Lycopene

Handling

Identification of Petitioned Substance

Chemical Name:
2,6,10,14,19,23,27,31-Octamethyl-dotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene

Other Names:
4,4-carotene
ψ,ψ-carotene
Lycopin
(all-E) lycopene
all-*trans* lycopene

Trade Names:
Lycosource
LycoVit®
Lycopene 10 Cold Water Dispersion (CWD)
Lycopene Dispersion 20
Lyconat

CAS Number:
502-65-8

Other Codes:
EC Number: 207-949-1

Characterization of Petitioned Substance

__Composition of the Substance__:

Lycopene ($C_{40}H_{56}$) is a member of the carotenoid family of phytochemicals (i.e., chemical compounds that occur naturally in plants) and consists of a linear chain of hydrocarbons with 13 carbon-carbon double bonds. There are two central methyl groups at the 1,5-position and additional methyl groups at the 1,6-position. The extended system of alternating double bonds is critical to the biological activity of lycopene, which includes its susceptibility to oxidative degradation (Mazza, 2002).

The all-*trans* isomer[1] of lycopene is the predominant form found in tomatoes and other red-colored fruits and vegetables. Food processing, cooking, storage, and exposure to light may cause isomerization of some of the all-*trans* isomer to different *cis* isomers, including the 5-*cis*, 9-*cis*, 13-*cis*, and 15-*cis*. Synthetic lycopene generally consists of the all-*trans* isomer (>70%), 5-*cis*-lycopene (up to 20%), and minor amounts of other *cis* isomers (EFSA, 2008). The chemical structure of the all-*trans* isomer of lycopene is provided in Figure 1.

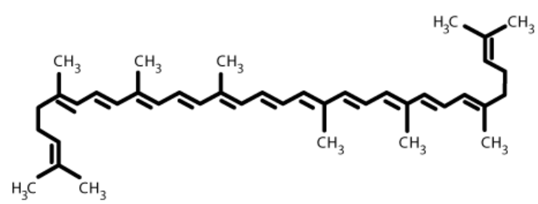

Figure 1. Chemical Structure of Lycopene (all-*trans* isomer) (Source: Lycocard, 2006)

[1]Isomers are compounds with the same molecular formula but different structural formulas. An all *trans* isomer consists of substituents that are oriented in opposing directions.

Properties of the Substance:

Lycopene is an antioxidant[2] that is synthesized by many plants and microorganisms but is not produced in humans or animals (Rao and Rao, 2007). It is a bright red carotenoid pigment that is found naturally in significant quantities in tomatoes, watermelon, and other fruits and vegetables that are red in color (Mazza, 2002; Olempska-Beer, 2006a). Lycopene extracted from tomato is a dark-red viscous liquid (Rath et al., 2009). Lycopene is lipophilic (i.e., oil soluble) and is insoluble in water. Commercial preparations of lycopene are available in a powder or crystalline form.

Lycopene absorbs light easily and is naturally fluorescent. In organic solution, lycopene is easily oxidized and destroyed, so precautions are needed to prevent its oxidative destruction during extraction from natural sources (Caballero, 2009).

Physicochemical properties of lycopene are provided in Table 1.

Table 1. Physical and Chemical Properties of Lycopene

Physical or Chemical Property	Value
Physical state	Solid (powder or crystal)
Appearance	Red or reddish brown
Molecular weight	536.88 g/mol
Boiling point	660.9°C at 760 mmHg
Melting point	172°C–175°C
Solubility	Insoluble in water; soluble in oil
Density	0.888 g/cm^3
pH	3.8-4.0 (in aqueous dispersion)

Source: Furia, 1972

Specific Uses of the Substance:

Dietary antioxidants such as lycopene are reported to be associated with a decreased risk for chronic disease and certain cancers in humans. Carotenoids, including lycopene, are not synthesized in humans. They are obtained through the consumption of fruits and vegetables that naturally contain them. Many processed foods are fortified with synthetic lycopene to increase total dietary intake of this carotenoid. In the United States, the typical dietary intake of lycopene is about 2–5 milligrams (mg) per day, which is likely the result of a diet high in tomatoes and tomato products (Krinsky and Johnson, 2005).

Lycopene is found naturally at high concentrations in many fruits and vegetables that are red in color, including tomatoes, red carrots, papayas, watermelon, pink grapefruit, pink guava, apricots, rosehip, wolfberry, and the Southeast Asian fruit, gac (*Momordica cochinchinensis)* (ERNA, 2007). Lycopene can also be found in certain algae and fungi. Tomatoes and tomato-based products, including sauces, juices, and ketchup, account for more than 85% of the dietary intake of lycopene for the average American. The processing of tomato-based products increases the bioavailability of lycopene (Rao and Rao, 2007). The process of heating tomatoes in oil was determined to be linked with an increase in lycopene absorption when compared with the absorption for unprocessed tomato juice (Krinsky and Johnson, 2005).

Nonsynthetic lycopene (commonly referred to as 'lycopene juice') is used as colorant in many food preparations, dairy products, nonalcoholic flavored drinks, cereal products, bread, fish and meat products,

[2] An antioxidant is a molecule that is able to inhibit the oxidation of other molecules. An oxidation reaction involves the transfer of hydrogen or electrons from one compound to an oxidizing agent. This reaction produces free radicals, which can start chain reactions capable of causing cellular damage. Antioxidants remove free radical intermediates and inhibit other oxidation reactions, thereby terminating the chain reaction and preventing cellular damage.

and spreads (IARC, 1998). Colorants are typically used to increase the visual appeal of food products. Nonsynthetic lycopene provides color shades from yellow to red. The use of natural lycopene as a colorant has increased in recent years as more information has surfaced regarding the potentially harmful effects associated with the use of artificial food colorants (Hakala and Heinonen, 1994). In addition, application of lycopene juice extracted from tomatoes has a distinct tomato flavor making application as a food colorant limited (EFSA, 2008).

Synthetic lycopene is added to some food and beverages as a dietary supplement. Synthetic lycopene is considered as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) and is commonly added to infant formula, breakfast cereals, instant soup, low-fat dressing, nutrient bars and meal replacements, yogurt, meatless meat products, crackers, salty snacks, and drinks (i.e., juice drinks, dairy fruit drinks, and energy drinks) at levels ranging from 5 to 70 milligrams per kilogram of food (Olempska-Beer, 2006a; WHO, 2007). Synthetic lycopene also serves as a coloring agent in many of the same food preparations that use nonsynthetic lycopene (International Formula Council, 2011; Olempska-Beer, 2006a).

Lycopene is added to infant formulas to simulate the nutritional quality of human breast milk. Although lycopene is a highly concentrated carotenoid in human tissue and breast milk, it is not synthesized in humans and must be obtained through the diet. Infants who consume dairy-based formulas have low to no intake of lycopene because this nutrient is not found naturally in cow's milk (International Formula Council, 2011; Krinsky and Johnson, 2005).

Approved Legal Uses of the Substance:

Lycopene is not currently included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) of nonagricultural (nonorganic) substances 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). The current petition is for the addition of synthetic crystalline lycopene to the National List for use in processed foods and specifically to infant formula in order to enhance their nutritional quality (i.e., carotenoid level) (International Formula Council, 2011).

In 2007, a petition to the National Organic Standards Board (NOSB) was filed for the inclusion of lycopene juice to the National List at 7 CFR 205.606 as a nonorganic color derived from an agricultural product. The NOSB rejected the petition to permit the use of lycopene from tomato as a coloring agent. The material was rejected as a colorant because the petitioner did not provide credible information regarding the lack of supply of organic raw materials and the ability to process it as organic. The use of lycopene juice as a colorant is not being reconsidered by NOSB at this time (USDA, 2007).

FDA has received petitions from multiple manufacturers of various lycopene products asserting that their products be regarded as GRAS. For example, in 2002, BASF Corporation notified FDA that synthetic lycopene (i.e., the crystalline form of lycopene produced by chemical synthesis) should be regarded as GRAS, and this determination was based on scientific review and estimates of dietary exposure, method of production, and product specifications as well as published and unpublished studies. Based on this information, BASF's GRAS panel concluded that synthetic lycopene at levels ranging from 5 to 70 mg per kilogram (kg) of food, and meeting established food-grade specifications, is GRAS under the conditions of its intended use as a direct food ingredient in breakfast cereals (ready-to-eat and cooked), drinks (juice drinks, energy drinks, and dairy fruit drinks), instant soup, low-fat dressings, meal replacements, meatless meat products, nutrient bars, salty snacks, crackers, and yogurt. BASF Corporation also noted that synthetic lycopene has the potential to impart color and its use as a direct food ingredient may constitute the use of a color additive. FDA had no questions regarding the manufacturer's conclusion that synthetic lycopene is GRAS under the intended use. However, FDA has not made its own determination regarding the GRAS status of that subject use of lycopene (FDA, 2005a).

In 2005, Vitatene S.A. (Vitatene) notified FDA that its lycopene manufactured from *B. trispora* should be considered GRAS for its use as an ingredient in a number of food categories—baked goods, baking mixes, beverages and beverage bases, breakfast cereals, cheeses, condiments and relishes, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings and fillings, gravies and sauces, milk products, plant protein products, processed fruits and fruit juices, snack foods—at levels up to 50 parts per million (ppm) and in soups and soup mixes that do not contain tomatoes at levels up to 575 ppm. FDA noted in its response to Vitatene that lycopene from *B. trispora* when used in food products has the potential to impart color and that the use of lycopene from *B. trispora* may constitute the use of a color additive. Following review of all the information provided by Vitatene, FDA had no questions regarding the manufacturer's conclusion that lycopene from *B. trispora* is GRAS under the intended use. However, FDA has not made its own determination regarding the GRAS status of that subject use of lycopene from *B. trispora* (FDA, 2005b).

FDA regulates infant formulas for sale in the United States under 21 CFR 107. This regulation does not include specifications for the use of lycopene in infant formula. In addition, GRAS petitions received from BASF Corporation and Vitatene do not include specific provisions for the use of synthetic crystalline lycopene or lycopene from *B. trispora* in infant formula.

FDA exempts the certification of tomato lycopene extract and tomato lycopene concentrate as a color additive.[3] FDA affirms that "tomato lycopene extract and tomato lycopene concentrate may be safely used for coloring foods generally in amounts consistent with good manufacturing practice, except that they may not be used to color foods for which standards of identity have been issued under section 401 of the act, unless the use of added color is authorized by such standards" (21 CFR 73.585).

Lycopene can be used legally as a human dietary supplement, but it is not registered with FDA for this use. FDA does not regulate human dietary supplements in the same way as drugs or animal feed additives; generally, manufacturers do not need to register their products with FDA or get approval before producing and selling supplements for human consumption. The product manufacturer is responsible for ensuring the safety of the product. FDA is responsible for taking action regarding an unsafe product after it reaches the market and to make sure the supplement's label is accurate and not misleading (FDA, 2005c).

Action of the Substance:

Lycopene is an antioxidant found naturally in many fruits and vegetables and is found in particularly high quantities in tomatoes. Lycopene acts as a potent antioxidant and is able to protect cells against oxidative damage, which can decrease the risk of chronic diseases such as cardiovascular disease. Antioxidants like lycopene counteract the activities of free oxidants which can react with substances and destroy important cells (Rao et al., 1999; Rao and Rao, 2007).

In addition to its antioxidant properties, lycopene has also been shown to exert effects on other mechanisms in the body including induction of cell-to-cell communication; modulation of hormonal, immune systems, and other metabolic pathways; carcinogen metabolism; and gene function regulation (Rao et al., 1999; Rao and Rao, 2007). Lycopene is also nearly twice as effective as beta (β)-carotene, another common antioxidant and carotenoid, in protecting lymphocytes from NO_2 radical death and membrane damage. It is also a peroxyl radical scavenger, or a molecule that locates free radicals and removes them, which prevents them from binding with other molecules. Additionally, lycopene may have an indirect antioxidant effect by inducing endogenous antioxidant defense enzymes like glutathione peroxidase, glutathione-S-transferase, and glutathione reductase (ERNA, 2007).

Lycopene is a bioactive red-colored pigment that is sometimes used as a natural coloring agent in food. The color characteristic of tomatoes and other foods high in lycopene is directly linked to the presence of a high concentration of carotenoid molecules. The coloring ability of lycopene depends on its concentration, method of dispersion, and formulation. One method for coloring food involves simply adding the lycopene-containing foods to the desired food product. However, this method is ineffective in large

[3] Color additives, in general, cannot qualify for GRAS status because GRAS only applies to food additives and not color additives.

industrial food production because a high concentration of pigment might be needed to obtain the desired shade, thereby requiring that a large amount of the lycopene-containing product be used. Unwanted flavors could also result. To resolve these issues, pigment is extracted from lycopene using organic solvents, which are subsequently removed, yielding an oleoresin rich in pigments, but also containing other material such as triglycerides, sterols, wax, and other lipid-soluble compounds (Mortensen, 2006).

Combinations of the Substance:

Lycopene is petitioned for addition to organic infant formula. Organic infant formula contains a number of nutrients (e.g., riboflavin, niacin, pantothenic acid, iodine, copper, potassium) included on the National List through the listing of nutrient vitamins and minerals, in accordance with 21 CFR 104.20, Nutritional Quality Guidelines For Foods (7 CFR 205.605). Moreover, a mixture of food ingredients comprising carbohydrates, proteins, fats, and stabilizers are expected to be included in infant formula to which lycopene is added. These ingredients vary with the type of product and manufacturer

According to the petitioner, lycopene is also known to interact with other fat soluble carotenoids, including beta-carotene and lutein, to enhance the oxidative stability of edible oils. Lutein is commonly added to infant formula in conjunction with lycopene (Higdon et al., 2009; International Formula Council, 2011; Rubin et al., 2011).

Many commercially available lycopene products contain proprietary formulations of various forms of lycopene (nonsynthetic or synthetic) and other additive ingredients. These additives may act as stabilizers, carriers, or diluents.

- *Stabilizers*: Many lycopene products including Lyconat cold water dispersion (CWD) (produced from *B. trispora* by Vitatene) and LycoVit® 10% (synthetic crystalline lycopene) are formulated with a class of stabilizing agents called tocopherols, a group of closely related, fat-soluble alcohols that behave similarly to vitamin E. Other stabilizing agents and flow aids frequently added to lycopene products include modified food starches, sodium ascorbate, ascorbyl palmitate, and tricalcium phosphate (AIC; 2008; BASF, 2006).

- *Carriers*: Gelatin used in natural lycopene tablets containing nonsynthetic lycopene extracted from tomatoes and LycoVit® 10% CWD (synthetic crystalline lycopene) acts as a carrier in lycopene preparations (Douglas Laboratories, 2003; EFSA, 2008). Natural lycopene tablets are also formulated with ingredients such as rice bran and oil (Douglas Laboratories, 2003). Sunflower oil acts as a carrier in LycoVit® Dispersion 10% lycopene in sunflower oil (BASF, 2005).

- *Diluents*: Water and glycerin are commonly used as diluents and are added to natural lycopene products (Douglas Laboratories, 2003)..

Status

Historic Use:

The Aztecs and Incas first cultivated tomatoes in 700 AD. Tomatoes were then introduced to Europeans by the Mexicans in the middle of the 16th century. The red-colored pigment now known as lycopene was first discovered in the tomato in 1876. It was later named lycopene by scientist, C.A. Schunck (Kong et al., 2010). The biological effects that are now traditionally associated with lycopene were not studied extensively until the 1980s. At this time, researchers began examining the potential effects associated with a diet high in lycopene, including anti-tumor effects, hypolipidemic effects, and effects on the initiation and progression of chronic illnesses. Notably, in 1985, Harvard University researchers observed a significant trend in decreased cancer risk associated with an increased dietary intake of carotene-containing fruits and vegetables (Hsiehs Biotech, 2010).

The use of lycopene in organic handling has involved some uncertainty due to its nutritional status. Because it is neither a vitamin nor a mineral, there are conflicting opinions regarding its necessity in human nutrition. In 1995, the NOSB made the following recommendation in "The Use of Nutrient Supplementation in Organic Foods" (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," lycopene and other nutrients not specifically listed in the regulation were permissible. However, after further discussion with 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 lycopene. See "OFPA, USDA Final Rule" for more information.

OFPA, USDA Final Rule:

Synthetic lycopene is not currently listed under 7 CFR 205.605(b) as a synthetic substance allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))."However, organic sources of lycopene would be allowed in organic handling and processing.

The NOP final rule limits "vitamins and minerals" allowed for use in organic products to those in FDA Nutritional Quality Guidelines for Food (21 CFR 104.20(d)(3)), which does not include lycopene. There has been confusion over the interpretation of the NOP regulations with regard to certain nutritive supplements as described above 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, methionine, and lycopene.

To clarify this situation, the NOP published a proposed rule in January 2012 (77 FR 1980) that would clarify the required nutrients that could be added to organic foods. Other nutrients, including lycopene, would need to be individually petitioned for consideration by the NOSB. If promulgated as a final rule, this amendment would clarify that lycopene is not one of the required nutrients currently allowed in organic products (USDA, 2012).

International:

In 2009, the European Economic Commission (EEC) agreed to permit the use of both lycopene oleoresin from tomatoes (nonsynthetic lycopene) and synthetic lycopene as novel food ingredients as specified in Table 2.

Natural sources of antioxidants (presumably including nonsynthetic lycopene) are permitted for use as food additives and processing aids in organic food production by the CODEX Alimentarius Commission in the *Codex General Standard for Food Additives* (CODEX GL 32---1999; CODEX Alimentarius Commission, 2010).

The Canadian Organic Production Systems Permitted Substances List for Processing does not include lycopene (CGSB, 2011). The International Federation of Organic Agriculture Movements (IFOAM) does not list lycopene within its "Norms for Organic Production and Processing" (IFOAM, 2006). Lycopene is not specifically listed as an allowed food additive in organic processed foods in the most recent revision of the

Japanese Agriculture Standard for Organic Processed Foods (Japanese Ministry of Agriculture, Forestry and Fisheries, 2006).

Table 2. EEC Permissible Lycopene Content by Food Category

Food Category	Maximum Content of Lycopene
Fruit/vegetable juice-based drinks (including concentrates)	2.5 mg/100 g
Drinks intended to meet the expenditure of intense muscular effort especially for sportsmen	2.5 mg/100 g
Foods intended for use in energy-restricted diets for weight reduction	8 mg/meal replacement
Breakfast cereals	5 mg/100 g
Fats and dressings	10 mg/100 g
Soups other than tomato soups	1 mg/100 g
Bread (including crispy breads)	3 mg/100 g
Dietary foods for special medical purposes	In accordance with the particular nutritional requirements
Food supplements (synthetic lycopene only)	15 mg per daily dose as recommended by the manufacturer

Source: European Economic Community (EEC) Council Regulation EC No. 258/97, 2009

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: 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)).

Both natural and synthetic forms of lycopene are currently used in commercial food production as a nutritional supplement and as a colorant. A brief description of the process to extract nonsynthetic lycopene from tomatoes is provided. The processes used to manufacture synthetic lycopene from chemical synthesis and from *B. trispora* are also described.

Nonsynthetic lycopene is found naturally in high concentrations in tomatoes and in other fruits and vegetables that are red in color (ERNA, 2007). Typically, the nonsynthetic lycopene used in food applications is extracted from tomatoes. The ripe fruits of tomato (*Lycopersicon esculentum* L.[4]) are generally used for the extraction of lycopene for commercial use; this strain possesses high lycopene content in the range of 150 to 250 mg/kg (EFSA, 2008; Rath et al., 2009).

Lycopene extract from tomato is produced by crushing tomatoes into a crude juice. The pulp and serum are then separated, and the pulp is extracted using the solvent ethyl acetate. The solvent is then removed by evaporation under a vacuum at 40–60°C and the final product remains. The final product consists of tomato oil containing lycopene and additional natural constituents of tomatoes including acylglycerols, fatty acids, water soluble matter, unsaponifiable matter, phosphorous compounds, and phospholipids. This form of nonsynthetic lycopene is used as both a nutritional supplement and as a colorant in food applications. The lycopene content of tomato extract may range from 5 to 15% and is dependent on the

[4] Genetically modified tomatoes (*Lycopersicon esculentum*, L.) were made commercially available in 1994 and were designed to maintain a longer shelf-life. Currently, no genetically modified tomatoes are being grown commercially in Europe or North America. The use of genetically modified tomatoes is not approved in Europe and all commercially available tomatoes in the European Union are not from genetically modified sources (GMO Compass, 2006; ISAAA, 2012).

amount of tomato seed oil that is used in the extract and the original lycopene content of the tomatoes used to create the extract (Rath et al., 2009).

BASF, a manufacturer of synthetic lycopene, describes a three-stage process for the chemical synthesis of lycopene. According to the petition to include synthetic crystalline lycopene on the National List, stage 1 produces an organic solution of C_{15} phosphonium methanesulfonate in the solvent dichloromethane (DCM), and stage 2 produces an organic solution of C_{10} dialdehyde in the solvent toluene. During stage 3, the intermediates produced in stages 1 and 2 are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. Glacial acetic acid and deionized water are added; the mixture is stirred vigorously; the aqueous and organic phases are allowed to separate; and the organic phase containing DCM and crude lycopene is extracted with water. Methanol is then added to the organic phase, and DCM is removed via distillation under reduced pressure. The crude methanolic lycopene solution is heated and then cooled to a crystalline slurry that is filtered and washed with methanol. The lycopene crystals are then re-crystallized and dried under heated nitrogen. BASF notes that synthetic lycopene is stored under nitrogen or suspended in an aqueous solution containing antioxidants to prevent oxidation and isomerization of lycopene (FDA, 2005a).

Lycopene can also be produced from the microorganism *B. trispora*. Vitatene manufactures a form of lycopene from *B. trispora* (Lyconat®) that is formulated as suspensions in edible oils or as water-dispersible powders (referred to as cold water dispersions) and are stabilized with antioxidants. Lycopene from *B. trispora* is affirmed by the manufacturer as void of genetically modified components and is produced through a co-fermentation process using the two sexual mating types of the organism. Although each strain is capable of producing low levels of carotenoids, the co-cultivation of both strains enhances the synthesis of these compounds and lycopene is an intermediate in the biosynthetic pathway of β-carotene (Olempska-Beer, 2006b). Vitatene states that both mating types are stable cultures and are preserved under conditions consistent with food good manufacturing practices. Lycopene is extracted from the fungal biomass and purified by crystallization and filtration using isopropanol and isobutyl acetate. The extraction solvents, isopropanol and isobutyl acetate, may be present in the final product at levels below 0.1% and 1%, respectively. Imidazole used during fermentation may be found in lycopene at levels below 1 mg/kg (Olempska-Beer, 2006b). This crystalline lycopene is then formulated into either a suspension in a cold water dispersible product containing modified food starch and 10% or 20% lycopene or a high oleic sunflower oil containing 5% or 20% lycopene. Both of the formulation types also contain up to 2% tocopherol as an antioxidant. To manufacture the oil suspensions, lycopene crystals are mixed and milled with high oleic sunflower oil and tocopherol. To manufacture the cold water dispersible product, lycopene crystals and tocopherol are first dissolved in a food grade solvent and then this solution is mixed with an aqueous modified food starch solution until a homogenous emulsion is formed. The solvent is evaporated under vacuum and the remaining liquid is dried (FDA, 2005b).

Evaluation Question #2: 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)).

Lycopene is commercially available in synthetic and nonsynthetic forms. The nonsynthetic form of lycopene is found naturally in many fruits and vegetables. Nonsynthetic lycopene extracted from tomatoes is not considered synthetic because the practices used during manufacture align with the requirements set forth by the NOP for distinguishing between synthetic and nonsynthetic substances (NOP, 2006). Specifically, all solvents used during lycopene extraction from tomatoes are removed, the final product has not been transformed using a chemical change, no functional properties have been altered, and the substance has not been altered into a chemical form that does not occur in nature.

Lycopene can be produced using a natural biological process, fermentation. Lycopene from *B. trispora* is produced by co-fermentation of two sexual mating types (plus and minus). Each strain is capable of producing low levels of carotenoids, but the co-cultivation of both strains enhances the synthesis of these compounds. The production process of lycopene from *B. trispora* is nearly identical to that used to manufacture β-carotene from the same fungus. Lycopene is an intermediate in the biosynthetic pathway of β-carotene. Following extraction from the biomass of the fermentation broth, the substance is then purified by crystallization and filtration using the solvents isopropanol and isobutyl acetate (Olempska-Beer, 2006b). Lycopene from *B. trispora* is a nonagricultural substance as it is not a direct product of agriculture. It is not specifically included in the petition (International Formula Council, 2011) to add synthetic crystalline lycopene to the National List.

Synthetic lycopene is prepared from synthetic intermediates that are commonly used in the production of other carotenoids used in food. Synthetic lycopene is produced by the Wittig condensation of two intermediate compounds, one of which is usually C10-dialdehyde, and the other is either lycopyl salt or another similar compound. Residuals of volatile solvents may be present in the final lycopene product (Olempska-Beer, 2006a). Batches of the crystalline material and the formulated product are generally analyzed for these solvents, namely, methanol, acetone, n-heptane, methylene chloride, and isopropanol. BASF Corporation reports that chemical characterizations of batches of products containing synthetic lycopene crystalline material identify 100 ± 1.6% of the components in the crystalline material. Approximately 98% of the identified components are lycopene, including both ***cis*** and trans-isomers. An additional 0.9% is identified as lycopene-related substances (e.g., rhodopin) and the remainder includes approximately 0.3% residual solvents (FDA, 2005a). Only the synthetic form of lycopene produced by chemical synthesis is discussed in the petition for the inclusion of synthetic, crystalline lycopene on the National List (International Formula Council, 2011).

See Evaluation Question #1 for more details on the manufacture of synthetic and nonsynthetic forms of lycopene.

<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)).

Nonsynthetic sources of lycopene are found in the carotene extracts from many plants. Lycopene is the predominant carotenoid in reddish and deep-orange fruits and vegetables including tomatoes, papayas, watermelon, pink grapefruit, apricots, pink guava, wolfberry, gac, red carrots, and rosehip. The tomato is the major source of natural lycopene (ERNA, 2007; IARC, 1998). Lycopene is primarily obtained from tomatoes by solvent extraction (see Evaluation Question #1). The extraction solvent is removed by evaporation, leaving lycopene and other tomato constituents dissolved and suspended in the tomato's natural lipid phase (EFSA, 2008).

<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?

Petitions from multiple manufacturers have been submitted to FDA requesting declaration of several forms of synthetic lycopene as GRAS (see Approved Legal Uses of the Substance). FDA has not issued objection to these self-affirmed GRAS petitions, but has also not made its own determinations regarding the GRAS status of these subject uses.

Vitatene, a manufacturer of lycopene produced from the fungus *B. trispora*, regards lycopene manufactured from *B. trispora* as GRAS at levels up to 50 ppm when used as an ingredient in a large number of food categories—baked goods, baking mixes, beverages and beverage bases, breakfast cereals, cheeses, condiments and relishes, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings and fillings, gravies and sauces, milk products, plant protein products, processed fruits and fruit juices, snack foods—and GRAS at levels up to 575 ppm in soups and soup mixes that do not contain tomatoes. Infant formulas are not specifically mentioned in the GRAS petition to FDA. FDA has not made its own determination regarding the GRAS status of this subject use of lycopene from *B. trispora* (FDA, 2005b). Table 3 describes the GRAS uses of lycopene produced by *B. trispora* in foods and beverages.

Table 3. GRAS Uses of Lycopene Produced by *B. trispora* in Foods and Beverages

Food Category	Maximum Use Levels (ppm)
Baked Goods and Baking Mixes	50
Beverages and Beverage Bases	25
Breakfast Cereals	50
Cheeses	5.0
Condiments and Relishes	50
Confections and Frostings	25
Fats And Oils	20
Frozen Dairy Desserts and Mixes	25
Gelatins, Puddings, and Fillings	25
Gravies and Sauces	50
Hard Candy	25
Milk Products	50
Plant Protein Products	50
Processed Fruits and Fruit Juices	25
Snack Foods	30
Soft Candy	25
Soups And Soup Mixes	575

Source: FDA, 2005b

BASF Corporation, a manufacturer of synthetic crystalline lycopene (i.e., LycoVit® 10%, Lycopene 10 CWD, and Lycopene Dispersion 20), also submitted a petition to FDA for declaration of synthetic lycopene manufactured by chemical synthesis as GRAS. BASF Corporation concluded that synthetic lycopene that meets its established food-grade specifications is GRAS under the conditions of its intended use as a direct food ingredient in a variety of foods including breakfast cereals, drinks, instant soups, low-fat dressings, etc. at levels ranging from 5 to 70 mg per kg of food. Infant formulas are not specifically mentioned in the GRAS petition to FDA. BASF Corporation acknowledged that synthetic lycopene has the potential to impart color, and its use may constitute the use of a color additive. FDA has not made its own determination regarding the GRAS status of this subject use of lycopene (FDA, 2005a). Table 4 describes the GRAS uses of synthetic crystalline lycopene in foods and beverages.

Table 4. GRAS Uses of Synthetic Crystalline Lycopene in Foods and Beverages

Food Category	Maximum Use Level[a] (mg synthetic lycopene[b] per 100 g food as prepared)
Breakfast cereals (Ready-To-Eat and cooked)	0.5, 2.0, 3.5, or 7.0[c]
Drinks (energy drinks, juice drinks, and dairy fruit drinks)	2.5
Instant soup	2.0
Low fat dressings	2.0
Meal replacements	2.5
Meatless meat products	5.0
Nutrient bars	5.0
Salty snacks and crackers	3.0
Yogurt	2.0

[a] The maximum use level of synthetic lycopene per 100 g food may be provided by any of the three synthetic lycopene-containing products (LycoVit®10%, Lycopene 10 CWD, or Lycopene Dispersion 20)

[b] Total synthetic lycopene (*cis* + trans isomers)

[c] 7.0 mg synthetic lycopene per 100 g RTE for cereals weighing less than 20 g per cup, e.g. plain puffed cereal grains; 3.5 mg synthetIC lycopene per 100 g RTE for cereals weighing 20 g or more but less than 43 g per cup; 3.5 mg synthetic Iycopene per 100 g RTE for high fiber cereals containing 28 g or more of fiber per 100 g; 2.0 mg synthetic lycopene per 100 g RTE for cereals weighing 43 g or more per cup or biscuit types; 0.5 mg synthetic Iycopene per 100 g cooked cereals

Source: FDA, 2005a

FDA exempts the certification of nonsynthetic tomato lycopene extract and tomato lycopene concentrate as a color additive[5] because it affirms that "tomato lycopene extract and tomato lycopene concentrate may be safely used for coloring foods generally in amounts consistent with good manufacturing practice, except that they may not be used to color foods for which standards of identity have been issued under section 401 of the act, unless the use of added color is authorized by such standards" (21 CFR 73.585).

<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)).

The primary uses of lycopene in food are as a nutritive additive and colorant. It is not used as a preservative, although it contains some antioxidant properties.

<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)).

Lycopene is an antioxidant that is found naturally in many foods and can be used to provide supplemental nutritional benefits. Synthetic lycopene is petitioned for use in infant formula in order to create a nutritive value similar to the level found in human breast milk. In the first months of life, breast milk and/or formula preparations are the only nutrition for infants. Limited data have shown that carotenoid levels in breast-fed term infants have been higher than levels in infants fed infant formula, and the concentration of carotenoids in human milk is highly variable among women because it is linked to the mother's dietary intake (International Formula Council, 2011). However, lycopene is not used to replace the nutritive value lost in processing and is only used to introduce lycopene to the infant formula mixture.

Tomato lycopene extract and lycopene juice have been used as coloring agents in food processing. Lycopene is a bright red carotenoid pigment and is capable of modifying other orange-red carotenoids and broadening the spectrum of carotenoid food colors already available. Lycopene imparts a yellow-orange to red color at concentrations ranging from 5 to 500 mg/kg food. However, because lycopene extracted from tomatoes has a distinct tomato flavor, application as a food color is limited (EFSA, 2008). In 2007, the lycopene juice as a colorant in organic food production was petitioned for use and was not approved by the NOSB, and its use as a colorant is not included in the petition currently under review (USDA, 2007).

<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)).

Lycopene has an effect on the nutritional quality of foods because it has antioxidant properties. The antioxidant properties of lycopene are reported to be associated with a decreased risk for chronic disease and certain cancers in humans. See Action of the Substance for more information on the potential benefits of lycopene on human health.

Tomatoes and tomato-based products (e.g., salad dressings, ketchup, sauces, and juices) are an integral part of the human diet and act as major dietary sources of natural lycopene. As mentioned earlier (see Specific Uses of the Substance), the processing of tomato-based products increases the bioavailability of lycopene (Rao and Rao, 2007). The cooking (heating) of tomatoes has been linked with increased lycopene absorption when compared with the absorption for unprocessed tomato juice (Krinsky and Johnson, 2005).

Because of its antioxidant properties, synthetic lycopene is commonly used to fortify foods that do not contain lycopene such as dairy products and cereals (Rao and Rao, 2007; EFSA, 2008). Synthetic lycopene is also added to dietary supplements. Synthetic lycopene is specifically petitioned for use in fortifying infant

[5] Color additives, in general, cannot qualify for GRAS status because GRAS only applies to food additives.

formula in order to create a level of lycopene that would be found in human breast milk. Carotenoids, including lycopene, are considered antioxidants and have been linked to a decreased risk for eye disease, a variety of cancers, and other chronic diseases. The health effects associated with lycopene are discussed in detail in Evaluation Question #10.

According to dietary surveys conducted as part of a risk assessment completed by the European Food Safety Authority, exposure to lycopene from natural dietary sources in different populations is estimated to be on average 0.5–5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, and especially tomato products, could produce occasional exposures of 20 mg/day or more (EFSA, 2008). It is estimated that levels of lycopene ranging from 35–75 mg/day may be required before the health benefits associated its antioxidant properties would be exhibited in individuals with cancer and other chronic diseases. Some studies have suggested that daily intake levels of 5–7 mg/day lycopene in healthy humans may be sufficient to prevent some chronic disease and combat oxidative stress. Although the beneficial role of carotenoids, including lycopene, has been acknowledged, carotenoids are not considered essential nutrients and do not have an assigned dietary reference intake value (Rao and Rao, 2007).

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)).

The use of pesticides is a typical practice in conventional tomato farming. However, many of the pesticides used in conventional farming practices are prohibited in organic tomato farming (ATTRA, 1999). No reports of residues of heavy metals or other contaminants in excess of FDA tolerances have been identified for lycopene.

Lycopene produced by chemical synthesis may contain low levels of triphenyl phosphine oxide (TPPO), *apo*-12′-lycopenal (known as lycopene C25-aldehyde), and other lycopene-related substances, such as 1,2-dihydro-1-hydroxylycopene (rhodopin) or 1,2-dihydro-1-acetyllycopene (acetylrhodopin). Synthetic lycopene may also contain residues of volatile solvents. However, no information has been identified to indicate that any reported residues of heavy metals or other contaminants in excess of FDA tolerances have been identified (Olempska-Beer, 2006a).

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

Synthetic lycopene is prepared from synthetic intermediates that are commonly used in the synthesis of other carotenoids used in food and the final production step involves a Wittig-type condensation reaction. The final synthetic lycopene product may contain low levels of triphenyl phosphine oxide, a common catalyst in the Wittig reaction, and residues of volatile solvents. Triphenyl phosphine oxide, if accidentally released to the aquatic environment, has the potential to harm aquatic organisms or cause long-term adverse effects in the aquatic environment (Fisher Scientific, 2008). No specific reported adverse effects on the environment associated with the manufacture of synthetic lycopene were identified.

Nonsynthetic lycopene is derived from tomatoes, which are farmed both conventionally and organically in North America. Synthetic lycopene is produced by various manufacturers using methods of chemical synthesis and extraction from manipulated fungi (see Evaluation Question #1). The use of synthetic lycopene is not likely to cause adverse effects on the environment or biodiversity because it is an antioxidant naturally found in many fruits and vegetables, and it is regularly consumed and easily digested (ERNA, 2007; Rao and Rao, 2007).

The tomato is one of the most commonly grown fresh market vegetables. However, because tomatoes are labor and water intensive and high yielding, quarter-acre, half-acre, and one-acre production units are common with market gardeners. The farming of tomatoes can have an impact on soil conditions, so agricultural practices including crop rotation and methods for replenishing soil nutrients (e.g., the addition of lime, rock minerals, green manures, etc.) are vital for maintaining biologically active soils. Sustainable production practices have been identified and practiced in the organic farming of tomatoes. Adherence to sustainable production practices is predicted to cause little harm to the environment or biodiversity (ATTRA, 1999).

The environmental impacts of conventional tomato cultivation are explored in a study conducted in the Netherlands by Pluimers et al. (2000). In conventional tomato cultivation, fuel use and related CO_2 emissions are relatively high and CO_2 is regarded as the most important greenhouse gas. Additionally, tomato cultivation may contribute to acidification through NO_X emissions from gas use and the use of fertilizers (Pluimers et al., 2000).

<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)).

No information was found to indicate that use of lycopene may have adverse human health effects. Because lycopene is a nutrient, positive health effects are expected to result from its use. Lycopene has been hypothesized to prevent carcinogenesis and atherogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins and DNA. Specifically, oxidation of low-density lipoproteins, which transport cholesterol into the blood stream, are suspected to be linked to atherosclerosis. Antioxidant nutrients, including lycopene, are believed to slow the progression of atherosclerosis because of their ability to inhibit damaging oxidative processes. The beneficial effects of lycopene on oxidative stress, cardiovascular disease, hypertension, atherosclerosis, cancers, diabetes, and other chronic diseases in humans have been suggested (Agarwal and Rao, 2012). More recently, the effect of lycopene on bone health and osteoporosis has been investigated. While more clinical studies are required before drawing any significant conclusions, it appears that lycopene may have a stimulatory effect on cell proliferation and inhibitory effects on the formation and resorption of osteoblasts, or bone cells that break down and remove bone tissue (Rao and Rao, 2007).

Giovannucci (1999) and Agarwal and Rao (2012) have published reviews of epidemiological studies that focused on linking beneficial health effects to a diet high in lycopene. Key conclusions summarized by these authors include the following:

Cancer:

- Dietary intake of tomatoes and tomato products has been found to be associated with a lower risk of cancers, including total cancer, lung, digestive tract, and pancreatic, prostate in several epidemiological studies in multiple countries (Giovannucci, 1997).
- Epidemiological evidence suggests that a Mediterranean diet that is rich in tomatoes may be associated with a lower rate of cancer, as is observed in the Mediterranean region (La Vecchia, 1997; Agarwal and Rao, 2012).
- In a case-control study, the dietary intake of tomatoes was found to be protective against digestive-tract cancers (Agarwal and Rao, 2012).
- A decreased cancer risk of 50 percent of total cancer was observed in a prospective cohort study involving an elderly population in Massachusetts (Colditz et al., 1985; Agarwal and Rao, 2012).
- The U.S. Health Professionals Follow-up Study evaluated the intake of various carotenoids and retinol using a questionnaire to evaluate the relationship between dietary lycopene intake and risk of prostate cancer. An inverse relationship between the estimated intake of lycopene from various tomato products and the risk of prostate cancer was observed. This relationship was not observed with any other carotenoid. A reduction in risk of almost 35 percent was observed for a consumption frequency of ten or more servings of tomato products per week, stronger protective effects were observed with more advanced or aggressive prostate cancer (Giovannucci et al., 1995; Agarwal and Rao, 2012).
- Following review of 72 epidemiological studies (including ecological, case–control, dietary and blood-specimen-based investigations of tomato based products, tomatoes, lycopene and cancer), an

inverse association between tomato intake or circulating lycopene levels and risk of several types of cancer was reported in 57 studies. In 35 of these studies, the association was considered statistically significant. No reports of adverse effects of high tomato intake or high lycopene levels were reported in these epidemiological studies (Giovannucci, 1999; Agarwal and Rao, 2012).

Cardiovascular disease:

- Some epidemiological studies have shown a reduced risk of cardiovascular disease is associated with the consumption of tomatoes and tomato products containing lycopene (Rao and Rao, 2007).
- In a multicenter case–control study with subjects recruited from ten European countries, the association between antioxidant status and acute myocardial infarction was evaluated. Antioxidant status was evaluated by using adipose tissue antioxidant levels as a biomarker and adipose tissue biopsies were taken directly after infarction and analyzed for various carotenoids. Following adjustment for a range of dietary variables, only lycopene levels were found to be protective while beta-carotene levels were not found to contribute to protection (Kohlmeier et al., 1997; Agarwal and Rao, 2012).
- One study that compared the Lithuanian and Swedish populations showed lower lycopene levels to be associated with increased risk and mortality from coronary heart disease (Rao and Rao, 2007).
- In another small study, lycopene was shown to reduce serum total cholesterol levels, thereby lowering the risk of cardiovascular disease (Rao and Rao, 2007).

Epidemiological evidence of the role of lycopene in cancer prevention and a reduced risk of cardiovascular disease is persuasive; however studies to support these assertions are limited and this role remains unproven (Agarwal and Rao, 2012; Kong et al., 2010).

Generally, the roles of major non-provitamin A carotenoids (i.e., lutein, zeaxanthin, and lycopene) in infant health are not well-established (Dancheck et al., 2005). While lycopene is present in human breast milk, its role in infant development is not scientifically supported (Aetna, 2005). Nonsynthetic lycopene from tomatoes has been linked to lower child mortality in an epidemiological study conducted with Sudanese children (Fawzi et al., 2000). No scientificdata on the direct association of synthetic lycopene with beneficial health effects in infants was identified.

<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)).

The commercial use of nonsynthetic lycopene in infant formula, rather than synthetic lycopene, has not been reported, and no evidence indicating that nonsynthetic lycopene would act as a viable substitute for synthetic lycopene in infant formula has been identified. Approximately 3% of adolescents have a fruit or vegetable allergy, and tomatoes, a source of most nonsynthetic lycopene, are not generally introduced to a baby's diet until after approximately the first ten months of life. Tomatoes may be associated with skin reactions in babies and cause rashes around the mouth and buttocks. Most adverse reactions reported in infants are likely due to the acidity associated with tomatoes (Family Education, 2012; Sydney Children's Hospital, 2011). However it is unclear whether or not an allergy to tomatoes would also indicate an allergy to lycopene.

Although no direct substitute for lycopene is currently available, there are other natural antioxidants that may exhibit similar properties and may decrease the incidence of chronic diseases by minimizing the effects of free radicals on the human body (Caballero, 2009). Synthetic versions of the substances discussed below are generally used for food applications (i.e., as dietary supplements). These antioxidant substances are identified below.

- Beta-carotene—found primarily in fruits containing yellow-orange pigments (e.g., apricots, cantaloupe), juices, and vegetables
- Lutein—found primarily in dark-green vegetables (e.g., spinach, broccoli, peas)

- Selenium—a mineral that is not technically an antioxidant, but is an important component of most antioxidant enzymes; found primarily in plant foods; the content of selenium in food is dependent on the selenium content of the soil where plants are grown or animals are raised
- Vitamin A—found in liver, sweet potatoes, egg yolks, carrots, milk, and mozzarella cheese
- Vitamin C—detected in high concentrations in many fruits and vegetables; also found in cereals, beef, poultry, and fish products
- Vitamin E—component of many oils (e.g., wheat germ, corn, safflower, soybean oils); also found in mango, nuts (almonds), broccoli, among other foods

Sources: Caballero, 2009; NIH, 2011

The National List includes nutrient vitamins and minerals as allowed synthetics when used in accordance with 21 CFR 104.20, Nutritional Quality Guidelines for Foods. It is assumed that Vitamins, A, C, and E as well as the mineral, selenium, would be permitted for use as dietary supplements in foods including infant formulas that are labeled as organic.

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