Ethanol

Livestock

Identification of Petitioned Substance

Chemical Name:
Ethanol

Other Name:
Ethyl Alcohol

Trade Names:
Anhydrous Alcohol
Denatured Alcohol

CAS Numbers:
64-17-5

Other Codes:
200-578-6 (EINECS No.)

Summary of Petitioned Use

The National Organic Program (NOP) final rule currently allows the use of ethanol in organic livestock production under 7 CFR 205.603(a)(1)(i) as a disinfectant and sanitizer for surface and topical use only. The substance is prohibited for use as a feed additive in organic production. In addition, ethanol is also allowed for use in organic crop production under 7 CFR 205.601(a)(1)(i) as an algicide, disinfectant, and sanitizer, including irrigation system cleaning. In this report, updated and targeted technical information for ethanol is compiled to augment the original 1995 Technical Advisory Panel (TAP) Report for Alcohols, which included methanol, ethanol, and isopropanol.

Characterization of Petitioned Substance

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

The exact composition of industrial alcoholic substances generally depends on the ethanol concentration, purity, and the addition of any denaturing agents. Absolute alcohol refers to pure ethanol containing only small quantities of water (one percent or less). Although it is not possible to produce anhydrous (water free) ethanol via fermentation, modern dehydration techniques can minimize the water content in ethanol to only a few parts per million. Ethanol may also be diluted with various quantities of water for industrial, academic, and medical uses as well as the production of alcoholic beverages. Alternatively, denatured alcohol consists of ethanol at varying concentrations spiked with a denaturing agent, which renders the resulting ethanol mixture unfit for consumption as a beverage (Merck, 2006). The main denaturing agent has traditionally been 10 percent methanol; other typical additives include isopropyl alcohol, acetone, methyl ethyl ketone, and denatonium (ODN, 1993). These substances may be added to ethanol either alone or in combination, depending on the requirements of the end use product. See "Combinations of the Substance" below for additional information regarding the formulation of denatured ethanol products and the NOP status of these denaturing additives.

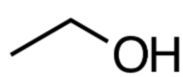

Figure 1. Ethanol structural formula

<u>Source or Origin of the Substance:</u>

Both fermentation and chemical synthesis procedures are used in the commercial production of ethanol for the preparation of disinfectant solutions, spirits, and industrial fuel sources. A variety of methods are available for the fermentative production of ethanol from carbon sources such as starch, sugar, and cellulose using natural and genetically engineered strains of yeast or bacteria (Merck, 2006; Logsdon, 2004).

Ethanol can also be produced synthetically through the direct or indirect hydration of ethylene ($H_2C=CH_2$), and as a by-product of certain industrial operations. As of 2001, fermentation accounted for 90 percent of the ethanol production in the U.S., Western Europe and Japan (Logsdon, 2004). Considering the continued advancements in fermentation-based technologies and increasing global demands for fuel ethanol, it is not surprising that this figure for all ethanol produced in 2013 is estimated to be 95 percent (Berg, 2013). See Evaluation Questions #2 and #3 for a detailed discussion of the fermentative and synthetic methods potentially used in commercial ethanol production.

Properties of the Substance:

Ethanol is a volatile, flammable, colorless liquid with the molecular formula CH_3CH_2OH. A summary of the chemical and physical properties of pure (absolute) ethanol is provided in Table 1.

Table 1. Chemical and Physical Properties for Ethanol

Property	Value/Description
Color	Clear, colorless
Physical State	Very mobile liquid
Molecular Formula	CH_3CH_2OH (C_2H_6O)
Molecular Weight, g/mol	46.07
Freezing Point, ºC	–114.1
Boiling Point, ºC	78.32
Density, g/mL	0.7893
Dissociation constant (pK_a)	15.9
Solubility in water at 25 ºC, mg/L	1,000,000 (highly soluble)
Solubility in organic solvents	Miscible in many organic solvents, including ethyl ether, acetone, and chloroform; soluble in benzene
Viscosity at 20 ºC, mPa•s	1.17
Soil Organic Carbon-Water Partition Coefficient (K_{oc}), mL/g	1.0 (Mobile in soils)
Aerobic Soil Half-life (DT_{50})	Literature suggests DT_{50} is 1–3 days
Hydrolysis	Stable to hydrolysis
Photodegradation	Photochemical oxidation in the presence of atmospheric nitrogen oxides and sulfur oxides
Octanol/Water Partition Coefficient (K_{ow})	0.4898
Vapor Pressure at 25 ºC, mm Hg	59.3
Henry's Law Constant, atm•m^3/mol	5×10^{-6}

Data Sources: HSDB, 2012; EC, 2010; UNEP, 2005; Logsdon, 2004.

Specific Uses of the Substance:

From its role as the active ingredient in antimicrobial solutions and wipes to its use as a transportation fuel, industrial solvent, and chemical precursor and inclusion in alcoholic beverages, the commercial applications of ethanol are both diverse and numerous. Because the use of ethanol as a sanitizer and disinfectant in organic livestock production is the subject of this report, primary consideration is given to the agricultural uses of ethanol.

Agricultural uses of ethanol include the disinfection of production tools and surfaces, topical disinfection, and plant regulation (ripening). Currently, the National List of Allowed and Prohibited Substances permits the use of ethanol as a disinfectant, sanitizer, and algicide in organic crop production. Organic livestock producers may use ethanol for sanitizing and disinfecting surfaces (e.g., production implements, troughs, and floor drains) and during medical treatments as a topical disinfectant (Jacob, 2013; Dvorak, 2008). Indeed, a protocol for the disinfection of methicillin-resistant *Staphylococcus aureus* (MRSA) on sows and their piglets using alcohol solutions was recently reported in the open literature (Pletinckx, 2013). Rubbing alcohol is also used to disinfect production implements such as livestock tagging applicators (OSU, undated). Antiseptic products containing ethanol and isopropanol are available for use on cattle, sheep and swine; for details, see the product label for Barrier® Livestock Wound Care (NIH, 2013). Regarding crop production, ethanol may be effectively used to decontaminate the lines of irrigation systems and remove bacteria, viruses and fungi from cutting tools (Benner, 2012). Crop producers may also convert ethanol to ethylene by dehydration in an ethylene generator for produce ripening (US EPA, 1995).

In addition to antimicrobial uses in agriculture, ethanol is also widely used in commercial and household products including hand sanitizers, medical disinfectants, and swimming pool water cleaning systems. Alcohols, including ethanol and isopropanol, are capable of providing rapid broad-spectrum antimicrobial activity against vegetative bacteria, viruses and fungi, but lack activity against bacterial spores (McDonnell, 1999). Indeed, the CDC recommends against the use of ethanol or isopropanol as the principal sterilizing agent because these alcohols are insufficiently sporicidal (i.e., spore killing) and cannot penetrate protein-rich materials (CDC, 2008). Notwithstanding these limitations, ethanol has been used to disinfect thermometers, hospital pagers, scissors, and stethoscopes. Commercial towelettes and other wipes saturated with ethanol have also been used to disinfect small surfaces in medical settings. As a general disinfectant, ethanol is generally applied through surface wipes, sprays, mop-on, sponge-on, wipe-on or pour-on treatments, and by immersion. Ethanol is also used to disinfect closed commercial/industrial water-cooling systems (EPA, 1995).

Ethanol is also used in large quantities as a fuel or fuel additive, an industrial solvent, a raw material in chemical synthesis, and in alcoholic beverages. Arguably, the most significant application of ethanol is as fuel, both as an oxygenate additive to gasoline and a gasoline extender (Kosaric, 2011). As a solvent, the major commercial applications of ethanol involve the manufacture of toiletries and cosmetics, detergents and disinfectants (discussed above), pharmaceuticals surface coatings, anti-freeze formulations, and in food and drug processing. The synthetic processes of numerous commercial chemicals, such as acetaldehyde and ethyl acetate, utilize ethanol as the chemical feedstock (Kosaric, 2011). Lastly, ethanol is the primary active constituent in alcoholic beverages produced through fermentation (e.g., beer and wine) and fermentation followed by distillation (e.g., hard liquor). In the past, ethanol produced through fermentation has generally been reserved for beverages and specialty chemicals, whereas ethanol produced by chemical synthesis has been used for industrial purposes. However, recent developments in ethanol production and the growing demand for ethanol-based fuels has led to increasing amounts of industrial grade ethanol being generated via fermentation (Kosaric, 2011).

Approved Legal Uses of the Substance:

The United States Food and Drug Administration (FDA) regulations allow a number of uses for ethanol in food preparation/storage for humans and animals. For humans, FDA considers ethanol to be "Generally Recognized As Safe" (GRAS) when added directly to human food (21 CFR 184.1293). The rule states "the ingredient is used as an antimicrobial agent…on pizza crusts prior to final baking at levels not to exceed 2.0 percent by product weight." The GRAS status of ethanol on other processed foods have also been reviewed; for example, ethanol is GRAS when used as a preservative in the filling of croissants at a concentration of 3,000 parts per million (FDA, 2004). Ethanol is also allowed for use as a diluent in color additives for marking foods and coloring shell eggs (FDA, 2013). According to 21 CFR 583.200, ethanol containing small amounts of ethyl acetate is a food substance affirmed as GRAS in the feed and drinking water of animals. Specifically, the rule states:

> *The feed additive ethyl alcohol containing ethyl acetate meets the requirements of 27 CFR 21.62, being not less than 92.5 percent ethyl alcohol, each 100 gallons having had added the equivalent of 4.25 gallons of 100 percent ethyl acetate. It is used in accordance with good feeding practices in ruminant feed supplements as a source of added energy.*

The United States Environmental Protection Agency (US EPA) regulates all non-food applications of ethanol, including its use as a pesticide and plant growth regulator. According to the Reregistration Eligibility Decision (RED) for Aliphatic Alcohols, ethanol and isopropanol were registered in the US as early as 1948 as active ingredients in indoor disinfectants (US EPA, 1995). Approximately 48 ethanol products were registered for use as hard surface treatment disinfectants, sanitizers and mildewcides as of 2012 (US EPA, 2012a). Ethanol is also the active ingredient in certain plant growth regulator products. Specifically, ethanol is used for "stored commodity fumigation" as a ripening agent on citrus fruits, pears, avocado, banana, papaya, melons, and tomatoes.

In addition to the legal uses of ethanol in pesticide products, statutory requirements mandate that transportation fuel consist of a minimum percentage of ethanol and other renewable fuels. US EPA oversees the implementation of the Renewable Fuel Standard (RFS), which originated with the Energy Policy Act of 2005 and was expanded and extended by the Energy Independence and Security Act (EISA) of 2007 (US EPA, 2013a). As part of the expansion, EISA increased the required volume of renewable fuel (e.g., ethanol) that must be blended into transportation fuel from nine billion gallons in 2008 to 36 billion gallons by 2022. Each year US EPA reevaluates and proposes stepwise increases in the ethanol-equivalent volume of biofuels that must be blended with conventional, petroleum-based fuels based on biofuel supply projections provided by the Energy Information Administration (EIA).

Action of the Substance:

Ethanol functions as a disinfectant by denaturing proteins and dissolving lipid membranes. Because proteins are denatured more quickly in the presence of water, enhanced bactericidal activity is generally observed for mixtures of ethanol and water when compared to absolute ethanol, which functions as a strong dehydrating agent (CDC, 2008). This crude observation provides qualitative support for the proposed mechanism, which relies heavily upon the ability of ethanol to denature proteins. Ethanol is able to effectively destroy many types of bacterial and viral cells due to this mode of action; however, ethanol is ineffective against bacterial spores because the substance evaporates before it can effectively penetrate the membrane and lead to protein denaturation (CDC, 2008).

Combinations of the Substance:

A number of natural and synthetic substances, ranging from colorants and denaturing agents to moisturizers and fragrances, are added to commercial products containing ethanol as the active ingredient. Ethanol-based topical antiseptics may include low levels of other biocides (e.g., chlorhexidine), which remain on the skin following ethanol evaporation, or excipients, which extend the lifetime of ethanol on skin and thus increase product efficacy (McDonnell, 1999). For denatured alcohol, one or more denaturing agents are generally added to absolute or diluted ethanol for the purpose of making the resulting products unpalatable and therefore undesirable for human consumption. This attribute allows denatured alcohol to remain exempt from the duty requirements of beverage grade alcohol. Denatured alcohol is used both industrially and domestically as a solvent, disinfectant, and fuel for camping stoves. Historically, ethanol was denatured with 10 percent methanol, rendering the alcohol unpalatable and effectively poisonous to humans. Numerous formulations of denatured alcohol formulations have been developed to meet the needs of diverse ethanol applications while also avoiding the toxic effects of methanol.

In addition to methanol, some of the more commonly used alcohol denaturants include 1–5 percent of isopropyl alcohol, acetone, methyl ethyl ketone, methyl isobutyl ketone, and denationium (ODN, 1993). The FDA also maintains a full list of denaturants authorized for the production of denatured alcohol (21 CFR 21.151).

The majority of authorized denaturants are synthetic substances that are not included on the National List. Denaturing agents derived from natural sources could be used to generate denatured alcohol solutions for applications in organic livestock production. Authorized denaturing agents that are naturally derived include essential oils (Bergamot essential oil, cinnamon oil, clove oil, lavender oil, peppermint oil, pine oil, rosemary oil, sassafras oil, spearmint oil, thyme oil, and turpentine oil). Naturally derived substance and pure chemicals, such as camphor, eugenol, menthol, and vinegar, are also listed as authorized denaturants. In addition, the following synthetic substances authorized by FDA as denaturing additives are currently listed on various sections of the USDA National Organic Program's National List:

- **Iodine.** Approved for use in organic livestock production as a disinfectant, sanitizer, and medical treatment. May also be used as a topical treatment, external parasiticide or local anesthetic (7 CFR 205.603(a)(14) and (b)(3)).
- **Isopropanol.** Approved for use in organic crop production as an algicide, disinfectant, and sanitizer, including irrigation system cleaning systems (7 CFR 205.601(a)(1)(ii)). Also approved as a disinfectant only in organic livestock production (7 CFR 205.603(a)(1)(ii)).
- **Potassium Iodide.** Nonagricultural (nonorganic) substance allowed as an ingredient in or on processed products labeled as "organic" or "made with organic" (7 CFR 205.605(a)).

Status

Historic Use:

Ethanol solutions have been used for disinfecting surfaces and farming implements in both organic and conventional agricultural operations. Although historical information documenting these uses are not available, it is likely that ethanol was the principal disinfectant prior to the advent of chemical sanitizers such as quaternary ammonium salts, peroxides, chlorine dioxide and bleach. In addition, modern sanitation standards and understanding regarding the spread of deleterious microorganisms through contaminated farm instruments likely increased the agricultural use of ethanol and other disinfectants.

Organic Foods Production Act, USDA Final Rule:

Neither of the terms "alcohol" or "ethanol" are mentioned in the Organic Foods Production Act of 1990 (OFPA). Ethanol is an approved synthetic substance on the National List for organic livestock production when used as a disinfectant and sanitizer only; ethanol is prohibited as a feed additive (7 CFR 205.603(a)(1)(i)). In addition, ethanol is an approved synthetic substance on the National List for organic crop production when used as an algicide, disinfectant, and sanitizer, including the cleaning of irrigation systems (7 CFR 205.601(a)(1)(i)). The current USDA organic regulations also permit the use of ethanol as an inert ingredient in pesticide products due to its inclusion on EPA List 4B (7 CFR 205.601(m) and 205.603(e)(1)). According to the 1995 Technical Advisory Panel Report, "alcohols are allowed as solvents and carriers in brand name products with allowed active ingredient(s). Also as disinfectant and in plant extracts" (USDA, 1995).

International

A number of international organizations provide guidance on the application of synthetic ethanol in organic crop and livestock production as well as the processing of organic foods. Among these are international regulatory agencies (EU, Canada, and Japan) and independent organic guidelines and standards organizations (Codex and IFOAM). Below, international regulations and standards regarding the use of ethanol in any form of organic production are summarized.

Canadian General Standards Board

Canadian organic production standards permit the use of ethanol for a number of agricultural applications. According to the "Organic Production Systems Permitted Substances Lists," ethanol may be used in organic livestock production as a production aid; specifically, ethanol is an allowed disinfectant and sanitizer only. Both synthetic and non-synthetic ethanol may also be used as a processing aid for organic foods and as a food-grade cleaner, disinfectant, and sanitizer on equipment (CAN, 2011a). The Canadian General Principles and Management Standards additionally stipulate the following for the disinfection of tapholes and tapping equipment in maple syrup procurement (CAN, 2011b):

> *The use of any types of germicide, including paraformaldehyde tablets, or denatured alcohol (a mixture of ethanol and ethyl acetate), in tapholes and on tapping equipment, is prohibited. Only food-grade ethyl alcohol may be used as a disinfectant during tapping by sprinkling it on spouts and on drill bits only.*

Codex Alimentarius

Ethanol is allowed under Annex 2 (table 2) of the Codex Guidelines when mechanical, physical and biological methods are inadequate for pest control. Further, the Guidelines require that an organic certification body or authority recognize the need for any pest control treatments using ethanol. Ethanol is also listed as an allowed processing aid "which may be used for the preparation of products of agricultural origin." Specifically, ethanol may be used as a solvent in these preparatory operations (Codex, 2013).

European Economic Community Council

Commission Regulation (EC) No 889/2008 provides rules for two different uses of ethanol in organic production in European Union member states. Alcohols, presumably including ethanol, may be used for cleaning and disinfecting livestock building installations and utensils under Annex VII of the regulations. In addition, Annex VIII stipulates the use of ethanol in Section B—Processing aids and other products, which may be used for processing of ingredients of agricultural origin from organic production. This regulation specifically allows the use of ethanol as a solvent in the preparation of foodstuffs of both plant and animal origin.

Japan Ministry of Agriculture, Forestry, and Fisheries

According to the Japanese standards for organic plant production, ethanol may be used in the processing, cleaning, storage, packaging and other post-harvest processes when physical or methods using naturally derived substances are insufficient. The specific crop uses of ethanol are for: (1) controlling noxious animals and plants, and (2) quality preservation and improvement (JMAFF, 2005a). Likewise, ethanol may also be used in the manufacturing, processing, packaging, storage and other processes associated with organic livestock feed when physical or methods utilizing biological function are insufficient for disease and pest control (JMAFF, 2005b). Similar provisions exist for the use of ethanol in the slaughter, dressing, selection, processing, cleaning, storage, packaging and other processes associated with organic livestock products. In addition, "alcohols" are listed as allowed cleaning and disinfecting agents for livestock housing (JMAFF, 2005c). It should be noted that ethanol use is not permitted for the purpose of pest control for plants and agricultural products. For processed foods, ethanol may be used as an additive in the processing of meat products only (JMAFF, 2005d).

International Federation of Organic Agricultural Movements

Under the IFOAM Norms, synthetic ethanol is an approved additive and processing/post-harvest handling aid when organic and natural sources are not available. Synthetic ethanol may be used under the category "crop protectants and growth regulators." Finally, ethanol is approved for use as an equipment cleaner and equipment disinfectant (IFOAM, 2012). As a naturally derived substance, non-synthetic ethanol is always approved for these purposes.

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) There are a number of home, commercial and agricultural uses of ethanol as a sanitizer and disinfectant. Therefore, ethanol falls in the category of "equipment cleansers."

(B) Ethanol may be considered an active or inert ingredient depending on the ethanol concentration and intended use for a specific product. As an inert, ethanol is listed on the US EPA List 4B—Other ingredients for which EPA has sufficient information to reasonably conclude that the current use pattern in pesticide products will not adversely affect public health or the environment (US EPA, 2004). Ethanol is also exempt from the requirement of tolerance when applied to: growing crops or raw agricultural commodities after harvest (40 CFR 180.910); animals (40 CFR 180.930); or antimicrobial pesticide formulation (40 CFR 180.940). These exemptions consider the use of ethanol as in inert (solvent or cosolvent) as well as an active ingredient in food-contact surface sanitizing products (US EPA, 2006).

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)).

Commercial methods for the industrial production of ethanol include chemical synthesis from ethylene and fermentation of sugar, starch or other biomass using either yeast or genetically modified bacterial strains. Other synthetic methods have been demonstrated in the laboratory but not fully developed to commercial scale. These include the hydration of ethylene in the presence of dilute acids, the oxidation of acetylene (H_2C_2) to acetaldehyde (C_2H_4O) followed by hydrogenation of the aldehyde to ethanol, and the Fischer-Tropsch process for converting pressurized synthesis gas (mixtures of carbon monoxide and hydrogen) to various organic compounds. For the purposes of this report, focus is given to commercial production methods currently in practice, with incorporation of relevant insights and developments from the independent literature. Technical information is compiled below for the two main commercial processes, chemical synthesis and fermentation, as well as the final distillation/purification step for industrial ethanol.

Chemical Synthesis

Two main processes exist for the chemical synthesis of ethanol: indirect and direct hydration of ethylene. The indirect hydration process, developed in 1930 by Union Carbide Corp., was the first commercially utilized method for generating ethanol from ethylene. Direct hydration, developed by Shell Chemical Company in 1948 and designed to eliminate the use of sulfuric acid, completely replaced the indirect hydration process for commercial ethanol production in the United States by the early 1970s. However, the old sulfuric acid process is potentially still used in Russia (Logsdon, 2004). Although both the indirect and direct hydration processes are described below, attention should be given to the materials and methods used in the direct hydration of ethylene for the purposes of this report.

Indirect Hydration of Ethylene. This general method, known as the indirect hydration, esterification—hydrolysis, or sulfuric acid process, is based on the initial absorption of large volumes of ethylene ($H_2C{=}CH_2$) in concentrated sulfuric acid (H_2SO_4) (Logsdon, 2004; Kosaric, 2011). The absorption step is carried out by countercurrent passage of ethylene through 95–98% sulfuric acid in a column reactor. Once absorbed, ethylene reacts with the sulfuric acid molecules to form monoethyl sulfate and diethyl sulfate (equations 1 and 2). Cooling is required because the overall absorption/transformation process is exothermic. The reaction mixture is then passed through hydrolyzers where the mixed ethyl sulfate intermediates react with water molecules (H_2O) to yield the desired product, ethanol, and dilute sulfuric acid (equations 3 and 4). In addition, diethyl ether [$(CH_3CH_2)_2O$] is formed as a byproduct via the reaction of diethyl sulfate and in situ generated ethanol. The resulting hydrolysis mixture is separated in a stripping column to give a bottom layer of dilute sulfuric acid and a gaseous ethanol, water, and diethyl ether mixture in the overhead space. Following this separation, the overhead mixture is washed with water or dilute sodium hydroxide and purified by distillation to provide pure ethanol.

Absorption of ethylene in concentrated sulfuric acid and formation of mixed ethyl sulfate intermediates:

$$H_2C{=}CH_2 + H_2SO_4 \rightarrow CH_3CH_2OSO_3H \qquad \text{(eq 1)}$$

$$2\ H_2C{=}CH_2 + H_2SO_4 \rightarrow (CH_3CH_2O)_2SO_2 \qquad \text{(eq 2)}$$

Hydrolysis of ethyl sulfates to ethanol:

$$CH_3CH_2OSO_3H + H_2O \rightarrow CH_3CH_2OH + H_2SO_4 \qquad \text{(eq 3)}$$

$$(CH_3CH_2O)_2SO_2 + 2\ H_2O \rightarrow 2\ CH_3CH_2OH + H_2SO_4 \qquad \text{(eq 4)}$$

$$(CH_3CH_2O)_2SO_2 + CH_3CH_2OH \rightarrow CH_3CHOSO_3H + (CH_3CH_2)_2O \qquad \text{(eq 5)}$$

Direct Hydration of Ethylene. There are two main process categories for production of ethanol through direct hydration of ethylene. Whereas gaseous reactant molecules contact solid or liquid catalysts in vapor-phase processes, liquid or gaseous reactants interact with solid or liquid catalysts in mixed-phase processes. Primary consideration is given to the vapor-phase process since ethanol is generally produced via the vapor-phase hydrolysis of ethylene.

The vapor-phase, direct hydration of ethylene takes place over a catalyst support impregnated with an acidic substance (Logsdon, 2004; Kosaric, 2011). Although the technical and patent literature describes a number of catalysts for ethylene hydration, only phosphoric acid catalysts supported by diatomaceous earth, montmorillonite, bentonite, silica gel, or Volga sandstone are industrially relevant. The use of phosphoric acid (H_3PO_4) on a charcoal support is claimed in one of the earliest patents on vapor-phase hydration of olefins (carbon–carbon double bonds). Shell has used a catalyst composed of phosphoric acid on a porous inert support such as Celite diatomite (diatomaceous earth) in its commercial production of ethanol. To prepare the catalyst, the support material is impregnated with aqueous phosphoric acid concentrations of less than 70% followed by drying to give a final acid concentration of 75–85%.

Ethanol production via the direct hydration of ethylene takes place via a series of chemical reactions (eq 6). Ethylene and deionized water are initially heated to 250–300 ºC at high pressure (6–8 MPa) by passage through a heat exchanger and a superheater. These gaseous reactants are then passed through the reactor, where ethylene adsorbs to the phosphoric acid-impregnated catalyst support. Following adsorption, the phosphoric acid catalyst protonates ethylene, generating a highly reactive species that rapidly reacts with a vapor-phase water molecule. This final transformation affords the desired product, ethanol, with regeneration of the phosphoric acid catalyst. Small amounts of phosphoric acid become incorporated in the gaseous product mixture and are generally neutralized through injection of a dilute solution of sodium hydroxide (NaOH). Crude product mixtures contain 10–25 percent by weight ethanol and are purified via distillation.

$$H_2C{=}CH_2 + H_2O \xrightarrow{catalyst} CH_3CH_2OH \qquad \text{(eq 6)}$$

Fermentation

It is possible to generate ethanol through the fermentation of any material that contains sugar or complex compounds (i.e., carbohydrates) that can be converted to sugar (Logsdon, 2004; Kosaric, 2011). The raw materials used in the manufacture of ethanol via fermentation are generally classified as one of three types of agricultural feedstocks: sugars, starches, and cellulose-based feedstocks. Sugars derived from sugar cane, sugar beets, molasses or fruit can be converted directly to ethanol without an intermediate processing step. Alternatively, starches obtained from grains, potatoes, or root crops must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or microorganisms. Cellulose derived from wood, agricultural residues, or aqueous effluent from pulp and paper mills must likewise be converted to sugars through reaction with strong mineral acids. Once the starches and cellulose materials are transformed to simple sugars, enzymes from yeast and certain bacterial strains can readily ferment these sugars to ethanol. Advancements in bioethanol production and distillation continue to appear in the patent literature (Walker, 2013). Targeted technical information from industry reviews and the independent literature is provided below for the fermentation of starches, cellulosic materials, and sugars using yeast and engineered bacteria.

Starches. Grain products are being increasingly employed as feedstock materials in the fermentative production of ethanol. As such, this section provides technical information on the current state of industrial ethanol fermentation and an outlook of potential methods based on a review of the scientific literature.

Industrial Production

All potable alcohol, most fermentation industrial alcohol, and the vast majority of fuel alcohol are made principally from grains in the United States. The generation of ethanol from starch-based materials such as grain requires two steps: conversion of complex carbohydrates to simple sugars (saccharification) and fermentation of these sugars to ethanol. Industrial processes convert starch to glucose enzymatically using the enzyme, diastase, present in sprouting grain or fungal amylase. Glucose is then fermented to ethanol with the aid of yeast, producing carbon dioxide (CO_2) as a byproduct (Logsdon, 2004). The yeast *Saccharomyces cerevisiae* is exclusively used in fuel and beverage alcohol production. Although genetically engineered yeasts are not currently employed in the ethanol industry, optimization of experimental strains and increasing ethanol demand pressures may lead to future adoption of GM microorganisms for ethanol production (Ingledew, 2011).

Experimental Methodologies

Laboratory-scale ethanol production from starch has been demonstrated using three genetically modified *Saccharomyces cerevisiae* (yeast) strains (Birol, 1998). Two of the strains produce the *Aspergillus awamori* glucoamulase (enzyme that decomposes starch into glucose) together with either the *Bacillus subtilis* or mouse alpha-amylase (enzyme that catalyzes the hydrolysis of starch into sugars) as separately secreted polypeptides. The third strain secretes a particular protein that contains both the *B. subtilis* and *A. awamori* glucoamylase activites. Higher growth rates were observed for all three yeast strains when grown on glucose. However, the yeast strain secreting *B. subtilis* alpha amylase for saccharification showed the most efficient utilization of starch for ethanol production with the lowest levels of accumulating sugars in the medium. It was also observed that ethanol production was comparable for this optimized yeast strain in both glucose- and starch-containing media.

A number of research developments on the engineering of yeast strains for ethanol production have been reported in the open literature since the late 1990s. For example, strains of *S. cerevisiae* were transformed with different combinations of foreign yeast amylase genes (e.g., *Lipomyces kononenkoae*) and *S. fibuligera* glucoamylase gene in an effort to improve the hydrolysis and fermentation of starch using *S. cerevisiae* (Knox, 2004). Optimization studies evaluating the effect of initial glucose supply, colony selection methodology prior to inoculation, and medium formulation on the ethanol yield of these experimental *S. cerevisiae* yeast strains have also been conducted and reported in the independent literature (Altıntaş, 2002; Ülgen, 2002).

In addition to starch and yeast extract, the following substances are commonly added to laboratory-scale fermentation media: citric acid; ammonium sulfate (a common fertilizer agent); potassium phosphate buffering salts (e.g., KH_2PO_4), sulfuric acid (H_2SO_4), and potassium hydroxide (KOH), and a number of trace elements (e.g., calcium and magnesium). Control of bacterial contamination in industrial starch fermentation media is currently accomplished using antibiotics (Ingledew, 2011). For additional information on the use of antibiotics and other antimicrobial agents, see the section below for antimicrobial agents used in the fermentation of raw sugars.

Cellulosic Materials. Both cellulose and starch are polymers of glucose. However, cellulose is much more difficult to hydrolyze due to its crystalline structure and lignin content. Lignocellulose feedstocks for ethanol production include wood chips, waste cereal materials (straw, leaves, stalks, hulls), spent brewers' and distillers' grains, and sugarcane bagasse, and corn stover (Parachin, 2011). High temperature and acid/base/organic solvent treatment are used in combination with a variety of enzyme mixtures for lignocellulose pretreatment and hydrolysis of carbohydrates to monomers (i.e., sugars). Because of the complex nature of carbohydrates present in lignocellulosic biomass, microorganisms capable of fermenting both six-carbon sugars (e.g., glucose) and five-carbon sugars (e.g., xylose) are required for the efficient production of ethanol from these hydrolyzed waste materials materials (Parachin, 2011).

Cellulosic ethanol production is limited to laboratory-scale processes and therefore is not sufficiently developed for industrial purposes. Recent research developments include ethanol production from the simultaneous saccharification and fermentation (SSF) of steam-pretreated corn stover using regular *S. cerevisiae* (Ohgren, 2006) and SSF of whey and rice byproduct substrates (Rocha, 2013). Genetic engineering of several microorganisms, including the bacterium *Clostridium thermocellum*, is being investigated for the combined pretreatment, hydrolysis, and fermentation of lignocellulosic biomass (Parachin, 2011). A variety of other laboratory-scale processes are available in the independent literature. As of 2011, there are no commercial biorefineries in the United States for the conversion of lignocellulosic biomass to fuels such as ethanol (NRC, 2011).

Sugars. Blackstrap molasses, a byproduct of cane sugar manufacture, was the most widely used sugar for ethanol fermentation prior to the late 1970s (Logsdon, 2004). Fermentation is preceded by dilution of molasses to a mash containing ~10–20 weight percent sugar and adjustment of the mash pH to about 4–5 with a mineral acid, typically sulfuric acid. The prepared mash is then inoculated with yeast or bacteria designed to produce large quantities of ethanol. Fermentation is carried out at 20–32 °C for about 1–3 days, depending on the microorganism used. In the United States, molasses fermentation is generally carried out for the production of alcoholic beverages, not industrial sources of ethanol. However, a brief survey of molasses fermentation methods is provided below, along with a discussion of commercially employed antimicrobial agents.

Ethanol production from sugars, both for alcoholic beverages (United States) and industrial purposes (Brazil), involves the fermentation of diluted molasses, cane juice or pure glucose followed by distillation of the fermented media. As a byproduct of cane sugar manufacturing, molasses has been the primary source of fermentable sugars for the rum industry since the 16^{th} century. Yeast strains of the genus *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Candida*, and *Toulopsis* are traditionally used to perform the alcoholic fermentation of diluted molasses (Fahrasmane, 1998). *Saccharomyces cerevisiae*, for example, has provided ethanol yields of 53 g L^{-1} in a medium containing 250 g L^{-1} total reducing sugars (Roukas, 1996). Recently, methods utilizing the bacterial strain *Zymomonas mobilis* have been developed for ethanol production, achieving yields of 55.8 g L^{-1} at a lower sugar concentration of 200 g L^{-1} (Cazetta, 2007).

Molasses is generally less contaminated with bacterial flora than cane juice, as a large portion of the non-sporulated bacteria (i.e., bacteria that do not produce spores) is destroyed during sugar production. Notwithstanding, dry must components are frequently subjected to bacteriostatic or sterilizing thermal (steam) treatments to control any bacterial flora that may otherwise excrete undesired organic compounds into the fermentation medium (Fahrasmane, 1998). The molasses-based fermentation medium may also be treated with small quantities (~0.3 mg/L) of antibiotics, such as penicillin (Borzani, 1957) and tetracycline (Aquarone, 1960). However, the extent of this practice in current ethanol production is uncertain. Bacteriosides such as chlorine dioxide (Sumner, 2011), ammonium bifluoride or quaternary ammonium compounds may also be used to control bacterial contamination (Murtagh, 1999). Finally, acidification of the media to a lower pH (i.e., pH = 4–5) using sulfuric acid (H_2SO_4) generally precedes the fermentation step as a protective measure against microbial contamination (Fahrasmane, 1998). As a result of the distillation step, residues of these antimicrobial substances do not persist in industrial sources of ethanol.

<u>Evaluation Question #3:</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)).

Ethanol may be considered synthetic or natural (nonsynthetic) depending on the commercial process used for its production. The term "synthetic" is defined by the NOP 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, except that such term shall not apply to substances created by naturally occurring biological processes" (7 CFR 205.2). According to this definition and the classification of fermentation as a naturally occurring biological process, ethanol would constitute a nonsynthetic (natural) substance when generated through biological fermentation. However, the potential use of genetically engineered microorganisms and chemical substances not allowed on the National List during the fermentation of starches and sugars should be weighed in determining the status of ethanol from fermentation as nonsynthetic (natural) or synthetic. Ethanol produced through chemical synthesis would be considered a synthetic substance due to the application of synthetic chemicals (reagents and solvents) in both the production as well as the purification/processing of crude ethanol. It is unlikely that residues of chemical precursors/substrates will persist in the final product due to the distillation step (fermentation and synthesis) and chemical/physical properties of the chemical precursors (synthesis).

<u>Evaluation Question #4:</u> 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 of ethanol in soil, water, and the atmosphere. Although ethanol is a volatile organic compound and potentially contributes to the formation of ozone and photochemical smog, large-scale releases of ethanol under the prescribed use pattern in organic livestock production are unlikely. The compiled data also indicate that ethanol is readily biodegradable in all three environmental compartments.

Ethanol may enter the environment as a result of its manufacture, solvent and chemical intermediate uses, and release during the fermentation and alcoholic beverage preparation. Likewise, ethanol is naturally emitted as a plant volatile, microbial degradation product of both plant and animal wastes, and biological fermentation product. Larger production sites minimize the release of ethanol using engineering controls and end-of-pipe abatement systems. Organic wastes from manufacture are also typically incinerated on site or professionally treated using waste contractors. Smaller, farm-scale fermentation manufacturers may not have extensive emissions controls in place, but the volume of ethanol emitted will be low and dispersed for these producers. It is anticipated that the emissions to the environment will likely result from the use of ethanol-containing products, such as commercial sanitizers and disinfectants for consumer use, where applications are open and engineering controls are not utilized for the recovery of released ethanol. Ethanol released to the environment will be predominantly distributed between air and water (UNEP, 2005; HSDB, 2012; US EPA, 2012a; US EPA, 1995).

If released to soils, ethanol may be degraded through volatilization and biodegradation processes. Ethanol is expected to have very high mobility in soils based on its K_{oc} of 2.75. Further, the Henry's Law constant for ethanol (5.0×10^{-6} atm•m^3/mol) indicates that volatilization from moist soil surfaces is likely to be an important fate process. Ethanol may also volatilize from dry soil surfaces based on its vapor pressure. Biodegradation of ethanol occurred with half-lives on the order of a few days in microcosms constructed with low organic sandy soil and groundwater. This result indicates that, in addition to volatilization, biodegradation is an important environmental fate process in soil (UNEP, 2005; HSDB, 2012).

Volatilization and biodegradation are also primary mechanisms for removal of ethanol from water. In agreement with the fate of ethanol in soils described above, ethanol is not expected to adsorb to suspended solids and sediment based on the K_{oc}. The Henry's Law constant for ethanol also indicates that dissolved ethanol is likely to rapidly volatilize from water surfaces. Calculated volatilization half-lives for a model river and lake are five and 39 days, respectively (HSDB, 2012). Rates of aerobic (with oxygen) and anaerobic (without oxygen) microbial ethanol biodegradation are rapid enough that ethanol is not expected to persist in ground or surface waters to any great extent. For example, the biodegradation of ethanol in surface water proceeds with half-lives ranging from hours to a day if the temperature ranges are appropriate (MDEP, 2011). The estimated Bioconcentration Factor (BCF = 3) suggests that there is low potential for bioaccumulation of ethanol in aquatic organisms, such as fish (HSDB, 2012). Based on these collective attributes, it has been concluded that ethanol meets the criteria for being considered readily biodegradable in water (UNEP, 2005).

If released to the air, ethanol will exist as a vapor in the atmosphere due to its relatively high vapor pressure (59 mm Hg at 25 ^{o}C). Ethanol is capable of absorbing radiation and is therefore subject to direct photolysis; however, the primary mechanism for degradation of vapor-phase ethanol is through photochemical oxidation in the presence of atmospheric pollutants (nitrogen and sulfur oxides). Half-lives of 14–15 hours have been determined for nitrous oxide- and sulfur dioxide-mediated photolysis, signifying rapid ethanol degradation in atmospheres polluted with nitrogen and sulfur oxides. Photochemically produced hydroxyl radicals are capable of degrading atmospheric ethanol with a calculated half-lives ranging from 10 hours to three days, depending on the hydroxyl radical concentration and radiation wavelength (UNEP, 2005; HSDB, 2012). As a volatile organic compound (VOC; carbon-based compound that contributes to ozone formation), industrial emissions of ethanol to the atmosphere are regulated by US EPA (US EPA, 2012b) and state agencies, such as the Air Resources Board of California EPA (ARB, 2008).

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)).

This section summarizes ethanol toxicity to eight taxa groups, including mammals, birds, fish, terrestrial and aquatic invertebrates, terrestrial and aquatic plants, and soil microorganisms. Overall, it can be concluded that ethanol is slightly toxic to practically non-toxic to most taxa groups evaluated in the literature.

According to US EPA, ethanol is practically non-toxic (Category IV) based on acute oral and inhalation toxicity tests as well as primary eye and dermal irritation studies (EPA, 1995). High LD_{50} values (i.e., ethanol doses at which 50 percent mortality of test subjects is observed) were determined, which points to the low toxicity of ethanol under these exposure routes. Although there are many repeat dose studies (subchronic and chronic toxicity) reported in the literature for ethanol, the vast majority of these studies were conducted to determine the risk associated with consumption of alcoholic beverages. Most of these studies are therefore based on the oral route of exposure and employ high dosing schemes. The subchronic toxicity of ethanol is considered to be low, with a lowest reported NOAEL (No Observed Adverse Effect Level) of 2,400 mg/kg in rats. Decreased body weights as well as decreased activity and maze learning ability were observed in a chronic toxicity study using rats; however, no treatment related mortalities occurred during the study. Based on bacterial mutation assays, chromosome aberration tests, and cell mutation assays, there is very little evidence available to suggest that ethanol is a genotoxic agent. Likewise, there is no robust evidence of carcinogenicity from *in vivo* studies in laboratory animals (UNEP, 2005).

At high doses such as those from drinking alcoholic beverages, ethanol has been shown to cause adverse effects on the reproductive system, fertility and fecundability in males and females and can elicit developmental toxicity in females (UNEP, 2005). For example, fewer pregnancies were initiated when male rats were administered ethanol in the diet with 10 percent of calories being derived from ethanol for 15 days throughout the mating period. This study was confounded by general toxicity symptoms, including ataxia, lethargy and weight loss. Other studies demonstrated reduced testis and epididymis weights (males) and reduced ovary weight and reductions in oestradiol and progesterone (female) in rats receiving liquid diets containing five percent ethanol for extended periods. The results of developmental inhalation studies showed no indication of teratogenicity (capability of producing fetal malformation) at dose limiting concentrations. Skeletal, brain and heart abnormalities as well as learning impairment was observed in the offspring of maternal rats fed diets containing 25 percent or more ethanol-derived calories. Malnutrition may be a confounding factor in these and related studies since pregnant animals exposed to ethanol typically consume less food than non-alcohol subjects (UNEP, 2005). See Evaluation Question #10 for details regarding Fetal Alcohol Syndrome in humans.

Studies investigating the toxicity of ethanol to other terrestrial organisms are compiled in the US EPA Ecotox database and summarized in the MDEP report (US EPA, 2013b; MDEP, 2011). Ethanol applied to Douglas fir seedlings at concentrations of 10 percent or greater became lethal within a week, and adverse effects were also observed with five and one percent solutions. Ethanol at a concentration of two percent in drinking water had significant effects on blood, brain weight and growth of Japanese quail after seven days of exposure. Honey bees fed solutions of ethanol at five percent and greater exhibited behavioral effects, and mortality was observed with solutions of 50 percent ethanol. A study of ethanol toxicity in the little brown bat provided an LD_{50} range of 3,900–4,400 mg/kg, suggesting that ethanol is slightly to practically non-toxic to this receptor.

Acute toxicity data are available for fish, aquatic invertebrates, algae and microorganisms (UNEP, 2005; US EPA, 2012a). Static and flow-through studies of freshwater fish gave LC_{50} values greater than 1,100 mg/L. Specifically, the 96-hour LC_{50} for *Salmo gairdneri* (rainbow trout) ranges from 11,200–13,000 mg/L, and the same toxicity endpoint for *Pimephales promelas* (fathead minnow) is 13,500–14,200 mg/L. These relatively high lethal concentrations are in accord with ethanol being practically non-toxic to freshwater fish. Likewise, LC_{50} values derived from studies on *Daphnia magna* (freshwater water flea; 48-hour LC_{50} = 12,340 mg/L), *Ceriodaphnia* (freshwater water flea; 48-hour LC_{50} = 5,012 mg/L), *Artemia salina* (brine shrimp; 48-hour LC_{50} = 1,833 mg/L), and *Palaemonetes kadiakensis* (glass shrimp; 96-hour LC_{50} > 250 mg/L) suggest that ethanol is practically non-toxic to slightly toxic to freshwater and marine invertebrates. For aquatic plants, EC_{50} values (ethanol concentration inducing a response on growth rate halfway between baseline and maximum) range from 1,000–11,619 mg/L in a variety of algal species (green algae and marine diatoms) and vascular aquatic plants (duckweed), and a five-day NOEC (no observed effect concentration) in the range of 3,240–5,400 mg/L based on cell count was determined for marine algae. Under US EPA criteria, ethanol would be considered practically non-toxic to aquatic plants (US EPA, 2012a; UNEP, 2005).

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

Considering its volatile nature and long history of production and transportation, releases of ethanol to the environment are inevitable. As such, ethanol has been detected in the air and water surrounding manufacturing and municipal facilities (UNEP, 2005). For example, ethanol and methanol were detected at Point Barrow, Alaska in 68 percent of samples at an average concentration of 0.52 parts per billion over 24 hours. There have also been several instances of ethanol leakage from storage areas and industrial facilities. For example, ethanol has been detected in the groundwater suspected of leachate contamination at 190 ppb, landfill ground water at 58 ppb, and surface water in the Hayashida River, Japan near a leather factory at a concentration of 4,020 ppb (UNEP, 2005).

Large volume ethanol release incidents with substantial environmental impacts generally involve accidents related to transport by rail and boat as well as spills from distilleries (MDEP, 2011). Train derailments have resulted in the release of 60,000–700,000 gallons of ethanol with concomitant fires that burned over the course of 24 hours to several days. In some cases, no environmental impacts beyond fire damage were noted; however, some incident reports indicated impairment of nearby soils and waterways. Likewise, incidents involving spills from distilleries have led to the formation of damaging fires and adverse impacts to aquatic environments. One example in Kentucky involved a 980,000 gallon ethanol spill from a distillery in Lawrenceburg, KY, which resulted in the liquid travelling downhill to the river below and subsequent fish kills within two days of the spill. These fish kills are the result of oxygen depletion that accompanies the microbial (aerobic) degradation of ethanol in the impacted waterways. The toxicity of ethanol to fish, aquatic invertebrates due to oxygen depletion is thus significantly greater than the inherent toxicity of ethanol to these receptors. Lastly, ethanol spills from tanker ships at sea have not resulted in detectible environmental impairment (MDEP, 2011).

Aside from accidental spills, the risk of environmental contamination from released ethanol is minimal. The release of strong acids and bases used in the production of ethanol due to improper handling/disposal could lead to serious environmental impairments and ecotoxicity in both terrestrial and aquatic environments. However, no incidents involving the release of these chemical feedstocks from ethanol production facilities have been reported. Further, small amounts of ethanol are constantly released to the environment from animal wastes, plants, insects, forest fires, and microbes without causing environmental impairment (HSDB, 2012). It is therefore unlikely that large-scale spills and associated environmental contamination will occur under the allowed use of ethanol as a sanitizer and disinfectant in organic livestock production.

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)).

There are no reported chemical interactions between ethanol and other substances used in organic livestock production. As a solvent, ethanol may solubilize and thereby enhance the dermal absorption of various chemical residues (e.g., pesticides) deposited on the skin during agricultural production activities. However, technical information regarding this phenomenon was not identified.

In general, ethanol functions as a disinfectant by denaturing proteins and dissolving lipid membranes. Because proteins are denatured more quickly in the presence of water, enhanced bactericidal activity is generally observed for mixtures of ethanol and water when compared to absolute ethanol, which functions as a dehydrating agent (CDC, 2008). This empirical observation provides qualitative support for the proposed mechanism, which relies heavily upon the ability of ethanol to denature proteins. Ethanol is able to effectively destroy many types of bacterial and viral cells due to this mode of action; however, ethanol is ineffective against bacterial spores because the substance evaporates before it can effective penetrate the membrane and lead to protein denaturation (CDC, 2008).

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)).

The current technical evaluation concerns the use of ethanol as a sanitizer or disinfectant for livestock housing, surfaces and production implements as well as a topical antiseptic for medical treatments in organic livestock production. When used for these purposes, it is unlikely that ethanol will regularly interact with components of the terrestrial agro-ecosystem (i.e., agricultural land). Further, technical information regarding non-target wildlife toxicity resulting from the use of disinfectant products containing ethanol in livestock production is lacking. Any potential leakage of ethanol, particularly large-scale spills, near the agro-ecosystem would be neither routine nor widespread.

Toxicity toward soil-dwelling organisms may result from the use and manufacture of ethanol. Although limited information is available on the toxicity of ethanol on soil bacteria, it has been determined that dilute ethanol solutions can be used as a carbon source to stimulate growth of algae and sulfate reducing bacteria (UNEP, 2005; Pagnanelli, 2012). In contrast, the scientific literature is replete with information regarding the ability of more concentrated ethanol solutions (50–70 percent in water) to kill the bacterial pathogens *Staphylococcus aureus* (Peters, 2013) and *Salmonella* (Møretrø, 2009), among other bacterial and viral microorganisms (CDC, 2008). More concentrated solutions of ethanol are therefore likely to kill beneficial soil bacteria and small invertebrates, such as earthworms.

In addition to soil microorganisms, crops have displayed different responses to dilute ethanol treatments. Studies investigating root growth in onions, germination of lettuce seeds and coleoptile (protective sheath covering the emerging shoot) and respiration in corn plants demonstrated inhibitory effects when subjected to ethanol concentrations of 3,000 mg/L (approximately three percent in water). Other studies, including investigations of respiration in potato tuber tissue and plant growth in oats, girasole, sugar cane and potato, have produced stimulatory and inhibitory effects at low ethanol concentrations (UNEP, 2005). In general, ethanol exposure to terrestrial organisms will be limited to spill situations. The small volumes of ethanol used as a disinfectant should rapidly volatilize and biodegrade. It is therefore highly unlikely that the relatively small volume, controlled applications of ethanol in livestock production would lead to major spills and concomitant adverse effects on the agro-ecosystem.

Accidental release of chemical reagents during the production process may also lead to ecological impairment. Strong acids (e.g., sulfuric acid) and bases (e.g., potassium hydroxide) are used in the chemical synthesis and, to a lesser extent, the fermentative preparation of ethanol. Improper use or disposal of acidic and basic reagents during the production of ethanol could affect both the pH and chemical composition of the soil, potentially resulting in physiological effects on soil organisms. Likewise, improper treatment and subsequent release of synthetic wastes and fermentation broths could impair soil populations. These types of spill scenarios are unlikely due to manufacturing safeguards.

Large scale releases of ethanol-based disinfectants near rivers, ponds and lakes could lead to population level impacts due to oxygen depletion and subsequent fish kills (MDEP, 2011). Otherwise, technical information regarding the potential impacts of ethanol on endangered species, populations, viability or reproduction of non-target organisms and the potential for measurable reductions in genetic, species or ecosystem biodiversity, is lacking.

<u>Evaluation Question #9:</u> 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)).

Ethanol is not expected to be persistent or hazardous to the environment under the prescribed use pattern as a sanitizer or disinfectant in organic livestock production (US EPA, 1995; UNEP, 2005; MDEP, 2011; HSDB, 2012). Ethanol generally partitions between the atmosphere and water. It is readily biodegradable and is not expected to accumulate in soils, plant material or animal tissues. In the air, ethanol is predicted to degrade rapidly in atmospheres where nitrogen and sulfur oxides are present. Although infrequent, large spills of ethanol from transportation vessels (rail and boat) and distilleries have led to ecological impairment due to subsequent fires and oxygen depletion in nearby waterways. Spills of chemical feedstocks used in the production of ethanol, such as strong acids and bases, could adversely affect terrestrial and aquatic systems; however, specific occurrences have not been documented and are unlikely due to modern manufacturing safeguards.

According to US EPA and World Health Organization (WHO) literature reviews, ethanol is practically non-toxic to slightly toxic to most biological receptors (US EPA, 1995; UNEP, 2005; MDEP, 2011). For mammals, ethanol is practically non-toxic (Category IV) based on acute oral and inhalation toxicity tests as well as primary eye and dermal irritation studies. In addition, *in vitro* and *in vivo* animal studies have demonstrated that ethanol is not a mutagenic or carcinogenic agent. Laboratory rats exposed to extreme doses of ethanol (≥ 10 percent of calories derived from ethanol) exhibited adverse reproductive effects; however, malnutrition was identified as a likely confounding factor in these studies. With the exception of one study in Japanese quail, dilute ethanol solutions (≤ 10 percent in water) are non-toxic to slightly toxic to terrestrial organisms. Although ethanol is not particularly toxic to aquatic organisms, such as fish, aquatic invertebrates and aquatic plants, oxygen depletion due to large ethanol spills could lead to population-level toxicity and death for these receptors. It is unlikely that the current use pattern of ethanol in organic livestock production would lead to significant ethanol exposure in the agro-ecosystem.

Intensive corn farming for the production of fuel ethanol has also been linked to water quality impairment near agricultural areas. Specifically, nitrogen and phosphorous fertilizers that escape from farmland during rain events are a threat to water bodies because elevated levels of these nutrients stimulate the growth of algae through a process known as eutrophication (UCS, 2011; Kim, 2008). Potential consequences of this nutrient overload and concomitant algal bloom include the transformation of clear, healthy water to slimy green water, altered aquatic vegetation and fish kills. Much like the hypoxia (oxygen depletion) that accompanies large ethanol spills to rivers and lakes, oxygen in the water is consumed as the algal blooms die and decompose, which kills fish and other marine life. These blooms also block sunlight, resulting in the death and decomposition of submerged plant life, thus exacerbating the level of hypoxia. Scientists believe that large "dead zones," or areas deprived of oxygen, expanding downstream from corn production regions of the United States (UCS, 2011). Ethanol derived from the fermentation of cornstarch is primarily used in fuels. Therefore, it is unlikely that the small amount of ethanol produced for use in organic production would contribute to the environmental impairment through eutrophication.

<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)).

In general, ethanol is characterized as not acutely toxic to humans by the oral, dermal and inhalation routes of exposure (US EPA, 1995; UNEP, 2005; MDEP, 2011). This observation is not surprising considering the ubiquitous nature of ethanol in hygiene products, fragrances, cosmetics, adhesives, and other consumer products. Likewise, small amounts of ethanol are expressed naturally within the human body. Human volunteers continuously exposed to ethanol-saturated patches under occlusive patches did not exhibit any signs of dermal irritation through day 14; however, edema (fluid accumulation under skin) and erythema (skin redness) were observed from days 15–21 of exposure (US EPA, 1995). Ethanol is considered an eye irritant since direct contact of liquid ethanol on the human eye causes an immediate sensation of burning and stinging. Air concentrations of ethanol in excess of 5,000 parts per million (ppm) are likely to induce lacrymation and coughing. The vast majority of animal studies are conducted orally and designed to understand the toxicity of ethanol at quantities likely to be consumed by humans in alcoholic beverages. Although not entirely relevant to the evaluation of ethanol toxicity from exposure to ethanol-based disinfectants, these studies support the conclusion that ethanol is slightly to practically non-toxic to humans at moderate to low doses. See Evaluation Questions #5 for additional information regarding ethanol toxicity studies conducted in laboratory mammals.

Ethanol has also been evaluated for mutagenic and carcinogenic activity. Bacterial mutation and assays chromosome aberration tests suggest that ethanol does not directly react with DNA or lead to other chromosomal irregularities. However, chromosomal aberrations studies have been criticized for not including exogenous mammalian cells as the metabolic activation system. Weak mutagenic effects were detected in only one mammalian cell mutation assay at very high ethanol concentrations (UNEP, 2005). There is little evidence to suggest that ethanol is genotoxic, although it may have a limited capacity to induce genetic changes in humans only at very high doses achievable by deliberate oral ingestion.

Epidemiological studies clearly indicate that drinking alcoholic beverages is causally related to cancers of the oral cavity, liver and other organs comprising the digestive and respiratory systems. Indeed, ethanol in alcoholic beverages is considered a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) and was added to the California Proposition 65 List as a human carcinogen in 2011 (Bevan, 2009; CA EPA, 2013). The etiology of these cancers is likely to proceed via a mechanism involving persistent irritation of the target tissues from high local concentrations of liquid ethanol followed by hyperplasia (proliferation of cells) and ultimately tumor formation (UNEP, 2005; Bevan, 2009). Small amounts of ethanol are inhaled and therefore rapidly and effectively eliminated from the body. Considering the known information on uptake of ethanol by the inhalation and dermal routes in addition to the lack of genotoxicity, it has been concluded that occupational exposure to ethanol and use of ethanol in consumer products does not pose a cancer hazard. The potential for ethanol-induced carcinogenesis is summarized in the 2009 Occupational Exposure Risk Assessment (Bevan, 2009):

> *In 1998, IARC classified alcoholic beverages as Group 1 carcinogens, concluding that the occurrence of malignant tumors of the oral cavity, pharynx, larynx, esophagus, liver, colorectum, and breast is causally related to the consumption of alcoholic beverages. The cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus) are most likely produced by direct contact of epithelial cells with alcohol…As these cancers are most probably specific to oral consumption, they are not considered to be of specific relevance in assessing cancer risk due to occupational exposure to ethanol.*

Ethanol is recognized as a human developmental neurotoxicant, contributing to the development of Fetal Alcohol Syndrome. The effects of this syndrome include altered prenatal growth and morphogenesis, characterized by severe growth retardation, mental retardation and reduced brain size. In general, these effects are associated with high (several grams per day) maternal consumption of ethanol in the form of alcoholic beverages (US EPA, 1995). Since 1987, "ethyl alcohol in alcoholic beverages" has been listed as a human developmental toxicant on the California Proposition 65 List (CA EPA, 2013). Fetal exposure to ethanol is not expected under the prescribed use of ethanol as a disinfectant and sanitizing agent in agricultural settings and therefore is not a concern for the current evaluation of ethanol in organic livestock production.

<u>Evaluation Question #11:</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)).

Technical information regarding the efficacy of natural, nonsynthetic agricultural commodities or products that could substitute for ethanol as a disinfectant in organic livestock production is limited. Nonsynthetic (natural) sources of ethanol may substitute for synthetic ethanol disinfectants. Likewise, natural sources of organic acids (e.g., acetic acid, citric acid and lactic acid) may also be used for disinfection. Certain essential oils exhibit antiviral and antibacterial properties, and are commonly used in homemade hand sanitizers. Examples of the strongest and most commonly used antiseptic essential oils include clove oil, melaleuca oil, and oregano oil. In addition, pine oil, basil oil, cinnamon oil, eucalyptus oil, helichrysum oil, lemon and lime oils, peppermint oil, tea tree oil, and thyme oil are also used as antiseptic substances. Aloe vera contains six antispectic agents (lupeol, salicylic acid, urea nitrogen cinnamonic acid, phenols and sulfur) with inhibitory action on fungi, bacteria and viruses (Surjushe, 2008). Depending on the required potency and intended application, essential oils may be used in pure form or as a mixture in carrier, such as water. University agricultural extension publication repositories contained no articles related to the practice of using essential oils as disinfectants or any performance data for these oils relative to ethanol. It is therefore uncertain whether essential oil mixtures could serve as viable, naturally derived alternatives to ethanol-based disinfectants for livestock housing, equipment surfaces, and animal skin in livestock production.

A wide variety of synthetic substances are available for sanitizing and disinfecting livestock housing and production equipment, and for topical antisepsis during medical treatments. Acids (acetic acid), alcohols (ethanol and isopropanol), aldehydes (formaldehyde and glutaraldehyde), alkalis (sodium or ammonium hydroxide, sodium carbonate, calcium oxide), Biguanides (chlorhexidine), chlorine compounds (sodium hypochlorite), iodine compounds and complexes (iodophors), oxidizing agents (hydrogen peroxide and peracetic acid), phenols, and quaternary ammonium compounds are commonly used as part of disinfection regimens in veterinary and animal housing environments (Dvorak, 2008). In addition, many of these chemical disinfectants are used as disinfectant solutions in footbaths (i.e., boot-washing stations) and for the disinfection of equipment and other surfaces. Not all of these substances, however, are approved for use in organic livestock production. The USDA recommends sodium hypochlorite, acetic acid, sodium carbonate, and/or sodium hydroxide for controlling foot-and-mouth disease outbreaks (USDA, 2005). Additionally, hypochlorite or other suitable disinfectants are commonly used on automatic feeding machines and sodium hydroxide is used against classic swine fever in Chile (Fotheringham, 1995). Hydrogen peroxide is also a widely used topical antiseptic in medical operations. Utilizing a combination of disinfection chemistries is not only advantageous for addressing various situations (i.e., target pest, surface, etc.), but also necessary for preventing microbial resistance (Dvorak, 2008; USDA, 2005).

In addition to ethanol (7 CFR 205.603(a)(1)(i)), the National List of Allowed and Prohibited Substances permits the use of the following synthetic materials as disinfectants, sanitizers, and medical treatments in organic livestock production:

- **Isopropanol** ($(CH_3)_2CHOH$) 7 CFR 205.603(a)(1)(ii)
- **Chlorhexidine** 7 CFR 205.603(a)(6)
    - Allowed for surgical procedures conducted by a veterinarian. Allowed for use as a teat dip when alternative germicidal agents and/or physical barriers have lost their effectiveness.
- **Chlorine Materials**
    - Allowed for disinfecting and sanitizing facilities and equipment.

        - **Calcium hypochlorite** ($Ca(ClO)_2$) 7 CFR 205.603(a)(7)(i)
        - **Chlorine dioxide** (ClO_2) 7 CFR 205.603(a)(7)(ii)
        - **Sodium hypochlorite** ($NaClO$) 7 CFR 205.603(a)(7)(iii)
- **Hydrogen peroxide** (H_2O_2) 7 CFR 205.603(a)(13)
- **Iodine** 7 CFR 205.603(a)(14)
- **Peroxyacetic acid/peracetic acid** 7 CFR 205.603(a)(19)
    - Allowed for sanitizing facility and processing equipment.
- **Phosphoric acid** (H_3PO_4) 7 CFR 205.603(a)(20)
    - Allowed as an equipment cleanser, provided the substance does not directly contact organically managed livestock or land.

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

Sterilization methods are critical for preventing the spread of deleterious bacterial, fungal and viral pathogens on production surfaces (i.e., livestock housing and equipment) and animal skin. In addition to chemical disinfectants, heat, light and radiation may also be used to reduce or eliminate microorganisms in livestock housing environments (Dvorak, 2008). Heat is one of the most established physical controls against deleterious microorganisms and is a fairly reliable sterilization method. Moist heat is most effective (e.g., steam) and requires less time, but dry heat (e.g., flame or baking) may also be used for inactivating microorganisms. Ultraviolet light is also capable of inactivating viruses, bacteria and fungi, but is limited by its lack of surface penetration. Less frequently used forms of radiation include microwaves and gamma radiation. Although thermal treatments may be effective for disinfecting certain pieces of equipment, other strategies would be required for eliminating microbes from animal housing surfaces and animal skin. Frequently changing the animal's bedding and/or using inorganic bedding (i.e., sand) may also reduce bacteria levels in livestock housing (Dvorak, 2008; Fotheringham, 1995). Likewise, removing debris from the production areas and ensuring the cleanliness of equipment are important steps for minimizing microorganism populations on and around livestock.

Microbial control regimens that exclude chemical disinfection are generally not advised, particularly for pathogens potentially present on animal skins and equipment surfaces. Although alternative practices are not available, a variety of alternative substances are presented in Evaluation Question #11.

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