Choline

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
2-hydroxy-N,N,N-trimethylethanaminium
(2-hydroxyethyl)trimethylammonium
(2-hydroxyethyl)trimethylammonium chloride
(2-hydroxyethyl)trimethylammonium-L-(+)-tartrate salt

Other Names:
Choline ion
Choline chloride
Choline bitartrate

Trade Names:
Vitashure®
Vitacholine™
Memor-C™
C-Salt™

CAS Numbers:
62-49-7 (choline)
67-48-1 (choline chloride)
87-67-2 (choline bitartrate)

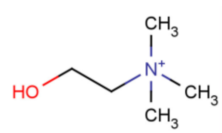
Other Codes:
EC# 200-535

Characterization of Petitioned Substance

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

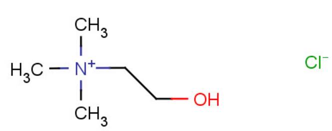
Choline is a positively charged ionic compound with the formula $[C_5H_{14}NO]^+$. It is a common dietary component and conditionally essential nutrient (essential depending upon life stage, gender, and other factors) for humans with many important functions in the body. Dietary choline is found in the form of free choline or choline-containing compounds such as phosphatidylcholine. Choline has been petitioned for use in processing of foods labeled as "organic" or "made from organic (specified ingredients or food group(s))" in its salt forms, choline bitartrate and choline chloride. The molecular structures of the choline ion, choline chloride, and choline bitartrate are shown in figures 1, 2, and 3, respectively.

Figure 1. Molecular Structure Choline (CAS# 62-49-7)



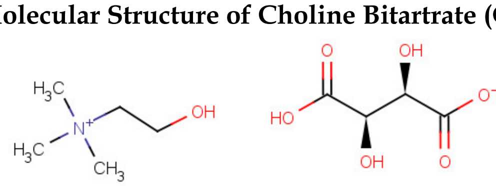
Source: ChemIDplus Advanced, 2011

Figure 2. Molecular Structure of Choline Chloride (CAS# 67-48-1)



Source: ChemIDplus Advanced, 2011

Figure 3. Molecular Structure of Choline Bitartrate (CAS# 87-67-2)



Source: ChemIDplus Advanced, 2011

Properties of the Substance:

Choline chloride is a colorless or white crystalline powder or crystals with a slight amine (fish-like) odor (Swanson and Evenson, 2002). It is hygroscopic (will absorb moisture from the air) and is very soluble in water. When dissolved in water, it dissociates into the positively charged choline ion and the negatively charged chloride ion (OECD, 2004). Choline bitartrate is a white crystalline powder with an acidic taste and faint amine odor (or odorless) (Swanson and Evenson, 2002). It is also hygroscopic and freely soluble in water (HSDB, 2008b).

Specific Uses of the Substance:

Choline compounds are widely distributed in common foods and are particularly high in liver, eggs, wheat germ, and human milk (Zeisel, 2006). The salt forms of choline (chloride and choline bitartrate) can be used as dietary supplements either alone or in processed foods. Choline chloride is petitioned, in part, for use as a partial replacement and flavor enhancer of sodium chloride in order to reduce the sodium content of snacks, baked goods, and other processed organic foods (Balchem Corporation, 2011; Fielding et al., 1992). Additional petitioned uses of choline salts in handling/processing of organic foods include but are not limited to the following products (Nestlé Infant Nutrition, 2011; Balchem Corporation, 2011):

- Infant formula and fortified infant and toddler foods;
- Beverages and beverage bases (nonalcoholic, including coffee and tea);
- Baked goods and baking mixes;
- Breakfast cereals;
- Milk and products of milk origin;
- Dairy product analogs;
- Egg products and egg dishes;
- Fats, oils, shortenings and dressings;
- Grain products and pastas;
- Meat, poultry, and fish products;
- Seasonings and flavorings;
- Fresh and processed vegetables and vegetable juices;
- Plant protein products, reconstituted; vegetable protein, and meat analogs and extenders;
- Fresh and processed fruits and fruit juices;
- Nut and nut products;
- Snack foods;
- Gravies and sauces;
- Soups and soup mixes;
- Condiments and relishes;
- Sweet sauces, toppings, and syrups;
- Jams and jellies; and
- Pet food.

Eating a varied diet should provide sufficient amounts of choline for the average, healthy adult. However, some individuals, in particular those who do not consume whole eggs (with yolks) or milk, may not consume enough choline in their diet (Linus Pauling Institute, 2008). Choline salts are added to foods and beverages based on the current dietary recommendations for choline set forth by the Institute of Medicine (IOM, 1998). The Adequate Intake (AI) values and Tolerable Upper Intake Levels (UL) for choline are summarized in Table 1.

Table 1. Adequate Intake (AI) and Tolerable Upper Intake Levels (UL) for Choline

Age Group		Choline AI	Choline UL
Infants, 0–6 months		125 mg/day	Not determinable
Infants, 7–12 months		150 mg/day	
Children, 1–3 years		200 mg/day	1 g/day
Children, 4–8 years		250 mg/day	
Children, 9–13 years		375 mg/day	2 g/day
Adolescents, 14–18 years	Boys	550 mg/day	3 g/day
	Girls	400 mg/day	
Adults	Men	550 mg/day	3.5 g/day
	Women	425 mg/day	
Pregnancy		450 mg/day	
Lactation		550 mg/day	

Source: IOM (1998)

According to the petition by Balchem Corporation (2011), the addition of choline chloride or choline bitartrate to infant formula typically falls within the range of 7 to 50 mg choline per 100 kilocalories of formula [equivalent to 47 to 335 mg choline per liter (Nestle Infant Nutrition, 2011)]. It should be noted that the American Society of Nutritional Sciences recommends a minimum choline level of 7 mg/100 kcal in infant formula based on the lower end of the range for the choline content of human milk; the recommended maximum level of choline is 30 mg/100 kcal based on based on extrapolation from adult data on the safe level of intake and potential age-related metabolic differences (Raiten et al., 1998). Human breast milk contains about 160 to 210 mg total choline per liter as choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin (IOM, 1998). According to studies by Holmes-McNary et al. (1996), cow milk and cow-based infant formulas not supplemented with choline salts contain similar choline component levels as human milk. Soy infant formulas contain more free choline and phosphatidylcholine but much less sphingomyelin than bovine or mature human milk (IOM, 1998). Choline salts are added to other infant foods such as cereals and pureés at levels to provide a "significant fraction of the Adequate Intake (AI)" of choline for infants over the age of six months (Nestlé Infant Nutrition, 2011). The European Society for Pediatric Gastroenterology and Nutrition and the American Academy of Pediatrics Committee on Nutrition have no specific recommendations for infant and child choline intake (Thureen and Hay, 2006).

According to one of the petitioners, Balchem Corporation (2011), when used as a partial replacement and flavor enhancer of sodium chloride, choline chloride is added at sufficient levels to replace 30 to 50% of the weight of sodium chloride normally present in certain processed foods (Balchem Corporation, 2011). No other sources of information discussing salt replacement levels have been identified.

Other possible applications of choline salts in organic handling/processing include dry and wet pet foods. As described in the "Historic Use" section below, choline chloride is currently used as a source of dietary choline in a variety of currently marketed organic dry and canned pet foods specifically designed for dogs and cats.

<u>Approved Legal Uses of the Substance:</u>

Choline chloride and choline bitartrate are affirmed as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA) when used as nutrients in food for human consumption in accordance with good manufacturing practice (21 CFR 182.8252, 8250). Based on authoritative statements made by the Institute of Medicine, FDA permits manufacturers to use nutrient content claims for choline on food labels (U.S. FDA, 2001). Non-milk-based infant formulas for sale in the U.S. must contain at least 7 mg choline per 100 kilocalories to use a nutrient content claim (21 CFR 107.100(a)); however there is no maximum level prescribed in this regulation. Choline addition to milk-based infant formulas is permitted but not required by FDA (21 CFR 107.100).

The use of choline chloride as a partial salt replacement and flavor enhancer of sodium chloride in processed foods is not covered under 21 CFR 182.8252 (i.e., not affirmed as GRAS). One of the petitioners, Balchem Corporation, has obtained a letter from USDA Food Safety and Inspection Service (FSIS) stating that FSIS has no objection to the use of choline chloride or the conditioned choline chloride product C-Salt™ (with 2% added magnesium stearate) as a direct replacement for sodium chloride in meat and poultry products (excluding eggs), including processed and ready-to-eat products, provided the use level of choline chloride does not exceed 1200 ppm (Balchem Corporation, 2011). This information could not be verified.

Lecithin (a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol) is a direct food substance affirmed as GRAS by FDA with no limitation other than good manufacturing practice (21 CFR 184.1400).

Choline chloride and choline bitartrate are also classified GRAS by FDA when used as nutrients and/or dietary supplements in animal drugs, feeds, and related products in accordance with good manufacturing or feeding practice (21 CFR 582.5250, 5252). In addition, choline xanthate may be safely used as a choline supplement in animal feed for poultry, ruminants, and swine in accordance with good feeding practice (21 CFR 573.300), and iron-choline citrate complex may be safety used as a source of iron in animal feed (21 CFR 573.580). Iron-choline citrate complex is permitted in conventional foods for special dietary use only (21 CFR 172.370). Choline in the form of choline bitartrate, choline chloride, ferric choline citrate, or choline xanthate may be used in organic livestock feed per 7 CFR 205.603(d)(3).

Several pharmaceutical drug products regulated by FDA contain choline compounds (U.S. FDA, 2011). Succinylcholine chloride, a skeletal muscle relaxant, is approved for use as an adjunct to general anesthesia, to facilitate tracheal intubation, and to provide muscle relaxation during surgery. Ophthalmic solutions containing acetylcholine chloride are approved for use during cataract surgery and other eye surgeries. Methacholine chloride is approved as a bronchoconstrictor for diagnostic purposes only when administered via inhalation. Choline fenofibrate capsules are approved for the treatment of elevated triglycerides, but the active portion of this drug product is fenofibric acid and not choline (U.S. FDA, 2011). Choline salicylate is permitted by FDA as an internal analgesic, antipyretic, and anti-rheumatic drug product for over-the-counter human use (21 CFR 201.326).

Action of the Substance:

Choline chloride and choline bitartrate are most often added to foods as a supplemental source of the nutrient choline. Choline chloride may also serve as a flavor enhancer and replacement for sodium chloride (Fielding et al., 1992). There is no indication that choline salts serve any other technical functions when added to foods; however, the food additive lecithin, which contains phosphatidylcholine, is commonly used as an emulsifier in processed foods (Song and Zeisel, 2005). Phosphatidylcholine is one of the surface-active components in lecithin that contributes to its emulsifying performance. Emulsifiers help to join together oily and aqueous phases of food because their molecules contain two parts: a hydrophilic part that is attracted to water molecules and a lipophilic part that is attracted to fats (Mahungu and Artz, 2002).

Dietary choline is absorbed into the body from the small intestine (IOM, 1998). Pancreatic enzymes can release free choline from choline compounds present in the diet (e.g., phosphatidylcholine, phosphocholine, glycerophosphocholine, and sphingomyelin). Choline is also acquired by *de novo* synthesis in the body (i.e., the synthesis of complex molecules from simple molecules). This pathway occurs mostly in the liver. Choline is transported to various tissues in the body where it accumulates, particularly in the liver and kidneys. It is transported across the blood-brain barrier. In the nervous system, choline accelerates the synthesis and release of acetylcholine, an important neurotransmitter for memory storage, muscle control, and other functions. Choline also functions as a precursor to phospholipids that have important functions in cell membranes, intracellular signaling, and the removal of cholesterol and lipids from the liver. Choline is also a precursor other biological molecules including sphingomyelin, platelet activating factor, and betaine. Betaine is used by the liver for metabolism by the kidney to balance osmotic pressure (IOM, 1998).

Choline is used as a methyl donor in the liver to aid in metabolism, as a precursor of the neurotransmitter acetylcholine which is important for memory and other nervous system functions (Song and Zeisel, 2005). Furthermore, choline is involved in lipid and cholesterol transport and metabolism, and it is a constituent of all cell membranes (Institute of Medicine, 1998; Song and Zeisel, 2005).

Choline also interacts with methionine, folate, and other methyl-group donors while being metabolized in the body. Research has shown that choline-deficient diets in rats lead to 31 to 40% decreases in hepatic folate content; which was reversible when choline was replaced. Additionally, rats fed diets deficient in both choline and methionine had folate levels half of controls after five weeks (IOM, 1998). This research indicates that the presence of choline is important in maintaining adequate levels of other essential nutrients like folate.

Combinations of the Substance:

Lecithin naturally contains phosphatidylcholine (one of the primary forms of choline), which means that choline is a component of lecithin. According to 7 CFR § 205.605, lecithin (bleached) is allowed as a synthetic substance in foods labeled as "organic" or "made with organic (specified ingredients of food group(s))." Unbleached, nonsynthetic, de-oiled lecithin is allowed as an ingredient in or on processed products labeled as "organic" (7 CFR § 205.606; NOSB, 2009B). Bleached lecithin is expected to be removed from the National List, according to a recent NOSB recommendation (NOSB, 2009a).

Another available combination of the substance is the conditioned choline chloride product C-Salt™ (with 2% magnesium stearate), which is used as a sodium chloride replacer (Balchem Corporation, 2011). Synthetic magnesium stearate is included on the National list "for use only in agricultural products labeled "made with organic (specified ingredients or food group(s))," prohibited in agricultural products labeled "organic" (7 CFR § 205.605(b))

Status

Historic Use:

Choline was first discovered in the 1860s (Swanson and Evenson, 2002); however its role in nutrition was not known until the 1930s (Balchem Corporation, 2005). It was officially recognized as a nutrient essential to life in 1998 by the Institute of Medicine (IOM, 1998). Since that time, there has been debate over whether choline is an essential component of the diet, because it is synthesized *de novo* in the body (OECD, 2004); however, recent studies have estimated that average intakes for several different U.S. populations are well below the Adequate Intake (AI) levels established by the IOM for older children, men, women, and pregnant women (Jensen et al., 2007).

The history of the legal use of choline in organic agriculture has revolved around uncertainty over the nutritional status of choline because it is neither a vitamin nor a mineral, and there are conflicting opinions regarding its necessity in human nutrition. In 1995, the NOSB wrote "The Use of Nutrient Supplementation in Organic Foods" for the Secretary of the USDA, which stated (USDA, 2011):

> *Upon implementation of the National Organic Program, the use of synthetic vitamins, minerals, and/or accessory nutrients in products labeled as organic must be limited to that which is required by regulation or recommended for enrichment and fortification by independent professional associations.*

The NOSB clarified that the term "accessory nutrients" meant "nutrients not specifically classified as a vitamin or a mineral but found to promote optimum health." However, confusion arose after the National List was established because an additional annotation (7 CFR §205.605(b)) stated, "Nutrient Vitamins and Minerals, in accordance with 21 CFR 104.20, Nutritional Quality Guidelines for Foods, would be allowed for organic agriculture (USDA, 2011)." Originally, the NOP interpreted that under 21 CFR 104.20(f), which states, "Nutrient(s) may be added to foods as permitted or required by applicable regulations established elsewhere in this chapter," choline salts and other nutrients not specifically listed in the regulation were permissible. However, after further discussion with the FDA, a memorandum (USDA, 2010) from NOP to the NOSB clarified that 21 CFR 104.20(f) pertained only to substances listed in 21 CFR 103.20(d), which does not include choline salts. See "OFPA, USDA Final Rule" for more information.

Choline chloride and choline bitartrate are ingredients currently used in many milk-based and non-milk-based organic infant formulas marketed in the U.S. For example, at least one of these choline compounds is used in Earth's Best Organic Infant Formula with Iron, Similac® Organic Infant Formula, Baby's Only Organic® Soy Formula, and Parent's Choice™ Organic Infant Formula (Earth's Best Organic, 2011; Abbott Laboratories, 2011; Nature's One, Inc., 2011; Parent's Choice Infant Formula, 2011).

Choline chloride is also an ingredient currently used in many organic dry and canned pet foods marketed in the U.S., for example, PetGuard® Organics Organic Lifepath™ dry dog food and Organic Chicken and Vegetable entrée canned dog food, Newman's Own® Organics premium dog and cat foods, and Karma Organic dry dog food (PetGuard Co., 2011; Newman's Own Organics, 2011; Natura Pet Products, Inc., 2011).

Choline chloride has been used as a livestock feed additive since the 1930s (OECD, 2004). According to the petitioner, Balchem Corporation (2011), choline chloride and choline bitartrate currently are used as livestock feed additives for all species in conventional and organic farming . Choline chloride is used as a livestock supplement more often than choline bitrartrate, and is either added to feed premixes, added directly in the feed, or added to water provided to the animals.

OFPA, USDA Final Rule:

Choline is not specifically included on the National List as a synthetic allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))" (7 CFR 205.605(b)). The NOP final rule limits "vitamins and minerals" allowed for use in organic products to those in the FDA Nutritional Quality Guidelines for Food (21 CFR 104.20(d)(3)), which does not include choline or its salts. There has been confusion over the interpretation of the NOP regulations with regard to certain nutritive supplements, as described in the "Historic Use" Section. Currently the allowed "vitamins and minerals" do not include several nutrients considered important in specific foods, such as arachidonic acid (ARA) single-cell oil, docosahexaenoic acid (DHA) algal oil, sterols, taurine, and choline.

Choline was listed as a vitamin for consideration in the 1995 Technical Advisory Panel (TAP) Report for Nutrient Vitamins (Montecalvo and Theuer, 1995); however the specific properties, manufacturing methods, uses, and actions of choline were not described in the TAP report and no comments were made by the TAP reviewers.

Choline is a synthetic nutrient that may be used in organic livestock production based on 7 CFR § 205.603(d)(3), which states that vitamins and minerals may be used in livestock feed for enrichment or fortification provided they are FDA approved; however, the producer must not provide feed supplements or additives in amounts above those needed for adequate nutrition and health maintenance for the species at its specific stage of life (7 CFR § 205.237(b)(2)). Organic livestock feed may be supplemented with choline bitartrate, choline chloride, ferric choline citrate, or choline xanthate (7 CFR § 205.603(d)(3); 21 CFR 573.300).

International

Choline is not specifically listed as a substance permitted for use in organic production by the Canadian General Standards Board (CGSB, 2011). However, because choline is a conditionally essential nutrient, it may be permitted as a non-organic ingredient in certain organic processed foods based on the following statement: "Minerals (including trace elements), vitamins and similar isolated ingredients shall not be used except where legally required or a dietary or nutritional deficiency can be demonstrated and shall be documented" (CGSB, 2011). The allowance for choline under this regulation may apply because recent studies have estimated that average intakes for some populations are well below the Adequate Intake (AI) levels established by the IOM, including for older children, men, women, and pregnant women (Jensen et al., 2007). Canadian Food and Drug Regulations require infant formula to contain at least 12 mg of choline per 100 kilocalories (Section B.25.054(1)(a)(vii) of the Food and Drug Regulations: Health Canada, 2011); therefore, organic infant formulas for sale in Canada contain supplemental choline.

Choline is not specifically listed as a permitted substance for use in the processing of organic food by the Commission of the European Communities. While minerals (trace elements included), vitamins, amino acids, and micronutrients are allowed in the processing of organic food, they are only authorized if their use is legally required in the foodstuffs in which they are incorporated (Commission of the European

Communities, 2008). For example, European regulations state that ready-to-use or reconstituted infant formula containing soy must contain at least 7 mg choline (and no more than 50 mg choline) per 100 kilocalories (Commission Directive 2006/141/EC: Commission of the European Communities, 2006). Choline chloride, choline citrate, and choline birtartate are listed as permitted forms of choline for use in infant formula.

Choline is not listed as a permitted substance for use in organic food by the CODEX Alimentarius Commission. Minerals (including trace elements), vitamins, essential fatty and amino acids, and other nitrogen compounds are permitted for use as food additives in organic processed foods only when their use is legally required in the food products in which they are incorporated (CODEX Alimentarius Commission, 2001). The Codex Standard for Infant Formula states that infant formula must contain a minimum of 7 mg/100 kcal and provides a guidance upper level of 50 mg/100 kcal (Codex Alimentarius, 1981). The most recent Codex General Standard for Food Additives, which applies to conventional foods, lists "choline salts and esters" (INS No. 1001) as food additives allowed in a variety of food categories under good manufacturing practices (CODEX Alimentarius Commission, 2011).

Choline is not specifically included on the International Federation of Organic Agriculture Movements (IFOAM) list of approved food additives and processing aids for use in organic processing (IFOAM, 2006). The IFOAM Norms state that, "Minerals (including trace elements), vitamins and similar isolated ingredients shall not be used unless their use is legally required or where severe dietary or nutritional deficiency can be demonstrated" (IFOAM, 2006).

The Japanese Agriculture Standard for Organic Processed Foods does not list choline as an allowed food additive in organic processed foods (Japanese Ministry of Agriculture, Forestry and Fisheries, 2006).

Evaluation Questions for Substances to be used in Organic Handling

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

Choline is synthesized in aqueous solution by a chemical reaction of trimethylamine and ethylene oxide (HSDB, 2008a). The petition by Nestlé Infant Nutrition (2011) states that this reaction takes place at 40ºC (104ºF) in a closed system and is followed by distillation and recovery of unreacted trimethylamine. The resulting choline hydroxide solution is treated with hydrochloric acid or tartaric acid to produce the salts choline chloride and choline bitartrate, respectively (HSDB, 2008a, 2008b; Nestlé Infant Nutrition, 2011). The process for manufacturing choline base (an intermediate in the synthesis of choline salts) and choline salts using these basic steps was patented by Blackett and Soliday (1956). Choline chloride can also be produced by reaction of trimethylene with chlorohydrin (HSDB, 2008a).

OECD (2004) reports that European production sites use a reaction of trimethylammonium chloride with ethylene oxide to produce choline chloride. The final product is free of ethylene oxide because the ethylene oxide is entirely used up during production. No further information was identified on the processes used to manufacture choline chloride and choline bitartrate.

<u>Evaluation</u> <u>Question #2:</u> Is the substance synthetic? 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)).

Choline is a naturally occurring nutrient synthesized by the body and available in a variety of foods (Zeisel, 2006). Choline chloride and choline bitartrate, petitioned for use as food additives, are synthetic substances. They are produced by chemical processes that involve reactions between synthetic substances (see the response to Evaluation Question #1).

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

Choline can be supplemented through diet by addition of organic liver, eggs, wheat germ, and other foods high in natural choline (Zeisel, 2006). Breast milk is another natural form of choline for infants whose mothers are able to breast feed.

While choline is a natural, nonsynthetic substance found in many foods, some people do not synthesize or consume enough choline in their diet (Linus Pauling Institute, 2008). It does not appear that there are natural or non-synthetic sources of the petitioned substances, choline chloride and choline bitartrate. One natural source of choline that may be used as a food additive in place of synthetic choline salts is unbleached lecithin, which contains phosphatidylcholine. Lecithin is defined by FDA as a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids; it is isolated as a gum following hydration of solvent-extracted soy, safflower, or corn oils (21 CFR 184.1400).

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

Choline chloride and choline bitartrate are both affirmed as GRAS by FDA when used as nutrients in foods for human consumption in accordance with good manufacturing practice (21 CFR 182.8252, 21 CFR 182.8250). The use of choline chloride as a partial salt replacement and flavor enhancer of sodium chloride in foods is not affirmed as GRAS by FDA.

Both choline salts (i.e., choline chloride and choline bitartrate) are affirmed as GRAS by FDA when used as nutrients and/or dietary supplements in animal drugs, feeds, and related products in accordance with good manufacturing or feeding practice (21 CFR 582.5250, 21 CFR 582.5252).

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

No information was found to indicate that choline functions as a preservative in foods. The primary purpose for addition of choline chloride and choline bitartrate to foods is to provide nutrient supplementation of choline. Choline chloride may also serve as a flavor enhancer and partial replacement for sodium chloride in foods (Fielding, 1992).

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

No information was found to indicate that choline chloride or choline bitartrate will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing. The primary effect on the nutritional quality of foods is to increase the choline content. Choline salts are used to fortify food and feed, but they are not intended to restore nutrients lost in processing.

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

The primary purpose of choline supplementation is to improve the nutritional quality of food by increasing the choline nutrient content (Linus Pauling Institute, 2008). When choline chloride is used as a replacement for sodium chloride, the sodium content of food is reduced (Balchem Corporation, 2011).

Choline interacts with methionine, folate, and other methyl-group donors while being metabolized in the body. Research has shown that choline-deficient diets in rats lead to 31 to 40% decreases in hepatic folate content; which was reversible when choline was replaced. Additionally, rats fed diets deficient in both choline and methionine had folate levels half of controls after five weeks (IOM, 1998). Therefore, the presence of choline is important in maintaining adequate levels of other essential nutrients like folate. The reverse is also true; folate deficiency will impact the availability of choline in the body. Research in human volunteers suggests that when deficient in both folate and choline, the body cannot synthesize enough choline to maintain necessary metabolic actions (Linus Pauling Institute, 2008).

The hygroscopic properties of choline chloride may make it less desirable than choline bitartrate for use in powdered infant formulas because choline chloride absorbs moisture from the air which can reduce the stability of other vitamins in the dry pre-mix (FAO, undated). Choline bitartrate is also hygroscopic, but does not absorb as much water as choline chloride (Balchem Corporation, 2011). However, several currently marketed powder infant formulas do contain choline chloride, including Earth's Best Organic Infant Formula, Similac® Organic Infant Formula, and Parent's Choice™ Organic Infant Formula (Earth's Best Organic, 2011; Abbott Laboratories, 2011; Parent's Choice Infant Formula, 2011). According to the Ohio State University, due to the hygroscopic properties of choline chloride in pre-mixes, these products should stored in a cool, dark, dry location and should be stored no longer than three months (Hogberg et al., 1998). No further information was found to indicate whether or not the presence of choline chloride in these formulations or any other product negatively affects the stability of other vitamins or nutrients commonly found in food products.

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

Excessive levels of heavy metals or other dangerous contaminants have not been reported in choline chloride or choline bitartrate. No substances listed on FDA's Action Levels for Poisonous or Deleterious Substances in Human Food have been reported as contaminants of concern in choline chloride or choline bitartrate. The requirements for FCC (Food Chemicals Codex) grade choline chloride and choline bitartrate indicate that these products cannot contain more than 2 ppm lead and must pass the test for acceptable levels of the organic impurity 1,4-dioxane (U.S. Pharmacopeia, 2010). The requirements for USP (U.S. Pharmacopeia) grade choline chloride and choline bitartrate necessitate that these products contain no more than 2 ppm arsenic, 0.3 ppm lead, 10 ppm total heavy metals, 10 ppm amines, or 10 ppm 1,4-dioxane (Balchem, 2011).

The organic compound 1,4-dioxane has been classified as 'possibly carcinogenic to humans' by the World Health Organization's International Agency for Research on Cancer (IARC, 1999). It may be present in choline salts due to the use of ethylene oxide in the manufacturing process (The Sapphire Group, 2007). No information was found to indicate any historic or current issues with dangerous levels of 1,4-dioxane in choline chloride or choline bitartrate products for use as food or feed additives.

There have been reports of harmful effects in laboratory rats associated with ingestion of choline bitartrate manufactured using the synthetic form of tartaric acid (DL-tartaric acid). Beginning in 2001, several research laboratories observed kidney and bladder stones in rats being fed standard laboratory diets which contained choline bitartrate (Klurfeld, 2002; Kankesan et al., 2003; Newland et al., 2005). The kidney and bladder effects were hypothesized to be the result of a change in the manufacturing process used to make the choline bitartrate contained in the diet. The supplier (Dyets, Inc.) reported that synthetic DL-tartaric acid had been substituted for the previously used natural L-tartaric acid isomer. It was believed that the kidney and bladder stones were caused by either the synthetic tartaric acid itself or by a toxic contaminant present at trace levels in the choline bitartrate that had been introduced into the product at some step in the process, possibly during the synthesis of DL-tartaric acid (Klurfeld, 2002).[1] Current FCC and USP

[1] Note that tartaric acid is the subject of a separate Technical Evaluation Report. However, the synthetic DL-tartaric acid is not discussed in the report, as it is not permitted for use in organic agriculture.

specifications only allow the natural L(+) form of tartaric acid to be used as a raw material in the manufacture of choline bitartrate (U.S. Pharmacopeia, 2010; Balchem, 2011; Nestlé Infant Nutrition, 2011). No other reports were found linking choline bitartrate with kidney or bladder stones in humans or laboratory animals.

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

Choline chloride and choline bitartrate are unlikely to cause harm to the environment or biodiversity if they are released into the environment during their manufacture or use. Choline is a naturally occurring substance that is readily biodegradable (OECD, 2004; Sunderland, 2009). Choline salts are readily soluble and the expected environmental distribution of choline chloride is almost 100% in water (OECD, 2004). If released into the atmosphere, choline chloride would rapidly degrade, and bioaccumulation in the environment is not expected (OECD, 2004). Choline is a dietary requirement for many animals and is unlikely to be harmful to mammalian, aquatic, and avian organisms in the environment (Sunderland, 2009). Furthermore, as explained in the "Action of the Substance" section, choline is a constituent of all cell membranes and performs a variety of important functions in the body (Institute of Medicine, 1998; Song and Zeisel, 2005).

The manufacture of choline salts may result in the release of trimethylamine and/or ethylene oxide to the environment (HSDB, 2009a); however no specific reports of pollution involving these substances and the manufacture of choline salts were identified. Trimethylamine is already widely distributed in nature because it forms during the decay of plants, animals, fish, sewage, and animal wastes (as a result of microbial degradation of choline and betaine which are common constituents of plants and animals) (HSDB, 2009a). If released into the air, trimethylamine is expected to degrade with an estimated half-life of nine hours. If released into the soil, it is expected to be mobile. Its potential for bioaccumulation in aquatic organisms is low. The degradation products of trimethylamine that are formed under aerobic conditions (i.e., with oxygen) include dimethylamine, formaldehyde, formate, and carbon dioxide, while products formed under anaerobic conditions (i.e., without oxygen) include dimethylamine, ammonium, and methane, all of which occur naturally and abundantly in the environment (HSDB, 2009a).

Ethylene oxide, if released into the air, is expected to degrade in the atmosphere with an estimated half-life of 57 days (HSDB, 2009b). If released into the soil, it is expected to have very high mobility. Its potential for bioaccumulation in aquatic organisms is low. In the environment, ethylene oxide hydrolyzes to ethylene glycol which is readily biodegraded (HSDB, 2009b).

<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)).

Many studies provide support for choline's status as an important nutrient for health and disease prevention. Inadequate intake of choline may lead to "fatty liver" disease (an accumulation of fat in the liver), liver damage, and/or muscle damage (Linus Pauling Institute, 2008). Inadequate choline also may result in low blood levels of low density lipoprotein (LDL, or "good") cholesterol. A recent study in men and women volunteers reported that choline deficiency can result in DNA damage and death of peripheral lymphocytes, which are important for immune system function (Linus Pauling Institute, 2008). Studies in rats have also reported that dietary choline deficiency may increase the risk of liver cancer, but the mechanism for this is unclear (Linus Pauling Institute, 2008). Research in rats also stresses that choline is likely important in neonatal and postnatal brain development (particularly in the hippocampus) (Zeisel, 2006).

High doses of around 10–16 grams choline per day may cause fishy body odor, vomiting, salivation, and increased sweating. Other human studies showed that a 7.5-gram dose of choline results in a slight lowering of blood pressure, which may result in dizziness or fainting (Linus Pauling Institute, 2008). Mild hepatotoxicity is associated with administration of choline magnesium trisalicylate; however, authors noted that the toxicity was likely due to the salicylate, rather than choline. Finally, some evidence indicates that choline bitartrate administered via the diet may induce urolithiasis (stones in the urinary tract) in rats and dogs. However, authors reported that the toxicity may not have been caused by choline, but rather synthetic tartaric acid or a toxic contaminant present at trace levels in the choline bitartrate (Newland et al., 2005; Klurfeld, 2002). See Evaluation Question #8 for more information.

Patients with trimethylaminuria (fish odor syndrome), renal disease, liver disease, depression, and Parkinson's disease may be more susceptible to the adverse effects of choline; thus, choline supplementation is usually not recommended for these populations (IOM, 1998). The IOM set an upper intake level (UL) of 3.5 grams/day of choline for adults, which was based primarily on the low blood pressure effects of higher doses (IOM, 1998). The IOM was unable to establish a UL for infants up to 12 months, but set ULs of 1.0 grams for children 1–8 years, 2.0 grams for children 9–13 years, and 3.0 grams for teenagers 14–18 years (IOM, 1998).

<u>Evaluation Information #11:</u> Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b)(1)).

An alternative to direct supplementation with synthetic choline would be supplementation of the diet with foods high in choline, such as organic eggs, liver, wheat germ, and beef. However, strict vegetarians that do not consume eggs or milk may not be able to obtain enough choline through diet alone (Linus Pauling Institute, 2008).

Another alternative is the use of lecithin as a dietary supplement. Lecithin contains phosphatidylcholine, which is a primary form of choline. Studies indicate that lecithin supplementation can help maintain plasma choline levels during intense exercise (Buchman et al., 2010). Hirsch et al. (1978) found that dietary choline chloride (3 grams) raised serum choline levels to a peak of 86% after 30 minutes, while choline levels after lecithin intake rose by 33% after 30 minutes, then continued to rise for at least 12 hours to 265% over control values ($p < 0.001$). The authors also stated that lecithin supplementation increased serum triglyceride levels and lowered serum cholesterol concentration (Hirsch et al., 1978). Wurtman et al. (1977) suggested that oral lecithin is more effective than choline chloride at raising serum choline levels and may "be the method of choice" for accelerating acetylcholine synthesis by increasing choline. However, most lecithin supplements only contain about 20–90% of phosphatidylocholine and contain less than 13% choline (Linus Pauling Institute, 2008). This indicates that large doses of lecithin may be needed to provide adequate amounts of choline. It should be noted that adults with varied diets should be able to obtain enough choline through foods; only vegetarians/vegans who do not consume milk or eggs may be at risk for inadequate intake (Linus Pauling Institute, 2008).

Natural lecithin made from soybeans, other plant products, or eggs are commercially available (Cargill, 2011). However, adding lecithin to food to supply nutrients may not be compatible with the manufacturing of certain foods, as soy lecithin tends to impart a bitter, "haylike" flavor and a sticky consistency (Stephan and Steinhart, 2000). Manufacturers reported that lecithin used for non-nutritive purposes rarely exceeds 1% by weight of the final food product (U.S FDA, 2006).

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