemical process resulting in a synthetic substance. Cetylpyridinium chloride is not created through natural biological processes and is not derived from agricultural sources.

<u>Evaluation Question #3:</u> If the substance is a synthetic substance, provide a list of non-synthetic or natural source(s) of the petitioned substance (7 CFR 205.600(b)(1)).

As described in Evaluation Questions #1 and #2, cetylpyridinium chloride is a synthetic substance that does not exist in nature. There are no natural or non-synthetic alternative sources of cetylpyridinium chloride.

<u>Evaluation Question #4:</u> Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to the FDA's good manufacturing practices (7 CFR 205.600(b)(5)). If not categorized as GRAS, describe the regulatory status.

The FDA has not categorized cetylpyridinium chloride as a GRAS substance. The FDA received an application from the Safe Foods Corporation in 1999 to apply for GRAS status for cetylpyridinium chloride "as an antimicrobial treatment in various types of raw and fully cooked food products that may include poultry, red meat, fish and shellfish, eggs, fruits, vegetables, cereal grains, nutmeats and dairy products at a level not to exceed 1 percent." In response to this petition, the FDA designated it as GRAS notice number GRN 000031. However, the GRAS petition for GRN 000031 was later withdrawn by the Safe Foods Corporation (FDA 2000a).

The Safe Foods Corporation submitted another petition for GRAS status for cetylpyridinium chloride "as an antimicrobial treatment in various types of raw and fully cooked food products, including meat and poultry products at a level not to exceed 1 percent." In response to this petition, the FDA designated it as GRAS notice number GRN 000038. Upon review, the FDA determined that the petition did not supply sufficient basis for cetylpyridinium chloride to receive GRAS status and therefore ceased to process GRN 000038 (FDA 2000b).

<u>Evaluation Question #5:</u> Describe whether the primary technical function or purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR 205.600(b)(4)).

The primary function of the substance is as an antimicrobial agent in food processing applications. As described in the "Action of the Substance" section, cetylpyridinium chloride deactivates bacteria through disruptions to their membrane structure and/or function. While the application of cetylpyridinium chloride reduces bacterial populations, it does not act as a preservative in poultry production.

<u>Evaluation Question #6:</u> Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR 205.600(b)(4)).

Cetylpyridinium chloride is not used to recreate or improve flavors, colors, textures, or nutritive values lost during processing. The chemical stability of cetylpyridinium chloride results in its use having no significant effects on these values in treated food products (Lim and Mustapha 2004, Singh et al. 2005, Lim and Mustapha 2007, Scott et al. 2015, Moore et al. 2017, Saucedo-Alderete et al. 2017, Nasila et al. 2021).

<u>Evaluation Question #7:</u> Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR 205.600(b)(3)).

When used as petitioned, cetylpyridinium chloride does not affect the nutritional quality of treated food or feed (Nasila et al. 2021). As described in Evaluation Question #6, the chemical stability of cetylpyridinium chloride does not result in any significant changes to the composition or nutritive values of treated foods.

<u>Evaluation Question #8:</u> List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR 205.600(b)(5)).

There are no reports of heavy metals in cetylpyridinium chloride. Pyridine left over from the production process (Equation 5) has been reported to be a possible contaminant. However, literature reports show the absence of pyridine at the instrumental detection limit (1 mg/L), even when the substance is subjected to elevated temperature (95°C) (EFSA 2012).

Evaluation Question #9: Discuss and summarize findings on whether the manufacture and use of the petitioned substance may be harmful to the environment or biodiversity (7 U.S.C. § 6517(c)(1)(A)(i) and 7 U.S.C. § 6517(c)(2)(A)(i)).

When used as petitioned, cetylpyridinium chloride is not expected to be released into the environment. Cetylpyridinium chloride solutions are recycled across applications throughout daily use, where >99% of the solution is recovered (EFSA 2012). Recycling cetylpyridinium chloride solutions has been reported to have no impact on the efficacy of the treatment and has not been reported to increase antimicrobial resistance (EFSA 2012, Cadena et al. 2019, Massey et al. 2020). Once the use has been completed (usually at the end of the day), the cetylpyridinium chloride solution is removed from processing effluent by filtration through activated carbon (Massey et al. 2020). Activated carbon is also an EPA-approved method of removing cetylpyridinium chloride (40 CFR 455.67). The cetylpyridinium chloride captured in the activated carbon is disposed of in the appropriate landfill facility or is incinerated (Massey et al. 2020).

If improperly used and released into the environment, cetylpyridinium chloride poses an environmental risk, especially to aquatic environments. Cetylpyridinium chloride is highly toxic to fish, crustaceans, molluscs, and other aquatic life, as shown in the toxicological data in Table 2 (Vallejo-Freire et al. 1954, Liao et al. 1990, Fisher 2007, Parchem 2015, ECOTOX 2021). The environmental toxicity of cetylpyridinium chloride is low for terrestrial plants and birds, with orchids showing growth inhibition and mortality at levels from 10–1000 ppm, and the northern bobtail quail having an LD_{50} of 175.6 mg/kg (Ernst et al. 1971, ECOTOX 2021).

Table 2. Aquatic toxicity values of cetylpyridinium chloride

Species	Endpoint	Concentration
Australorbis sp. (snail)	Lethal	5 mg/L
Penaeus monodon (jumbo tiger prawn)	LC_{50}	0.8 mg/L
Penaeus japonicus (Kuruma shrimp)	LC_{50}	3.1 mg/L
Penaeus semisulcatus (shrimp)	LC_{50}	1 mg/L
Fenneropenaeus penicillatus (redtail prawn)	LC_{50}	0.56 mg/L
Metapenaeus ensis (greasyback shrimp)	LC_{50}	2.1 mg/L
Macrobrachium rosenbergii (great river prawn)	LC_{50}	0.13 mg/L
Oncorhynchus mykiss (rainbow trout)	LC_{50}	0.15 mg/L
Cyprinus carpio (carp)	Not reported	0.01 mg/L
Daphnia magna (water flea)	EC_{50}	0.00736 mg/L

Sources: Vallejo-Freire et al. 1954, Liao et al. 1990, Fisher 2007, Parchem 2015, ECOTOX 2021

Quaternary ammonium salts have been reported to sorb in soils, especially when the soils contain sediments, sludges, or clays (PC 31239, Timmer et al. 2020). Cetylpyridinium chloride is expected to undergo rapid biodegradation in soil environments and has an environmental half-life of 9.7 days with studies showing 70.7% mineralization after 28 days (Timmer et al. 2020, ECHA 2021). Soils with high cation exchange capacity (CEC) are expected to produce more rapid biodegradation outcomes. Due to the bactericidal character of cetylpyridinium chloride, it may deactivate or inhibit the soil microbes that are responsible for its breakdown. However, when a soil has high CEC character, cetylpyridinium chloride becomes less available in the soil, which has been reported to reduce microbial inhibition and promote faster environmental degradation (Timmer et al. 2020).

As described in Evaluation Questions #1 and #8, a slight excess of pyridine is used in the production of cetylpyridinium chloride, making it a possible contaminant. The environmental fate of pyridine is determined by environmental conditions. Acidic soils (pH < 5) will convert pyridine to pyridinium, which increases adsorption onto mineral and clay surfaces. However, when applied to alkaline soils, adsorption was reported to be negligible. Pyridine is highly water soluble and may leach into water systems. Low concentrations of pyridine (< 20 mg/L) are expected to biodegrade in water and soil systems within 8 days and have not been shown to bioaccumulate (ATSDR 1998).

<u>**Evaluation Question #10:**</u> **Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517(c)(1)(A)(i), 7 U.S.C. § 6517(c)(2)(A)(i)) and 7 U.S.C. § 6518(m)(4)).**

There are limited data on the effects of cetylpyridinium chloride on human health (EWG, Flomenbaum et al. 2002, ACS 2021). Cetylpyridinium chloride has been reported to cause eye and skin irritation upon contact and may cause nausea and vomiting if ingested (EWG, PC 31239, Flomenbaum et al. 2002, Nasila et al. 2021).

Cetylpyridinium chloride is petitioned for use at low concentrations (≤ 1.0%) and is followed by immersion in a chiller solution or a potable water wash when used in post-chiller applications, as stipulated by FDA regulations (21 CFR 173.375). The continued processing and chiller immersion, or water wash, is expected to remove the majority of cetylpyridinium chloride from treated surfaces. However, residual cetylpyridinium chloride has been detected on treated surfaces at the processing endpoint and is expected to be found in concentrations of 2.9–25.9 mg/kg on poultry skin. The maximum reported concentration of 25.9 mg/kg found on the meat surface would result in an average concentration of 2.3 mg/kg of cetylpyridinium chloride on treated meat. However, cetylpyridinium chloride was not found in non-surface meat, with detection levels of 0.19 μg/g. Therefore, the use of cetylpyridinium chloride as petitioned is not expected to pose safety concerns for humans (EFSA 2012).

Concentrated cetylpyridinium chloride, however, is expected to be toxic to humans (PC 31239, Nasila et al. 2021). Cetylpyridinium chloride has been identified as a hazardous substance according to the Global Harmonized System of Classification and Labeling of Chemicals (GHS), as described in Table 3.

Table 3. GHS classification of cetylpyridinium chloride

Hazard class	Hazard statement	Pictogram
Acute toxicity, oral, category 4	H302—Harmful if swallowed	
Skin corrosion/irritation, category 2	H315—Causes skin irritation	
Serious eye damage/eye irritation, category 1	H318—Causes serious eye damage	
Acute toxicity, inhalation, category 2	H330—Fatal if inhaled	
Specific target organ toxicity, single exposure, respiratory tract irritation, category 3	H335—May cause respiratory irritation	

Sources: Parchem 2015, Vertellus 2015, SF 2019, ACS 2021

<u>**Evaluation Question #11:**</u> **Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518(m)(6)).**

Irradiation of food products offers an alternative intervention against bacterial infections and is regarded among the most effective bacterial inactivation methods. There are multiple methods of inactivation by irradiation, including ionizing radiation, ultraviolet radiation, and pulsed-light radiation. Radiative methods have broad-spectrum antimicrobial character and leave no chemical residues on treated food products (Ramos et al. 2013, Meireles et al. 2016). While irradiation provides an alternative to cetylpyridinium chloride within conventional agriculture, it is not allowed as an antimicrobial treatment within organic agricultural production.

<u>**Evaluation Question #12:**</u> **Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517(c)(1)(A)(ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518(m)(6)).**

Non-synthetic alternatives

There are relatively few non-synthetic alternatives to cetylpyridinium chloride, mainly water and organic acids. In addition to non-synthetic organic acids, synthetic organic acids have the same mode of action and antibacterial properties. Both water washes and organic acids are discussed in more detail below.

Water

Water washes provide an alternative bacterial disinfection protocol. Water is a desirable alternative to cetylpyridinium chloride as it does not need to be manufactured, results in no additional chemical inputs, and does not contribute to negative environmental outcomes. The efficacy of water washes varies based on the treated substance and type of bacteria. Lim and Mustapha (2007) reported significant reductions in bacterial populations of *E. coli* and *L. monocytogenes* on roast beef with water washes, although water washes resulted in increased *S. aureus* populations.

Water washes have been reported to be less effective than cetylpyridinium chloride and other chemical antimicrobial interventions for bacterial disinfection. Additionally, the lack of an active antimicrobial agent results in the increased potential for cross-contamination (Lim and Mustapha 2007).

As described in the "Properties of the Substance" section, cetylpyridinium chloride is chemically stable and is not prone to decomposition in long-term storage or at elevated temperatures (USDA 2008, EFSA 2012, USDA 2015, USDA 2016, Saucedo-Alderete et al. 2017, USDA 2018). Besides water, all antimicrobial alternatives are more chemically reactive than cetylpyridinium chloride. Therefore, they are more susceptible to breakdown during storage prior to their application than cetylpyridinium chloride (Saucedo-Alderete et al. 2017).

Organic acids

There are several organic acids that may be used as an alternative antimicrobial treatment, including citric acid, lactic acid, and tartaric acid (Moore et al. 2017, Sawyer and Stockel 2020, FSIS 2021a). Organic acids are able to penetrate cellular membranes and decrease the pH of the cytoplasm, which disrupts proton pumps and cellular function (Moore et al. 2017).

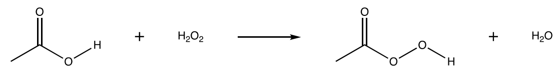
The acidic nature of these compounds results in higher chemical activity compared to cetylpyridinium chloride. The acidic environment may result in oxidation of food products, which may lead to lower quality meats, reduced nutritional quality, and changes to food colors and textures (Moore et al. 2017, Nasila et al. 2021). Unlike cetylpyridinium chloride, organic acids reduce the surface pH of food products following treatment (Moore et al. 2017). Organic acids have a reduced inhibitory effect against *L. monocytogenes* than cetylpyridinium chloride when stored for longer than 40 days (Singh et al. 2005).

Synthetic alternatives

In addition to the non-synthetic substances discussed above, there are several synthetic substances that have been approved for organic handling and processing (7 CFR 205.605). These substances are discussed in greater detail below.

Peroxyacetic acid

Peroxyacetic acid (also known as peracetic acid) is a mixture of acetic acid and hydrogen peroxide, which react *in situ* to form the acidic oxidant, as described in Equation 6 (USDA 2016, Moore et al. 2017, FSIS 2021a). Peroxyacetic acid may be applied as both a pre- and post-chill treatment as a spray or dip, with concentrations up to 2000 ppm (Thames and Sukumaran 2020, FSIS 2021a). Unlike organic acids, the primary mode of action for peroxyacetic acid is oxidation of the membrane structure. Additionally, the substance is also able to penetrate into the cytoplasm and denature protein structure due to its acidic nature (USDA 2016).



Equation 6

Peroxyacetic acid is an effective broad-spectrum antimicrobial agent that has shown greater efficacy against Gram-negative bacteria (e.g., *E. coli*) than cetylpyridinium chloride (Scott et al. 2015). In addition to initial reductions of bacterial populations, Scott et al. reported that peroxyacetic acid continued to reduce bacterial populations for 24 hours after the initial treatment. Cadena et al. (2019) considered peroxyacetic acid to be the best commonly used antimicrobial agent due to its broad-spectrum efficacy, while producing the smallest number of bacterial mutations.

The acidic and oxidizing nature of peroxyacetic acid results in higher chemical activity compared to cetylpyridinium chloride. The highly oxidizing nature of peroxyacetic acid makes it susceptible to deactivation when interacting with organic matter, which undergoes background oxidation (Moore et al. 2017). Like with organic acid disinfectants, the acidic environment may result in oxidation of food products, which may lead to lower quality meats, reduced nutritional quality, and changes to food colors and textures (Scott et al. 2015). However, Moore et al. (2017) reported that short applications of peroxyacetic acid (20 seconds at 0.1%) did not result in significant changes to color. Unlike cetylpyridinium chloride, peroxyacetic acid results in reduced surface pH following treatment (Moore et al. 2017, Nasila et al. 2021).

Acidified sodium chlorite

Aqueous acidified sodium chlorite is an antimicrobial agent used in red meat and poultry processing (Lim and Mustapha 2004, USDA 2008). Acidified sodium chlorite is an aqueous mixture of the ionic compound sodium chlorite and an acid, including sulfuric, hydrochloric, and citric acids, which produce chlorous acid as the active ingredient. Like peroxyacetic acid, the primary mode of action is the oxidation of bacterial membranes, making it a broad-spectrum antimicrobial (USDA 2008). Aqueous chlorine dioxide may also be produced in solution, which contributes as an additional oxidizing agent (Lim and Mustapha 2004, USDA 2008).

Acidified sodium chlorite may be applied as a spray or dip, with concentrations up to 1200 ppm (Thames and Sukumaran 2020, FSIS 2021a). Acidified sodium chlorite requires low pH values between 2.3 and 3.2 in order to be an effective disinfectant (Lim and Mustapha 2004, FSIS 2021a).

Lim and Mustapha (2004) reported that acidified sodium chlorite was more effective at reducing the populations of *E. coli* than cetylpyridinium chloride. However, acidified sodium chlorite was less effective than cetylpyridinium chloride against Gram-positive bacteria (e.g., *L. monocytogenes*, *S. aureus*), although it resulted in significant reductions in Gram-positive bacterial populations as well. The highly oxidizing nature of acidified sodium chlorite makes it susceptible to deactivation when interacting with organic matter, which undergoes background oxidation (Moore et al. 2017).

Saucedo-Alderete et al. (2017) reported that acidified sodium chlorite was less effective at reducing bacterial populations on rough surfaces (e.g., raw cantaloupe) than cetylpyridinium chloride. The acidic requirement for effective treatment results in higher chemical activity compared to cetylpyridinium chloride. Like with peroxyacetic acid and other organic acid disinfectants, the acidic environment may result in oxidation of food products, which can lead to lower quality meats, reduced nutritional quality, and changes to food colors and textures (Lim and Mustapha 2004, Lim and Mustapha 2007, Nasila et al. 2021). Unlike cetylpyridinium chloride, acidified sodium chlorite results in reduced surface pH following treatment (Lim and Mustapha 2004, Lim and Mustapha 2007).

Chlorine materials

Chlorine materials include chlorine dioxide and hypochlorous acid. As described above, chlorine dioxide is formed by the reaction of an acid, usually hydrochloric acid, with sodium chlorite, and may be applied as an aqueous or gaseous antimicrobial intervention (USDA 2008, USDA 2018). Hypochlorous acid and chlorine dioxide deactivate bacteria through oxidative processes that disrupt cellular membranes and inhibit bacterial respiratory and metabolic functions (USDA 2015, USDA 2018).

The highly oxidizing nature of chlorine materials makes them susceptible to deactivation when interacting with organic matter, which undergoes background oxidation (Moore et al. 2017, USDA 2018). Chlorine antimicrobials require acidic pH to be effective and are deactivated in basic solutions (Moore et al. 2017, Thames and Sukumaran 2020). Additionally, chlorine-based antimicrobial agents have been reported to have a high incidence of antimicrobial resistance (Thames and Sukumaran 2020).

Evaluation Information #13: Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR 205.600(b)(1)).

There are no organic agricultural products that are alternatives to cetylpyridinium chloride.

Report Authorship

The following individuals were involved in research, data collection, writing, editing, and/or final approval of this report:

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All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

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