Chlorhexidine

Livestock

Identification of Petitioned Substance

Chemical Names:
1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanidine

CAS Numbers:
55-56-1 (Chlorhexidine), 56-95-1 (Chlorhexidine diacetate), 18472-51-0 (Chlorhexidine gluconate)

Other Name:
Chlorhexidine diacetate, Chlorhexidine gluconate, Chlorhexidine hydrochloride

Other Codes:
200-238-7 (EINECS, Chlorhexidine)

Trade Names:
Nolvasan®, Cougar, Mint-A-Kleen®

Summary of Petitioned Use

The National Organic Program (NOP) final rule currently allows the use of chlorhexidine in organic livestock production under the corresponding synthetic substances list (7 CFR 205.603(a)(6)). According to this rule, chlorhexidine is allowed for surgical procedures conducted by a veterinarian, and is allowed for use as a teat dip when alternative germicidal agents and/or physical barriers have lost their effectiveness. This report provides updated and targeted technical information to augment the 2010 Technical Advisory Panel Report on chlorhexidine in support of the National Organic Standards Board's review of the substance under the sunset process.

Characterization of Petitioned Substance

Composition of the Substance:

Chlorhexidine is a member of the bisbiguanide class of chemicals, which are known for their bactericidal properties. When used in commercial pesticide products, chlorhexidine is commonly formulated as its diacetate, digluconate and dihydrochloride salts (US EPA, 2011a). Accordingly, one equivalent of chlorhexidine is treated with two equivalents of D-gluconic acid, hydrochloric acid or acetic acid to generate the commercially relevant chlorhexidine substance (Figure 1). With the molecular formula of $C_{22}H_{20}Cl_2N_{10}$, chlorhexidine is a synthetic compound composed of carbon, hydrogen, chlorine and nitrogen atoms. The structure of chlorhexidine consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain (Greenstein, 1986).

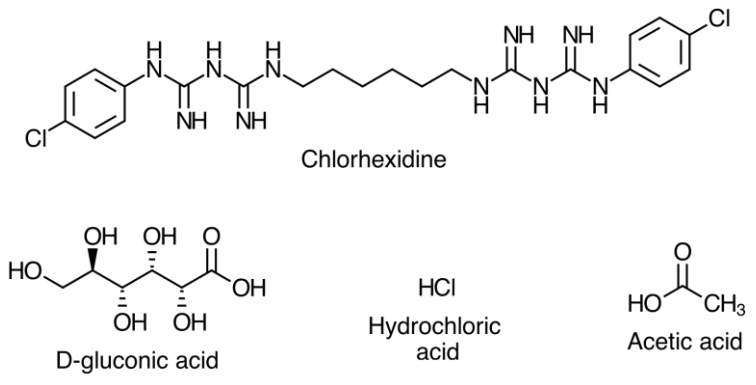


Figure 1. Structural formulas for Chlorhexidine, D-gluconic acid, and Acetic acid.

Source or Origin of the Substance:

Limited information is available regarding the manufacture of chlorhexidine for use in commercially available disinfectants, sanitizers, bactericides and virucides. The general procedure for industrial-scale chlorhexidine production involves initial synthesis of the 1,6-hexamethylenebis(dicyandiamide) intermediate followed by reaction of the intermediate with 4-chloroaniline hydrochloride (Güthner, 2006; Werle, 2013). Once purified, chlorhexidine is combined with acetic acid or D-gluconic acid to generate the commercially relevant diacetate or digluconate salts of chlorhexidine.

Properties of the Substance:

Chlorhexidine exists as a white to yellowish powdery solid with no distinct odor. A summary of the available chemical and physical properties of chlorhexidine is provided below in Table 1.

Table 1. Chemical and Physical Properties of Chlorhexidine.

Property	Description
Color	White to yellow
Physical state	Solid
Odor	Odorless
Molecular formula	$C_{22}H_{30}Cl_2N_{10}$
Molecular weight (g/mol)	505.45 (Chlorhexidine), 625.55 (Chlorhexidine diacetate), 897.8 (Chlorhexidine digluconate)
Melting point (°C)	134
Water solubility (mg/L) at 20 °C	800
Dissociation constant (pKa) at 25 °C	10.78
Octanol/water partition coefficient at pH 5.0 (K_{ow})	0.08
Soil organic carbon-water partition coefficient (K_{oc})	26
Vapor pressure at 25 °C (mm Hg)	2.0×10^{-14}
Henry's Law Constant at 25 °C (atm•m³/mol)	1.6×10^{-17}

Data sources: US EPA, 2011a; HSDB, 2004.

Specific Uses of the Substance:

Chlorhexidine is used in a variety of contexts, ranging from livestock production in agriculture to dentistry and home disinfection. This report focuses on the use of chlorhexidine as a bactericide in teat dip solutions to control and prevent mastitis in milk producing animals. Additional uses of chlorhexidine as a general disinfectant in agricultural, dental, surgical, residential and public settings are briefly described.

All of the established agricultural uses of chlorhexidine rely on the antimicrobial properties of the substance. In particular, chlorhexidine is used "for dipping teats as an aid in controlling bacteria that causes mastitis" both before and after milking in both conventional and organic production (Zoetis Inc, 2014). Chlorhexidine is effective against a broad array of pathogenic microorganisms, including the Gram-negative bacterium *Escherichia coli* and Gram-positive bacteria *Streptococcus agalactiae* and *Staphylococcus aureus*, associated with mastitis infections in dairy animals (Nickerson, 2001). USDA organic regulations permit the use of chlorhexidine-based teat dips "when alternative germicidal agents and/or physical barriers have lost their effectiveness" (7 CFR 205.603(a)(6)). Chlorhexidine solutions are occasionally applied via intramammary infusions to induce cessation of lactation in chronically infected mammary gland quarters in conventional dairies. When applied in this manner, the objective is to avoid milking that quarter for at least the remainder of the present lactation period (Smith, 2005).

In veterinary medicine, chlorhexidine is used as a general-purpose disinfectant for cleansing wounds, skin, instruments and equipment (EMA, 1996; OSU, 2015). These medical disinfectants are generally applied as dilute solutions of chlorhexidine gluconate in water at a concentration of approximately 1.5%

weight/volume (EMA, 1996). The skin of medical patients—including humans, pets and livestock—is a major source of pathogens that cause surgical-site infection (Darouiche, 2010). Specifically, most wound infections are caused by the host commensal bacteria, such as *Staphylococcus*, *Streptococci* and *Bacillus* species, which migrate to the skin surface during surgery (Evans, 2009). Cleansing products containing the active ingredients chlorhexidine (e.g., chlorhexidine digluconate) and iodine (e.g., povidone-iodine) are most commonly used as disinfecting surgical scrubs and pre-operative skin treatments (Darouche, 2010; Gibson, 1997). Recent reports also indicate that chlorhexidine may be used to protect newborn foals (i.e., small horses) from umbilical infections (House, 2008). In conventional agriculture, chlorhexidine diacetate can be used to control bacteria on agricultural premises and equipment, egg handling and packing equipment, meat processing plants, and for veterinary or farm premises to control viruses (US EPA, 2011a).

Beyond agricultural applications, a number of dental, surgical and other antimicrobial uses have been reported for chlorhexidine. One product (BioSurf) formulated with chlorhexidine digluconate as the active ingredient may be used for hard, non-porous surfaces (wheelchairs, metal bed frames, exteriors of toilets, countertops, metal surfaces, imaging equipment surfaces, metal, glass acrylic and porcelain) in hospitals, restrooms, schools, offices, gyms, and homes. Mint-A-Kleen®, a ready-to-use liquid product containing chlorhexidine digluconate, is used to control microbial contamination in dental unit waterlines (US EPA, 2011a). Chlorhexidine gluconate has also been used as the active ingredient in certain mouthwashes due to its plaque-inhibiting effects (Ogbru, 2014).

Approved Legal Uses of the Substance:

Products formulated with chlorhexidine diacetate as the active ingredient were first registered in the United States as early as 1955 for use as disinfectants and virucides on farm premises. Two manufacturing use products and three end-use products with chlorhexidine diacetate as an active ingredient are registered with US EPA for use as hard surface-treatment disinfectant/non-food contact surface sanitizer (floors & walls)/bactericides/virucides. Likewise, a product (BioSurf) formulated with chlorhexidine gluconate as an active ingredient was registered with US EPA in 1987 for use as a disinfectant for hard, non-porous surfaces, as described in "specific uses of the substance." The chlorhexidine digluconate product Mint-A-Kleen® became registered in 2010 for cleaning and control of microbial contamination in dental unit waterlines (US EPA, 2011a). US EPA has not established tolerances or tolerance exemptions for chlorhexidine in agricultural commodities (40 CFR 180).

United States Food and Drug Administration (FDA) regulations allow the use of chlorhexidine as an active ingredient in certain antiseptic ointments, washes and over-the-counter drug products. Numerous commercially available solutions consisting of 0.12% chlorhexidine gluconate are FDA-approved for use as antimicrobial mouth washes (FDA, 2014a). According to FDA regulations at 21 CFR 524.402, chlorhexidine acetate may be formulated at a concentration of one percent in ointment base for use as a topical antiseptic on the wounds of dogs, cats and horses. These products may not be used in horses intended for human consumption. Chlorhexidine may also be formulated at a rate of one gram chlorhexidine dihydrochloride per tablet or 28-milliliter syringe suspension in new animal drugs intended to treat and/or prevent metritis and vaginitis in cows and mares (21 CFR 529.400). FDA established a tolerance of zero for residues of chlorhexidine in the uncooked edible tissues of calves (21 CFR 556.120).

In addition to the allowed uses above, FDA has also removed several chlorhexidine products from the market for reasons of safety or effectiveness. Specifically, FDA withdrew the registrations for all tinctures of chlorhexidine gluconate formulated for use as human preoperative skin preparations (21 CFR 216.24).

Chlorhexidine teat dips are considered unapproved animal drugs according to FDA regulations. The FDA published a proposed regulation in the Federal Register of 1977 (42 FR 40217) which would designate teat dips as new animal drugs and require the evaluation of marketed teat dip products for safety and efficacy under the New Animal Drug Application (NADA) approval process (FDA, 2014b). However, the proposed regulation was never finalized. Teat dips and udder washes classified as animal drugs may currently be marketed for mastitis control and prevention without NADA approval. According to the FDA Grade A Pasteurized Milk Ordinance, "udders and teats of all milking animals are clean and dry before milking. Teats shall be cleaned, treated with a sanitizing solution and dry just prior to milking" (FDA, 2011).

Action of the Substance:

The antimicrobial mechanism of action for chlorhexidine at low concentration involves ATPase inactivation, whereas higher concentrations of the substance induce damage of the cytoplasmic membrane by precipitating essential proteins and nucleic acids (Saha, 2014). Under physiological conditions, chlorhexidine exists as a positively charged (cationic) molecule that binds to the negatively charged sites on the cell wall or membrane, thereby destabilizing the cellular surface and osmotic balance within the cell (Silla, 2008). Damage to the outer cell layers takes place, but is insufficient to induce cell death directly. Once the cell wall/outer membrane is damaged, chlorhexidine passively diffuses into the cell and subsequently attacks the bacterial cytoplasmic (or inner) membrane or the yeast plasma membrane (McDonnell, 1999). Damage to the delicate semipermeable membranes of the cytoplasm allows for leakage of cellular components (e.g., amino acids) and ultimately cell death. At sufficiently high concentrations, chlorhexidine causes the cytoplasm to congeal or solidify (McDonnell, 1999).

Combinations of the Substance:

Commercially available chlorhexidine teat dip products contain chlorhexidine diacetate or digluconate as the sole active ingredient with the remainder of the formulation listed as "other ingredients." The label for Dairyland's Sprayable CHG Teat Dip (animal drug) lists 0.45% chlorhexidine digluconate as the active ingredient as well as several other ingredients, including 4.25% isopropyl alcohol, 2.0% glycerin and FD&C Blue No. 1 (Dairyland, 2010). Some product labels direct dairy operators to mix 32 ounces of Nolvasan® concentrate (2% chlorhexidine diacetate) with six ounces of glycerin followed by dilution of the mixture with clean potable water to a final volume of one gallon (Zoetis Inc, 2014). Glycerin moisturizes the treated skin, and is allowed as a livestock teat dip for organic production when produced through the hydrolysis of fats or oils (Nickerson, 2001; 7 CFR 205.603(a)(12)). A ready-to-use disinfectant for household and bathroom floors consists of chlorhexidine diacetate (0.01%) and didecyl ammonium chloride (0.03%), while a hospital hard-surface disinfectant is formulated as ethyl alcohol (70.5%) with only 0.2% chlorhexidine digluconate (US EPA, 2014).

Labels for currently registered products list the appropriate chlorhexidine salt and any other active ingredient but do not always include the identity of "other ingredients." Product formulations are considered confidential business information, and manufacturers of chlorhexidine-based antimicrobial pesticides and animal drugs may occasionally reformulate products. As a result, it is rarely possible to know the identity of adjuvants and other inert ingredients.

Status

Historic Use:

In 2009, the National Organic Standards Board recommended that chlorhexidine be included on the National List as an allowed synthetic substance for use in teat dips when other approved disinfectants prove ineffective (USDA, 2010). Product formulations with chlorhexidine diacetate as an active ingredient were registered in the United States as early as 1955 for use as a farm premises disinfectant/virucide (US EPA, 2011a). However, it is uncertain when organic or conventional dairy operators began using chlorhexidine in disinfecting teat dips to control mastitis. It was discovered in 1958 that dipping teats in 0.1, 1, and 2.5% acidic iodine solutions significantly reduced the numbers of *Staphylococci* (bacteria) that were recovered from milking machine liners (Boddie, 2000). Not long after, manufacturers began incorporating iodine into commercially available teat dip products. Teat dip treatments using chlorhexidine were introduced to the dairy industry following development of iodine teat dips. Regarding surgical applications, chlorhexidine gluconate was introduced as a skin antiseptic in 1954 (Evans, 2009).

Organic Foods Production Act, USDA Final Rule:

The National Organic Program (NOP) final rule currently allows the use of chlorhexidine as a synthetic substance in organic livestock production (7 CFR 205.603(a)(6)) as a disinfectant, sanitizer and medical treatment. Specifically, chlorhexidine is allowed for use as a teat dip when alternative germicidal agents (e.g., iodine) and/or physical barriers have lost their effectiveness. Chlorhexidine is also an allowed disinfectant for surgical procedures conducted by a veterinarian.

International

A subset of the international organizations surveyed has provided guidance on the use of pre- or post-milking teat dip substances in organic livestock production. Among these are regulatory agencies (Canada, Japan, and the EU) and independent organic standards organizations (IFOAM). International organic regulations and standards concerning chlorhexidine and/or other teat dips and disinfectants are described in the following sub-sections.

Canadian General Standards Board

The Canadian General Standards Board allows the use of chlorhexidine under Section 5.3 (Health Care Products and Production Aids) of the Permitted Substances Lists for Livestock Production (CAN, 2011). Specifically, the rule states that chlorhexidine may be used in the following ways: (1) for surgical procedures conducted by a veterinarian, and (2) as a post-milking teat dip when alternative germicidal agents and physical barriers have lost their effectiveness.

European Union

According to Article 23 (4) of the Commission Regulation concerning organic production and labeling of organic products,

> *Housing, pens, equipment and utensils shall be properly cleaned and disinfected to prevent cross-infection and the build-up of disease carrying organisms. Faeces, urine and uneaten or split feed shall be removed as often as necessary to minimize smell and to avoid attracting insects or rodents.*

The list of approved substances for cleaning and disinfection of building and installations for animal production includes "cleaning and disinfection products for teats and milking facilities." However, the rule does not explicitly describe the restrictions of use for available teat dip substances (EC, 2008). It is therefore uncertain whether European regulations allow the use of chlorhexidine as a topical disinfectant (e.g., teat dip) in organic livestock production.

Japanese Ministry of Agriculture, Forestry and Fisheries

According to Table 4 of the Japanese Agricultural Standards for Organic Livestock Products, chlorhexidine is an allowed synthetic agent for cleaning and disinfecting livestock housing (JMAFF, 2012). However, chlorhexidine is not explicitly allowed for use in pre- or post-milking teat dips under Japanese organic regulations.

International Federation of Organic Agriculture Movements

Appendix 5 of the IFOAM Norms, which provides a list of "substances for pest and disease control and disinfection in livestock housing and equipment," includes iodine and "cleaning and disinfection products for teats and milking facilities." However, the standard does not explicitly describe the restrictions of use for available teat dip substances (IFOAM, 2014). It is therefore uncertain whether IFOAM guidelines permit the use of chlorhexidine as a topical disinfectant (e.g., teat dip) in the organic production of dairy animals.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

(A) Both antimicrobial pesticide products and specially formulated animal drugs containing the active ingredient chlorhexidine are used as teat dips in the dairy industry and topical cleansers during veterinary surgical procedures. Chlorhexidine would be considered a livestock medicine (animal drug) under these

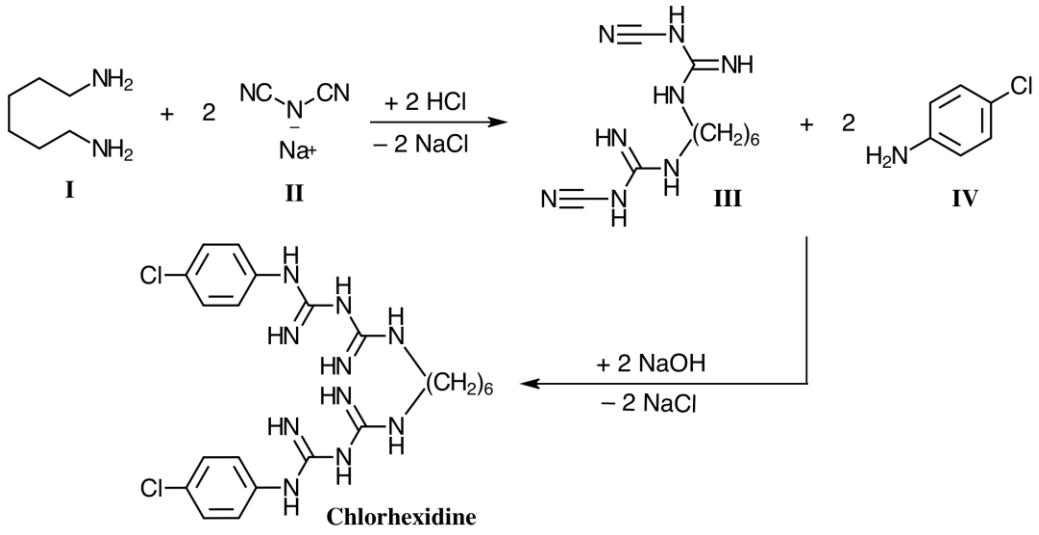
use patterns. In addition, chlorhexidine may be considered an equipment cleanser when used as a disinfectant during surgical procedures conducted by a veterinarian.

(B) Chlorhexidine is used solely as an active ingredient in pesticide products and thus would not be considered an inert. Further, US EPA has established no tolerances or exemptions from the requirement of a tolerance for chlorhexidine residues on agricultural commodities.

Evaluation Question #2: 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)).

Information regarding the manufacture of chlorhexidine used in commercially available disinfectants, sanitizers, bactericides and virucides is limited to the published patent literature. In general, industrial scale chlorhexidine production involves initial synthesis of the 1,6-hexamethylenebis(dicyandiamide) intermediate followed by reaction of the intermediate with 4-chloroaniline hydrochloride (Güthner, 2006; Werle, 2013). Once purified, chlorhexidine is combined with acetic acid or D-gluconic acid to generate the commercially relevant diacetate or digluconate salts of chlorhexidine (Sanchez, 2012).

Industrial syntheses of the chlorhexidine base occur in two steps, as shown below in Scheme 1. In the first stage of the process, hexamethylenediamine (I) is treated with two equivalents of hydrochloric acid (HCl) to generate the corresponding hydrochloride salt, hexamethylenediaminedihydrochloride, which is subsequently reacted with sodium dicyanamide (II). The resulting mixture is reacted under reflux conditions in alcoholic solvent (e.g., butanol) at temperatures greater than 110 °C to provide 1,6-hexamethylenebis(dicyandiamide) intermediate (III). Addition of triethylamine [$(CH_3CH_2)_3N$] establishes a pH of approximately 9, and may be necessary to achieve satisfactory yields in this first stage of the synthesis. In the second stage, intermediate III is treated with 4-chloroaniline (IV) under reflux conditions in an alcoholic solvent such as ethanol, n- or iso-propanol, or 2-ethoxyethanol to afford the desired chlorhexidine base. Addition of hot aqueous sodium hydroxide (NaOH) quenches the reaction and allows for separation of the chlorhexidine base from water soluble impurities. Details regarding the two-step synthesis of chlorhexidine are provided below in Scheme I (Werle, 2013). Variations of this methodology may be employed commercially.



Scheme 1. Chlorhexidine production involves a two-step synthetic route.

Upon completion of the synthetic reaction, chlorhexidine is typically extracted from the reaction mixture and purified by recrystallization from methanol (CH_3OH) to obtain chlorhexidine as colorless needles. However, this recrystallization method significantly reduces product yields and may not provide chlorhexidine free of the p-chloroaniline reagent (Sanchez, 2012). Other solvent systems for extraction and recrystallization, including mixtures of alcohols (e.g., methanol, ethanol, isopropanol) and ketones (e.g., acetone), have been employed to improve the yield and purity of chlorhexidine. The available data indicate

that small but significant amounts (500 to 1,000 parts per million) of p-chloroaniline will remain in the final product if the crude chlorhexidine is not washed several times with a suitable solvent extraction system (Sanchez, 2012). Commercially relevant chlorhexidine digluconate or diacetate salts are prepared through controlled reactions of the purified chlorhexidine base with gluconic acid (also existing in the glucono delta-lactone form) or glacial acetic acid, respectively (Sanchez, 2012). See Figure 1 for structures of these chemical reagents.

Evaluation Question #3: 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)).

According to USDA organic regulations, the NOP defines synthetic as "a substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources" (7 CFR 205.2). Chlorhexidine is not a naturally occurring chemical; therefore, chlorhexidine acetate used in commercially available teat dip products must be produced through chemical synthesis. Indeed, the primary industrial method used for the preparation of chlorhexidine involves the combination of chemical substances produced synthetically (i.e., hydrochloric acid, p-chloroaniline, hexamethylenediamine, and sodium dicyanamide). It therefore follows that chlorhexidine as well as its commercially relevant salts (diacetate and digluconate) are synthetic substances based on NOP definitions and the use of synthetic chemical reagents and solvents during production, processing and product formulation. See the discussion in Evaluation Question #2 for details regarding the two-step synthetic route, chlorhexidine salt formation, and extraction/purification methods.

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

This section summarizes technical information related to the persistence, fate and transport of chlorhexidine in the soil, water and atmospheric compartments of the environment. Although limited, the compiled data indicate that chlorhexidine is readily biodegradable in the atmosphere, with limited biodegradation in the terrestrial and aquatic compartments (HSDB, 2004). Chlorhexidine is not considered to be a persistent, bioaccumulative and toxic chemical (Evonik, 2011). Production and use of chlorhexidine as an antiseptic and disinfectant will necessarily result in releases of the substance to the environment through waste streams and spills.

Limited information is available regarding the mobility and biodegradation potential of chlorhexidine in soil. Chlorhexidine is expected to have very high mobility in soil based on the calculated soil organic carbon-water partition coefficient (K_{oc}) of 26. However, its pKa of 10.78 indicates that the compound will exist primarily in the protonated form in the environment; cations generally adsorb more strongly to organic carbon and clay than neutral compounds. Based on the Henry's law constant (1.6×10^{-17} atm•m^3/mole) and low vapor pressure (2.0×10^{-14} mm Hg), chlorhexidine is not expected to volatilize from moist or dry soil surfaces. Chlorhexidine dissolved in a mineral salts medium did not degrade over the 21-day period in a soil extract inoculum; therefore, biodegradation may not be an important fate process for chlorhexidine in soil (HSDB, 2004). An independent report states that "experimental data on biodegradability of chlorhexidine digluconate are inconclusive, but do not generally exclude biodegradability (Evonik, 2011).

When released to water, chlorhexidine is expected to adsorb to suspended solids and sediments based on its K_{oc}. Volatilization of chlorhexidine from water surfaces is not expected based on the Henry's law constant and vapor pressure. With a BioConcentration Factor (BCF) of 3, it is unlikely that chlorhexidine will bioaccumulate in the tissues of aquatic organisms. Hydrolysis is not expected to be an important environmental fate process due to the lack of hydrolysable functional groups in the chlorhexidine molecule (HSDB, 2004). According to an independent report, chlorhexidine gluconate "is highly absorptive to soil, sediment and sewage sludge but does not bioaccumulate in environmental organisms (Evonik, 2011).

Chlorhexidine released into the air will exist solely in the particulate phase in the ambient atmosphere based on the vapor pressure (2.0×10^{-14} mm Hg). Particulate-phase chlorhexidine may be removed from the air by wet and dry deposition. Because chlorhexidine molecules absorb light in the environmental range (i.e., greater than 290 nanometers), it is likely that chlorhexidine will be degraded by direct photolysis in the air, as well as the surface of water and soil (HSDB, 2004).

It should be noted that US EPA did not conduct an environmental fate assessment during the 1996 reregistration process because "it is unlikely for the environment to be exposed to the pesticide when it is used as labeled" (US EPA, 1996). More recently, the Agency determined that an environmental fate assessment was necessary for chlorhexidine as an example of "disinfectant/sanitizers used in animal premises that may potentially pass through wastewater treatment plants (WWPTs) and may be discharged into terrestrial and aquatic environments" (US EPA, 2011a). This assessment is not currently available.

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Acute toxicity testing has been conducted using both the diacetate and digluconate salts of chlorhexidine. In mammals, chlorhexidine diacetate is mildly to moderately toxic on an acute basis when administered via oral (Toxicity Category III), dermal (Toxicity Category III), and inhalation (Toxicity Category II) routes. Results for acute toxicity testing were consistent with Toxicity Category IV (slight toxicity) for oral, dermal and inhalation routes, as well as eye and dermal irritation (US EPA, 2011b). Chlorhexidine is suspected of being an acute pulmonary toxicant based on poisoning incidents in humans and laboratory studies in rats. Specifically, aspiration of chlorhexidine solutions directly into the lung has led to several cases of acute respiratory distress syndrome (ARDS) in humans, and direct injection of the chlorhexidine digluconate into the lungs of experimental rats induced an inflammatory response at the treatment site (Xue, 2011). A primary dermal irritation study conducted with chlorhexidine diacetate indicated mild toxicity (Toxicity Category IV). However, repeat primary eye irritation study suggest that the chemical is severely toxic/irritating via ocular exposure (Toxicity Category I). Chlorhexidine diacetate and digluconate salts were not found to be skin sensitizers when tested in guinea pigs (US EPA, 2011b).

The available literature suggests there is minimal concern for adverse reproductive, developmental, and genotoxic effects associated with subchronic and chronic exposure to commercially available products containing chlorhexidine active ingredients (US EPA, 2011b). As part of a reproductive/developmental study, experimental rats were dosed with chlorhexidine diacetate via gavage at 0, 15.6, 31.3, or 62.5 mg/kg-day (corrected for chlorhexidine base) from day six through 15 of gestation. The second highest dose of 31.3 mg/kg-day resulted in dose-related decreased body weight gain, rales (respiratory noise), and increased salivation of treated animals; however, no observable malformations or developmental toxicity were found at any dose level tested. Chlorhexidine diacetate was negative for genotoxicity/mutagenicity when tested under the following conditions:

- Up to cytotoxic levels (6 μg/mL in activated assays) in gene mutation testes with mammalian lymphoma cells *in vitro*;
- In *in vitro* cytogenetic assays with Chinese hamster ovary cells (negative for chromosomal breakage, with and without activation at test concentrations up to 10 μg/mL);
- In DNA damage/repair (unscheduled DNA synthesis) study using primary rat hepatocyte cultures *in vitro* with exposure levels up to 2.42 μg/mL.

Chlorhexidine is considered slightly toxic to practically non-toxic to avian species on an acute oral and subacute dietary basis. A no observed effect level (NOEL) of 292 mg/kg-day (slightly toxic) was determined in a study of Bobwhite quail administered chlorhexidine digluconate via oral gavage, while other subacute dietary exposure studies in Bobwhite quail and mallard duck provided NOELs of 1780–5620 ppm (practically non-toxic). In contrast, both the diacetate and digluconate salts of chlorhexidine are highly toxic to fish and aquatic invertebrates. Rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*) were highly sensitive to chlorhexidine digluconate exposure, with LC_{50} values (concentration lethal to 50% of test fish) ranging from 0.51 to 2.3 ppm. In addition, both salts of chlorhexidine have LC_{50} values of 63–84 parts per billion (ppb) for the freshwater water flea (*Daphnia magna*) and are therefore listed as "very highly toxic" to aquatic invertebrates (US EPA, 2011a).

Residues of chemical reagents used in the production of chlorhexidine are also associated with toxicity in various systems. Specifically, the 4-chloroaniline used as an intermediate in the synthesis of chlorhexidine is likely to be present as an impurity in the chlorhexidine base, the diacetate and digluconate salts of chlorhexidine, and the formulated products containing these active ingredients. Further, the decomposition

of chlorhexidine salts is likely to produce small amounts of 4-chloroaniline (Sanchez, 2012). Based on a review of the available literature, the World Health Organization (WHO) determined that 4-chloroaniline is highly toxic to red blood cells and DNA: "all chloroaniline isomers are haematotoxic and show the same pattern of toxicity in rats and mice, but in all cases 4-chloroaniline shows the most severe effects. 4-chloroaniline is genotoxic in various systems" (WHO, 2003).

Evaluation Question #6: Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

General use of commercially available chlorhexidine salts is unlikely to result in environmental contamination. As a potent microbiocide, the substance is frequently used to disinfect skin, equipment and various surfaces, thus minimizing the level of contamination with pathogenic microorganisms. Chlorhexidine teat dips are typically used in small amounts, at low concentrations (e.g., 0.5%) and under relatively controlled conditions (Zoetis Inc, 2014); however, medical, dental and consumer products likely contribute more significantly to the chlorhexidine load in wastewater. Indeed, surgical skin scrub formulations, hand cleanser wipes and mouth wash formulations contain respective chlorhexidine salt concentrations of 4, 0.5 and 0.12% (US EPA, 2011a). The Material Safety Data Sheet (MSDS) for pure chlorhexidine diacetate lists several environmental precautions for the product (Sigma Aldrich, 2014):

- Prevent further leakage or spillage if safe to do so,
- Do not let product enter drains, and
- Discharge into the environment must be avoided

The MSDS also states that "an environmental hazard cannot be excluded in the event of unprofessional handling or disposal" and the substance is "very toxic to aquatic life with long lasting effect" (Sigma Aldrich, 2014). Indeed, laboratory testing has demonstrated that low concentrations (less than or equal to 100 ppb) of chlorhexidine in water can be detrimental to certain species of aquatic organisms, including fish and aquatic invertebrates (Sigma Aldrich, 2014; US EPA, 2011a). As indicated above, however, the bulk of chlorhexidine released to the environment is likely a result of uses other than mastitis control in dairy operations. Further, neither US EPA nor other available data sources documented cases of environmental contamination associated with use of chlorhexidine products.

In addition to the active substances, the manufacture of chlorhexidine could lead to adverse effects on aquatic receptors. Specifically, reaction solutions containing strong acids (i.e., hydrochloric acid) and bases (i.e., sodium hydroxide) could alter the pH of receiving waters if released to the environment due to improper handling and/or disposal of these materials. Severe changes in the pH of natural waters could results in population-level effects such as fish kills in the affected areas. No reports of contamination due to the manufacture of chlorhexidine were identified, and the risk of such events is minimized when hazardous substances are treated according to state and federal law prior to disposal.

Evaluation Question #7: Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

Limited information is available regarding the potential for chemical interactions between chlorhexidine and other substance used in agricultural production. Known interactions involve the ability of cationic chlorhexidine compounds (i.e., diacetate and digluconate salts) to sequester the available chlorine content and form insoluble precipitation products (Rossi-Fedele, 2012). Chlorhexidine also forms precipitates when combined with chelating agents, such as ethylenediaminetetraacetic acid (EDTA) (Rasimick, 2008). Although unlikely, the interaction of cationic chlorhexidine with the hypochlorite anion could be problematic due to the use of calcium hypochlorite and sodium hypochlorite in organic crop (7 CFR 205.601(a)(2)(i), 205.601(a)(2)(iii)) and livestock (7 CFR 205.603(a)(7)(i), 205.603(a)(7)(iii)) production as disinfectants, sanitizers and algicides. A synergistic relationship also exists between chlorhexidine and the antifungal agent itraconazole (HSDB, 2004); however, the latter synthetic substance is not allowed for use in organic production.

Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

Chlorhexidine is a rapidly acting biguanide germicide. It is effective against a broad array of pathogenic microorganisms, including Gram-negative (e.g., *Escherichia coli*) and Gram-positive (e.g., *Streptococcus agalactiae* and *Staphylococcus aureus*) bacteria and numerous viral strains (Nickerson, 2001). The antimicrobial mode of action for chlorhexidine involves precipitation of cytoplasmic proteins and macromolecules, as well as damage to the inner cytoplasmic membrane and subsequent leakage of cellular components such as amino acids (McDonnell, 1999; Saha, 2014). Based on this general mode of action, chlorhexidine is potentially toxic to beneficial soil microorganisms, including nitrogen fixing bacteria and mycorrhizal fungi. Information regarding the toxicity of chlorhexidine to non-target soil organisms was not found in the available literature.

In addition to the active substances, the manufacture of chlorhexidine could lead to adverse effects on environmental receptors. Specifically, reaction solutions containing strong acids (i.e., hydrochloric acid) and bases (i.e., sodium hydroxide) could alter soil pH if released to the terrestrial environment due to improper handling and/or disposal of these materials. Drastic changes in soil pH could alter the bioavailability of macro- and micronutrients for plants and beneficial soil microflora. No reports of contamination due to the manufacture of chlorhexidine were identified, and the risk of such events is minimized when hazardous substances are treated according to state and federal law prior to disposal.

Information was not identified on the potential or actual impacts of chlorhexidine, commercially available chlorhexidine salts, or manufacturing methods on endangered species, population, viability or reproduction of non-target organisms and the potential for measurable reductions in genetic, species or eco-system biodiversity.

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

The available information indicates that chlorhexidine is readily biodegradable in the atmosphere, with limited biodegradation in the terrestrial and aquatic compartments (HSDB, 2004). However, chlorhexidine is not considered to be persistent, bioaccumulative or toxic to humans. Production and use of chlorhexidine as an antiseptic and disinfectant will result in releases to the environment through waste streams and spills. Chlorhexidine exists primarily in protonated (cationic) form in the environment, and thus is expected to adsorb strongly to organic carbon and clay despite its predicted high mobility in soil. Likewise, chlorhexidine is expected to adsorb to suspended solids and sediments when released to water (HSDB, 2004; Evonik, 2011).

Despite the relatively low risk associated with chlorhexidine, environmental hazards cannot be excluded for improper handling and disposal of chlorhexidine products. Specifically, chlorhexidine salts are highly toxic to aquatic life with long lasting effects (Sigma Aldrich, 2014). Registrant-submitted studies indicate that concentrations as low as 60 parts per billion are toxic to half of the freshwater water fleas in an acute toxicity test (US EPA, 2011a). Further, 4-chloroaniline used in the synthesis of chlorhexidine is highly toxic to red blood cells and DNA, and exposure to residues of this substance in contaminated chlorhexidine solutions may lead to toxic effects in terrestrial organisms (WHO, 2003). As a general antimicrobial agent, chlorhexidine is potentially toxic to beneficial soil organisms, including nitrogen fixing bacteria and mycorrhizal fungi.

Evaluation Question #10: 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)).

Studies suggest that chlorhexidine salts are acutely irritating to the eyes (Toxicity Category I), but mildly to moderately toxic on an acute exposure basis when administered via oral (Toxicity Category III), dermal (Toxicity Category III), and inhalation (Toxicity Category II) routes. In addition, chlorhexidine is suspected of being an acute pulmonary toxicant based on poisoning incidents in humans and laboratory studies in rats. Indeed, accidental ingestion of chlorhexidine in children and the elderly have occurred, and the development of acute respiratory syndrome (ARDS) was reported after accidental injection or ingestion of chlorhexidine (Xue, 2011). Very few human and animal incidents associated with chlorhexidine exposure

have been reported to the Incident Data System of the Office of Pesticide Programs (OPP). According to the 2011 US EPA Human Health Scoping Document for chlorhexidine derivatives:

> *The three human incidents reported to be associated with chlorhexidine exposure included: (1) tracheal edema in a woman following her visit to a veterinarian's office where a chlorhexidine solution had been used, (2) severe cold-like symptoms that progressed to bronchitis in a woman running a cattery housing six cats who used a chlorhexidine solution to disinfect cages, and (3) dermal sensitization symptoms occurring in one person after dermal exposure to a chlorhexidine cleaning solution.*

In addition, five poisoning incidents involving exposure to chlorhexidine diacetate were reported to the California Department of Pesticide Regulation (CDPR) through the Pesticide Illness Surveillance Program (PISP) between 1994 and 2011. Accidental eye exposure led to redness, pain and swelling of the eye with discharge, while dermal exposure resulted in severe rash and swelling of the hands (CDPR, 2011). The report noted that individuals reporting dermal irritation were not wearing proper personal protective equipment (PPE), such as gloves.

Few human exposure studies are available for chlorhexidine active ingredients and formulated products. However, one recent study evaluating the penetrability of 2% aqueous chlorhexidine digluconate in human skin found no detectable penetration through the full skin thickness (Karpanen, 2008). It was therefore concluded that systemic exposure to chlorhexidine as a result of dermal contact is minimal.

Residues of 4-chloroaniline in commercially available chlorhexidine solutions may present a toxicity concern for chronically exposed humans. Specifically, 4-chloroaniline increases the production of methemoglobin and sulfhemoglobin, reacts with red blood cells to form hemoglobin adducts, and results in cellular oxygen deprivation. The substance is also carcinogenic in laboratory animals, with the induction of unusual and rare tumors of the spleen in rats as well as liver cancer and hemangiosarcoma (tumor formation in blood vessels) in male mice (WHO, 2003). Based on a 1993 evaluation of the available data on 4-chloroaniline, the International Agency for Research on Cancer (IARC) determined that there is *inadequate evidence* in humans, but *sufficient evidence* in experimental animals, for the carcinogenicity of the substance (IARC, 1993). IARC therefore classified as *Group 2B – Possibly carcinogenic to humans* (IARC, 2014). Both 4-chloroaniline and its hydrochloride salt are also listed as carcinogens on the California Proposition 65 List (OEHHA, 2014).

Evaluation Question #11: 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)).

Information regarding the availability of natural, non-synthetic agricultural commodities or products that could substitute for synthetic teat disinfectants is limited. Nisin, a naturally occurring antimicrobial protein known as a bacteriocin, has been incorporated into pre- and post-milking teat dips and is highly effective against Gram-positive as well as Gram-negative bacteria (Nickerson, 2001). Formulated products containing nisin, such as Wipe Out® Dairy Wipes, are currently available for mastitis prevention (Jeffers, 2014). Nisin naturally present in milk is also instrumental in preventing milk spoilage due to bacterial contamination (Ahlberg, 2012). The antimicrobial mode of action for nisin involves lysis of the cytoplasmic membrane phospholipid components (Nickerson, 2001).

Nisin, generally considered a natural product, is not listed as a prohibited non-synthetic substance in organic livestock production (7 CFR 205.604). However, the NOSB classified nisin as synthetic during their 1995 review of the substance for organic processing (USDA, 1995a). Nisin was not recommended for inclusion on the National List for use in the processing of food labeled as "organic" and "made with organic ingredients" (USDA, 1995b; OMRI, 2014).

Small-scale milk producers use homemade udder washes containing lavender essential oil, water, and apple cider vinegar (i.e., acetic acid) as the active antimicrobial agent (Weaver, 2012). Other procedures for pre- and post-milking treatments include an udder wash (warm water or warm water with a splash of vinegar) in combination with a teat dip (1 part vinegar, 1 part water, plus 3–4 drops Tea Tree oil per ounce). Naturally derived acids (e.g., lactic acid) may be used as standalone germicides or further activated through the synergistic interaction with hydrogen peroxide to provide a bactericidal teat cleansing

treatment (Belsito, 2012). In addition to the natural substances mentioned above, a small number of synthetic substances are currently allowed as disinfectants, topical treatments, and external parasiticides in organic livestock production (7 CFR 205.603 (a) and (b)):

- **Iodine**: Disinfectant, topical treatment, and/or parasiticide. A broad spectrum germicide, which is fast-acting and effective against all mastitis-causing bacteria as well as fungi, viruses, and some bacterial spores. It is microbicidal due to the oxidizing reaction between iodine and organic matter. Iodophors are produced when iodine is dissolved in aqueous solutions containing water-soluble detergents or surfactants (Nickerson, 2001).
- **Ethanol**: Disinfectant and sanitizer only, prohibited as a feed additive.
- **Isopropanol**: Disinfectant only.
- **Sodium hypochlorite**: Commonly referred to as commercial bleach. On the National List as a disinfectant, not a topical treatment option. It has been noted that such solutions are not marketed as teat dips and their use violates federal regulations; however, its use has continued for both pre- and post-milking teat dips at a 4.0% hypochlorite concentration (Nickerson, 2001).
- **Hydrogen peroxide**: On the National List as a disinfectant, not a topic treatment option. Provides a wide spectrum of control against most mastitis-causing bacteria through its oxidizing action.

Suppliers of livestock and dairy products have indicated that iodine is traditionally the preferred germicide used as a teat dip for mastitis prevention. Recent natural disasters in Japan and water shortages in Chile led to increasing prices for iodophor products and resultant interest in alternative teat dips (Animart, 2012). Goodwin *et al.* (1996) demonstrated that post-milking teat dips using chlorhexidine reduced the total bacteria load in milk to a greater extent than similar treatments with a commercial iodophor; however, the small sample size (nine cows) is a limiting factor for this study. Other study results suggest that commercially available chlorhexidine digluconate is equally effective as iodine and iodophor products at controlling common mastitis pathogens. For example, chlorhexidine post-milking teat dips reduced *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections by 86–89% and 51–56%, respectively (Drechsler, 1993). Post-milking chlorhexidine teat disinfection significantly lowered new intramammary infections by *Streptococcus* species (50%), *Staphyloccocus* species (49%) and *Corynebacterium bovis* (65%) in a related natural exposure study (Oliver, 1990).

There are limitations associated with the use of chlorhexidine teat dip products. Although chlorhexidine germicides are effective against most Gram-positive and Gram-negative bacteria, chlorhexidine solutions that are heavily contaminated from repeated use may not be effective against *Serratia* and *Pseudomonas* species (Nickerson, 2001). Further, extension experts have suggested that *Serratia spp.* are commonly resistant to chlorhexidine digluconate disinfectants, regardless of the level of contamination (Petersson-Wolfe & Currin, 2011). It is therefore recommended that producers with herds experiencing *Serratia* mastitis choose a pre-milking teat disinfectant containing a different active ingredient. Continued use of a chlorhexidine disinfectant solution contaminated with resistant bacteria could results in the spread of mastitis pathogens throughout the herd.

Animal health researchers recently found that acidified sodium chlorite (ASC)-chlorine dioxide solutions are equally effective in preventing new intramammary infections (IMI) in lactating dairy cows naturally exposed to mastitis pathogens when compared to an established iodophor teat dip product (Hillerton, 2007). Alternatively, the results of experimental challenge studies (cows intentionally exposed to mastitis pathogens) suggest that ASC may actually provide enhanced antimicrobial activity against the mastitis bacteria *Staphylococcus aureus* and *Streptococcus agalactiae* relative to a commercial iodophor (Boddie, 2000; Drechsler, 1990). These studies also indicate that the tested ASC products had no deleterious effects on teat condition. Further, ASC components exhibit minimal persistence in the environment and are highly unlikely to contaminate the milk from treated animals (USDA, 2013). Commercial ASC teat dips are being increasingly used in conventional dairies, and the NOSB is considering a petition to add this substance to the National List (Ecolab Inc, 2012).

The available information suggests that commercial antimicrobial products containing oxidizing chemicals (e.g., sodium chlorite, hypochlorite, iodophor), natural products composed of organic acids (e.g., lactic acid), and homemade products using vinegar (i.e., acetic acid) as the active ingredient may all be equally effective teat dip treatments. For example, commercially available post-milking teat germicides containing

Lauricidin® (glyceryl monolaurate), saturated fatty acids (caprylic and capric acids), lactic acid and lauric acid reduced new intramammary infections (IMI) in cows inoculated with *Staphylococcus aureus* and *Streptococcus agalactiae* at levels approaching those achieved using iodophor products (Boddie & Nickerson, 1992). Aging the product solutions for five months at elevated temperature (40 ^{o}C) diminished the level of protection of Lauricidin® against new IMI. Although numerous active ingredients are formulated in pre- and post-dip products, iodine and iodophor products have a long history of supporting the health and productivity of milk-producing animals through effective mastitis control.

A wide variety of disinfectants are used alone or in combinations in health-care settings. These include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, *ortho*-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds (CDC, 2008). Chlorine materials (e.g., sodium hypochlorite and chlorine dioxide), quaternary ammonium compounds, phenolics (e.g., Lysol®) and peracetic acid/hydrogen peroxide/acetic acid solutions (e.g., Spor-Klenz®) are specific examples of hard-surface disinfectants that could substitute for chlorhexidine in veterinary settings (OSU, 2015). On the other hand, iodophors (e.g., Betadine®, Prepodyne® and Wescodyne®) are the only recommended substitutes for chlorhexidine used as surgical scrubs and pre-operative skin preparations. Ethyl alcohol and isopropyl alcohol are lower-level topical disinfectants that can be used in conjunction with chlorhexidine and iodophor products in medical contexts (OSU, 2015).

Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

A number of control measures for contagious mastitis pathogens have been developed and successfully implemented in the dairy industry. Mastitis, an inflammation of the breast tissue, is typically caused by environmental pathogens, such as Gram-negative bacteria *Serratia spp.* (Petersson-Wolfe & Currin, 2011). Since these pathogens are commonly found in soil and plant matter, cows on pasture or housed on organic bedding experience heighted exposure to mastitis-causing pathogens. Damage of the teat ends and poor udder cleanliness may also increase the risk of spreading the pathogens throughout the herd. The risk of mastitis incidents is significantly reduced when producers maintain a clean and dry environment for the animals. Frequently changing the animal's bedding material and/or using inorganic bedding (i.e., sand) may also reduce environmental contamination with these bacteria (Petersson-Wolfe & Currin, 2011). In addition, providing a healthy, balanced diet to the animal and ensuring the cleanliness of milking implements are important steps for maintaining healthy udders.

Alternative practices to teat dipping/spraying or udder washing are not advised, as the exclusion of a disinfecting step from a mastitis control program would significantly increase the likelihood of infection. Teat dips and udder washes are critical for preventing incidents of mastitis, and virtually all milk producers apply some form of teat disinfectant post milking. Any mastitis control program will incorporate disinfecting teat dips at milking to prevent new infections and reduce the duration of existing infections. Cessation of hygienic milking practices, and particularly teat dipping, will allow bacterial populations on teat skin to propagate, thus increasing the risk of infection (Poock, 2011). While pre-dipping can be beneficial to animal health, post-dipping with an effective sanitizer is essential for both removing milk residue left on the teat and killing harmful microorganisms (Bray & Shearer, 2012). Overall, dairy professionals agree that teat dipping using a safe and effective disinfectant is vital to maintaining the health and productivity of milk-producing animals.

Likewise, surgical procedures should always be conducted under aseptic conditions. Contamination may arise from instruments or implants, the surgical team, the environment, and the patient's (i.e., animal's) own skin. Equipment sterilization, gowning, masking and gloving are standard protocols used to reduce or eliminate the likelihood of contamination (Gibson, 1997). In addition, altering air flow, isolating the surgical site and minimizing surgical times may help lessen the incidence of surgical wound infections. Pre-operative patient skin preparation, such as clipping the hair/shaving and applying antiseptic scrubs, generally reduces the numbers of skin bacteria and resulting wound infections (Gibson, 1997; Evans, 2009).

Although no practice is a fully viable substitute for teat dipping and pre-operative skin antisepsis, a large number of alternative substances for chlorhexidine treatments used in dairy operations and surgical settings are presented in Evaluation Question #11.

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