Lutein

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
(1R)-4-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18[(1R,4R)-4-hydroxy-2,6,6-trimethylchyclohex-2-en-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl}-3,5,5-trimethylcyclohex-3-en-1-ol
β,ε-carotene-3,3′-diol
(3R,3′R,6′R)-β,ε-carotene-3,3′-diol

Other Names:
Xanthophyll
Bo-Xan
Lutein ester
Luteine
Vegetable lutein, vegetable luteol
all-trans-Lutein
all-trans-Xanthophyll

Trade Names:
FloraGLO® Lutein
FloraGLO® Crystalline Lutein
LUTENAT®

CAS Number:
127-40-2

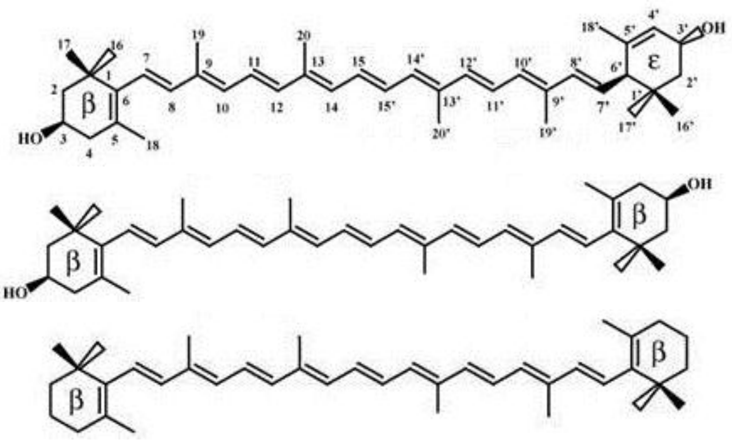
Other Codes:
204-840-0 (EINECS number)
E 161b (E number)

Characterization of Petitioned Substance

Composition of the Substance:

Lutein, also commonly referred to as xanthophyll, is a carotenoid (i.e., a naturally occurring organic pigment) related to beta-carotene and is a powerful antioxidant (Thorne Research, Inc., 2005). Lutein is present in many natural plant and animal products, such as egg yolks, yellow flower petals, and algae (NLM, 2011a). It is also naturally present in many vegetables—notably green vegetables like spinach, broccoli, kale, and green peas (Roodenburg et al., 2000; Thorne Research, Inc., 2005). It is abundant in marigold flowers, which serve as the most common source of lutein used as a coloring agent, nutritive food and feed additive, and nutritional supplement (JECFA, 2006; Bosma et al., 2003).

Lutein, with empirical formula $C_{40}H_{56}O_2$ (NLM, 2011a; 2011b), has two six-carbon rings connected by a chain of alternating single and double bonded carbons (known as a conjugated polyene chain)-see Figure 1A) (JECFA, 2006; Krinsky et al., 2003). A related substance, zeaxanthin, has the same molecular formula as lutein but differs in the double-bond placement and orientation of a hydroxyl group in one of the two carbon rings-see Figure 1(B) (Krinsky et al., 2003). Lutein and zeaxanthin naturally occur together in many plants, fruits, and vegetables, are often combined in nutritional supplements, and play similar roles in the human eye (see “Combinations of the Substance” and Evaluation Question #10) (Krinsky et al., 2003). The difference in double-bond location between the two molecules results in a different stereochemical orientation of the 3′ hydroxyl group. While lutein and zeaxanthin are often grouped together by researchers under the term xanthophylls, the subtle differences in stereochemistry translate to major differences in biomolecular roles and properties of the compounds (Krinsky et al., 2003). Beta-carotene differs from lutein in that it has two beta-ionone rings and contains no hydroxyl groups (see Figure 1[C]) (Krinsky et al., 2003). Zeaxanthin and beta-carotene are not included in the petition for lutein. Zeaxanthin is not specifically listed in OFPA or USDA Final Rule. Beta-carotene is currently allowed in organic products as a coloring agent under 21 CFR 205.606(d).



(A)

(B)

(C)

Figure 1. Molecular Structures of Lutein (A), Zeaxanthin (B), and beta-Carotene (C)
(Krinsky et al., 2003)

Properties of the Substance:

Lutein is one of two major constituents (the other being zeaxanthin) of the macular pigment of the retina and helps to function as a filter of high-energy blue light (Thorne Research, Inc., 2005; Krinsky et al., 2003). Lutein is highly light absorptive due to its polyene chain with nine conjugated double bonds (Krinsky et al., 2003). Compared with beta-carotene, lutein is more polar due to the presence of a hydroxyl group on each of its ionone rings (Krinsky et al., 2003). As a result of its polarity, lutein is lipophilic and accumulates in fatty tissues (Krinsky et al., 2003). Physicochemical properties of lutein are provided in Table 1.

Table 1. Physicochemical Properties of Lutein

Property	Value
Physical state	Solid
Appearance	Free-flowing powder, orange-red color[a]
Molecular weight (g/mol)	568.87144[b]
Melting point (˚C)	196[c]
Solubility in water	Insoluble[a]
Solubility in hexane	Soluble[a]
Log octanol/water partition coefficient (K_{ow}) (unitless)	14.82[c]
Atmospheric OH rate constant (cm^3/molecule-second at 25˚C)	6.84×10^{-10}[c]
Wavelength absorption maximum in ethanol (nm)	445[d]

[a]JECFA, 2006
[b]NLM, 2011b
[c]NLM, 2011a
[d]Krinsky et al., 2003

Specific Uses of the Substance:

Lutein is used in food handling and processing as a coloring agent and nutrient supplement, marketed for its important role in eye health and development (Bettler et al., 2010; Perry et al., 2009; JECFA, 2006; Krinsky et al., 2003). The petitioner seeks placement on the National List of Allowed and Prohibited

Substances (hereafter referred to as the "National List") of a food-grade lutein with a suitable status for use in infant formula (Kemin Health, L.C., 2011).

All lutein in the human body is acquired through the diet; it cannot be synthesized by the body (Bettler et al., 2010; Sujith et al., 2010). Lutein is naturally present at high levels in many unprocessed green vegetables like kale, spinach, broccoli, peas, and brussel sprouts. It is found at lower levels in corn, persimmons, tangerines, and orange juice. Table 2 provides a list of some foods that naturally contain lutein. Lutein can also be found naturally, albeit in low levels, in processed foods made from corn such as cornmeal, corn-based cereals, and corn-based chips. Eggs and egg products like mayonnaise also contain lutein (Perry et al., 2009; Thorne Research, Inc., 2005; Krinsky et al., 2003).

Lutein is added as a coloring agent and nutrient supplement to foods such as baked goods or baking mixes, beverages, cereals, gum, candy, dairy-based desserts or dessert mixes, prepared sauces, gravies, soups, and packaged mixes for sauces, gravies, and soups (JECFA, 2006). It is commonly added to corn- and alfalfa-based poultry feed to enhance the orange color of egg yolk and yellow color of chicken skin desired in poultry products intended for human consumption (Bosma et al., 2003). Table 3 lists human food additive uses for lutein and acceptable use levels in those foods as determined and approved by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 2006.

Table 2. Naturally Occurring Lutein in Selected Foods[a]

Raw Vegetables	**Concentration (µg/100 g)**	**Cooked Vegetables**	**Concentration (µg/100 g)**
Cucumber	361	Artichoke heart	62
Endive	399	Broccoli	72
Lettuce (iceberg)	171	Brussel sprouts	155
Lettuce (romaine)	3824	Kale	8884
Pepper (green)	173	Spinach	12,640
Pepper (orange)	208	Squash (butternut)	150
Pepper (yellow)	139	Squash (yellow)	150
Scallions	782	Zucchini	1355
Spinach	6608		
Squash (acorn, no skin)	47		
Fruit	**Concentration (µg/100 g)**	**Other Foods**	**Concentration (µg/100 g)**
Apple (red delicious, skin)	15	Cilantro	7703
Cantaloupe	19	Egg (cooked)	237
Grapes (green)	53	Egg (raw)	288
Grapes (red)	24	Egg yolk (cooked)	645
Honeydew	25	Egg yolk (raw)	787
Mango	6	Green Olive	79
Nectarine	8	Lima beans (cooked)	155
Orange juice	33	Mayonnaise	35
Peach	11	Parsley	4326
Tomato	32	Pistachios (shelled)	1405
Watermelon	4	Salsa	40

[a]Perry et al., 2009

Table 3. Food Uses and Use Levels for Lutein as Provided by JECFA (2006)[a]

Food Category	Food Use[b]	Use Level (mg/kg)[c]	Food Category	Food Use[b]	Use Level (mg/kg)[c]
Baked goods/ baking mixes	Cereal and energy bars	50	Hard candy	Hard candy	67
	Crackers/crisp breads	67	Infant and toddler foods[d]	Junior, strained, and toddler-type baby foods	5.9–140
Beverages and beverage bases	Bottled water	2.1	Milk products	Dry milk	13
	Carbonated beverages	8.3		Fermented milk beverages	2.6
	Meal replacements	8.3		Flavored milk and milk drinks	13
	Tea, ready-to-drink	2.6		Milk-based meal replacements	13
Breakfast cereals	Instant and regular hot cereals	8.3		Yogurt	13
	Ready-to-eat cereals	36–130	Processed fruits and fruit juices	Energy, sport, and isotonic drinks	8.3
Chewing gum	Chewing gum	330		Fruit-flavored drinks	8.3
Dairy product analogs	Imitation milks	8.3		Fruit juice	8.3
	Soy milks	6.3		Nectars	8.3
Egg products	Liquid, frozen, or dried egg substitutes	40		Vegetable juice	8.3
Fats and oils	Margarine-like Spreads	100	Soft candy	Chewy and nougat candy	25
	Salad dressings	50–100		Fruit snacks	25
Frozen dairy desserts/mixes	Frozen yogurt	8.3	Soups and soup mixes	Canned soups	2.6
Gravies/sauces	Tomato-based sauces	2.6			

[a]Table reproduced from JECFA (2006).
[b]Represents the food categorization system from the General Standard for Food Additives (GSFA).
[c]When a range of use levels (mg/kg) is reported for a proposed food use, particular foods within that food-use group may differ with respect to their serving size.
[d]Does not include infant formula.

Lutein is also sold as a nutrient supplement, as it is an antioxidant that plays an important role in eye health and development (Bettler et al., 2010; Perry et al., 2009; Krinsky et al., 2003). Supplemental lutein is typically in the form of lutein diester at levels of 6–25 mg per capsule. It is included in some multivitamins at levels of 0.25 mg per capsule (Krinsky et al., 2003).

Lutein is a natural component of human breast milk. In one recent study, it was measured at levels averaging 21.1 micrograms per liter (0.492 micrograms per gram of milk fat) when mothers consumed an average of 3363 mg per day of lutein (Bettler et al., 2010). Another study reported that average levels of lutein plus zeaxanthin in breast milk samples from 9 countries was 25 ± 19 micrograms per liter (Capeding et al., 2010). Due to differences in bioavailability between supplemental lutein in formula and natural lutein in breast milk, lutein would need to be added to formula at levels approximately 4 times higher than those observed in human breast milk to achieve similar serum lutein concentrations in formula-fed and breastfed infants (Bettler et al., 2010). It is currently unclear why lutein bioavailability is different when consumed via human milk versus lutein-fortified formula, as the lutein added to the formula is in the same form as the lutein present in human milk. Several studies have shown that factors such as the food matrix, fat intake, and nutrient-nutrient interactions may play a role in lutein bioavailability in foods (Bettler et al., 2010).

As of 2010, lutein was not added directly to infant formulas in the United States but was present in ingredients used in the manufacturing of infant formulas, such as skim milk powder and whey protein ingredients, resulting in some measurable lutein content in infant formulas (Bettler et al., 2010). Recently, infant formula manufacturers began marketing new higher-tier infant formula products (such as "advance" and "sensitive" formulations) that contain added nutrients for brain and eye development, including lutein. See "Historic Use" for more details.

Approved Legal Uses of the Substance:

Lutein is not currently included on the National List as a nonorganically produced agricultural product allowed as an ingredient in or on processed products labeled as "organic" (7 CFR 205.606). Nor is it listed as a nonagricultural (nonorganic) substance 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).

Lutein is not specifically included on FDA's Food Additives Status List (U.S. FDA, 2012); however, tagetes (marigold)—oil only—is listed as a substance that is allowed as a natural flavoring substance or natural substance used in conjunction with flavors (21 CFR 172.510). Lutein is not included on FDA's Color Additive Status List; however, tagetes (Aztec marigold) meal and extract (for chicken feed only) is included (U.S. FDA, 2011a; 21 CFR 73.295). FDA regulates infant formulas for sale in the United States under 21 CFR 107. This regulation does not include specifications for the use of lutein.

Lutein is not currently listed as GRAS under 21 CFR 182, 184, or 186. As described further in Evaluation Question #4, FDA has responded with nonobjection to several GRAS notices submitted by manufacturers of lutein products, such as lutein esters, (U.S. FDA, 2003), crystalline lutein (U.S. FDA, 2004), and suspended lutein (U.S. FDA, 2007), all of which are mixtures of the xanthophylls lutein and zeaxanthin. The GRAS notification for suspended lutein specifies its use in infant formulas at a maximum level of 250 micrograms per liter (U.S. FDA, 2007). However, no changes have been made to include lutein in the GRAS regulations at 21 CFR 182, 184, or 186, or 21 CFR 107.100 (the regulation for infant formula).

Lutein 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 it regulates drugs or animal feed additives; generally, manufacturers do not need to register these products with FDA or receive 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 making sure the supplement's label is accurate and not misleading (U.S. FDA, 2005).

Action of the Substance:

When used as a nutritional supplement, lutein functions as an antioxidant (Bowen et al., 2002; Perry et al., 2009; Fernandez-Sevilla et al., 2010). After ingestion, lutein is absorbed through the gastrointestinal tract into the bloodstream and accumulates in the macula region of the retina where it protects macular cells from oxidative stress (Bowen et al., 2002; Perry et al., 2009; Fernandez-Sevilla et al., 2010). There are many modes of antioxidant action; carotenoids function as singlet oxygen quenching antioxidants (Bowen et al., 2002). Singlet oxygen quenchers prevent oxidation by reacting with the singlet oxygen molecule before it has a chance to oxidize a different molecule, preventing free radicals or peroxides from being formed

(Buettner and Schafer, 2002). Carotenoids are effective singlet oxygen quenchers because they easily lose an electron from the polyene chain (Krinsky et al., 2003). Lutein also absorbs blue light, thereby decreasing the incidence of chromatic aberration that occurs when blue light impinges on photoreceptor cells in the retina and preventing generation of reactive oxygen species that damage photoreceptor cells (Krinsky et al., 2003).

Lutein also acts as a coloring agent. When extracted from marigold, it is red-orange in color (JECFA, 2006).

Combinations of the Substance:

Lutein is added to infant formulas, including some organic formulas (see "Historic Use"). Infant formulas contain a number of nutrients (i.e., protein, calcium, iron, thiamin, biotin, phosphorus, magnesium, zinc, riboflavin, niacin, pantothenic acid, iodine, copper, potassium, and vitamins A, C, D, E, B6 and B12) included on the National List in accordance with FDA's Nutritional Quality Guidelines for Foods (21 CFR 104.20, see "OFPA, USDA Final Rule" for further discussion). Furthermore, a mixture of food ingredients, including carbohydrates, proteins, fats, and stabilizers, are expected to be included in infant formula and other foods to which lutein is added. These ingredients will vary significantly with the type of product and manufacturer.

In dietary supplements, lutein is often combined with other marketed nutrient supplements such as zeaxanthin, beta carotene, ascorbic acid, tocepheryl acetate, zinc oxide, and cupric oxide. Supplement formulations often contain "non-active" ingredients such as corn oil, glycerin, gelatin, beeswax, soy lecithin, magnesium stearate, corn starch, hydroxypropyl methylcellulose, polyethylene glycol, titanium dioxide, sucrose, caramel color, riboflavin, and artificial colors (Walgreens Co., 2012).

Commercially available lutein products contain other ingredients such as safflower oil, zeaxanthin, and zeaxanthin esters (Abbott Nutrition, 2011; Soni & Associates, Inc., 2011; U.S. FDA, 2004; U.S. FDA, 2007).

Status

Historic Use:

Lutein is naturally present in many fruits and vegetables consumed by humans (see "Specific Uses of the Substance"). The presence of lutein in the macula of the human retina was discovered in 1945 although it was not until the 1990s that lutein's role in age-related macular degeneration was recognized (Krinsky et al., 2003).

As of 2010, lutein was not added directly to infant formulas in the United States (Bettler et al., 2010). In the early 2000s, formula makers such as Enfamil ® began marketing higher-tier infant formulas with added fatty acids such as arachidonic acid (ARA) and docosahexaenoic acid (DHA) intended to promote brain development (Trademarkia, 2012a). Shortly after, products with lutein added for eye development, such as Similac ® Advance ® EarlyShield ® came on the market (Trademarkia, 2012b). Lutein is currently added to many higher-tier infant formulas including nonorganic formulas such as Similac® soy, milk, and non-milk based formulas marketed with EarlyShield®, and Parent's Choice™ Advantage (Store Brand Formulas, 2012; Abbott Laboratories, 2012a; Parents Choice Infant Formula, 2012). Lutein is an added ingredient in Similac® Advance® Organic, which is marketed with EarlyShield® ingredients (including lutein) to promote eye and brain development and is labeled as certified USDA Organic (Abbott Laboratories, 2012b). Lutein is currently not added to most basic organic infant formulas marketed in the United States. For example, lutein is not used in Similac® Organic Infant Formula, Vermont Organics™ Infant Formulas (soy-based or milk-based), Baby's Only Organic® Soy Formula, or Parent's Choice™ Organic Infant Formula (Abbott Laboratories, 2012; Vermont Organics, 2012; Nature's One, Inc., 2012; Parent's Choice Infant Formula, 2012).

The history of the legal use of lutein in organic handling/processing has revolved around uncertainty over the nutritional status of lutein because it is neither a vitamin nor a mineral. In 1995, the National Organic

Standards Board (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 at 7 CFR 205.605(b) permits the use of "nutrient vitamins and minerals, in accordance with 21 CFR 104.20" (USDA, 2011). Originally, the National Organic Program (NOP) interpreted that under 21 CFR 104.20(f), which states that "nutrient(s) may be added to foods as permitted or required by applicable regulations established elsewhere in this chapter," lutein 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 104.20(d)(3), which does not include lutein. See "OFPA, USDA Final Rule" for more information.

<u>OFPA, USDA Final Rule</u>:

Lutein is not currently listed under 7 CFR 205.606, as a nonorganically produced agricultural product allowed as an ingredient in or on processed products labeled as "organic." It also is not listed under 7 CFR 205.605 as a nonagricultural (nonorganic) substance allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group[s])." The petitioner believes that lutein should be added to 7 CFR 205.606 (Kemin Health, L.C., 2011). Nonorganic ingredients listed on section 205.606 are permitted only when the product is not commercially available in organic form. Currently, some compounds that are chemically and functionally similar to lutein are included under 21 CFR 205.606(d), titled "colors derived from agricultural products," such as beta-carotene extract color derived from carrots (CAS #1393-63-1).

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, choline, inositol, and lutein.

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 lutein, would need to be individually petitioned for consideration by the NOSB. If promulgated as a final rule, this amendment would clarify that lutein is not one of the required nutrients currently allowed in organic products (USDA, 2012).

<u>International</u>

The International Federation of Organic Agriculture Movements (IFOAM) does not list lutein within its "Norms for Organic Production and Processing" (IFOAM, 2006).

The Codex Alimentarius Commission of the Joint FAO/WHO Food Standards Programme also does not list lutein within its guidelines for organically produced foods (Codex Alimentarius Commission, 2001). 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 world-wide standard for infant formula does not list lutein as an essential component or acceptable additive for infant formulas (Codex Alimentarius Commission, 1981).

The European Economic Community (EEC) Council Regulations do not list lutein as allowable for use in organic foods/food production (Commission of the European Communities, 2008). 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). European Commission Directive 2006/141/EC, the directive on infant formula, does not include specifications for the use of lutein in infant formulas (Commission of the European Communities, 2006).

The Canadian Organic Production Systems Permitted Substances List (CGSB, 2011) does not list lutein. Canadian Food and Drug Regulations do not require infant formula to contain lutein under Section B.25.054 (Health Canada, 2011).

The Japanese Agricultural Standard for Organic Processed Foods does not include lutein (JMAFF, 2006). The East African Organic Product Standard and the Pacific Organic Standard were both created using the IFOAM and Codex guidelines as models; both standards do not list lutein as allowed for use in organic foods (East African Community, 2007; Secretariat of the Pacific Community, 2008).

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)).

Lutein can be extracted from agricultural sources (e.g., marigolds) and also can be obtained through methods using fermentation and chemical synthesis (Cussler and Moggridge, 2011). Extraction from *Tagetes erecta* (Aztec marigold) petals is the most prevalent method for commercial manufacture worldwide (EFSA, 2008). While lutein is found in many plants, about 90% of the total carotenoid pigments in marigolds is lutein and 5% is the similar compound zeaxanthin (Attokaran, 2011); the lack of other pigments is a reason that marigolds are advantageous for commercial lutein production (Sujith et al., 2010). Still, marigolds are approximately 85% moisture, and lutein content is only between 0.1% and 0.15% (Attokaran, 2011). Marigold flower petals can contain nearly 10 g of lutein per kilogram of dried petals (Sujith et al., 2010) or anywhere from 10–30 kg of lutein per hectare of planted marigolds (Bosma et al., 2003). Other plants from which lutein can be extracted include grass, spinach, alfalfa (lucerne), and a variety of edible fruits (EFSA, 2008).

Marigolds are commercially grown in Mexico, Peru, India, Ecuador, Argentina, and Venezuela (Bosma et al., 2003; EFSA, 2008; Bechtold and Mussak, 2009). No information was found regarding the use of pesticides in marigold farming. The flowers are harvested by hand, slowly dried under low-temperature conditions (because the pigments are heat-sensitive), and pelletized (Bosma et al., 2003; Attokaran, 2011). About 60% of the total volume of dried, powdered marigold flowers, referred to as marigold meal, is used directly as a colorant, mainly in poultry feed (Bechtold and Mussak, 2009).

The traditional process to extract the lutein from marigold meal involves extraction of marigold oleoresin (which is a substance containing lutein esters, other carotenoids, and natural plant oils and waxes or "resins") using solvents, saponification to yield free lutein, and crystallization to purify the lutein (Sujith et al., 2010; JECFA, 2006). Saponification is a chemical reaction through which the fatty acids are cleaved from the xanthophyll esters (EFSA, 2008). This process is described in more detail below.

Marigold oleoresin is extracted from the marigold meal using a solvent, most commonly hexane (JECFA, 2006; EFSA, 2008). Approximately 300 kilograms of hexane must be used per kilogram of flowers (Cussler and Moggridge, 2011). Other solvents often used by manufacturers include isopropyl alcohol, acetone, methanol, and ethanol; dichloromethane and methyl ethyl ketone are also allowed for use (ESFA, 2008). The oleoresin contains lutein in the form of lutein esters, mainly lutein dipalmitate (50%), lutein dimyristate (30%), and lutein monoester (6%) (Attokaran, 2011). Marigold oleoresin does not contain any free lutein (Bechtold and Mussak, 2009). A percentage of the commercially produced oleoresin is mixed with vegetable oil and used directly in food applications or is mixed with corn or soy meal for poultry feed (Bechtold and Mussak, 2009).

Free lutein is then prepared from the remaining oleoresin through a process of saponification and crystallization. The commercial saponification method involves the use of multiple solvents—typically including propylene glycol or methanol—potassium hydroxide, and water under heated conditions (Sujith et al., 2010; JECFA, 2006; EFSA, 2008). Reaction times and temperatures vary depending on the manufacturer; reports range from 40 minutes to 10 hours at various temperatures for the full process; reaction for a period of 4 hours at 80°C can generally achieve 90% saponification (Cussler and Moggridge, 2011). The resulting free lutein crystals are washed with deionized water, recrystallized to purify them, and dried (JECFA, 2006; EFSA, 2008). An alternative method for saponification uses ethanol, water, and a 45% alkali solution heated at 45–80°C for 3–5 hours; however, because of the high amount of alkali needed, this method is less economical on a commercial scale (Sujith et al., 2010).

Alternative extraction methods include chemical synthesis and fermentation technology (which takes advantage of microbiota naturally associated with the marigold flowers such as *Flavobacterium IIb, Acinetobacter anitratus,* and *Rhizopus nigricans*); however these methods have disadvantages compared with the traditional method described above (Navarrete-Bolanos et al., 2004). Removal of toxic substances used during chemical synthesis methods has many practical limitations, and yields from traditional fermentation processes are generally low (Navarrete-Bolanos et al., 2004). Solid-state fermentation can result in greater yields than normal fermentation processes, up to 17.8 g/kg dry weight (Navarrete-Bolanos et al., 2004). Solid-state fermentation is a process defined by growth of microorganisms on moist solid materials in the absence of free water, in which a solid natural substrate is used as a carbon source or an inert substrate is used for solid support (Panday et al., 2008). Solid-state fermentation has been in use for food production since ancient history, as it was the process used for making bread in ancient Egypt and soy sauce by the Buddhists in the 7^{th} Century. However, it was not until the 20^{th} century that solid state fermentation was used to produce pigments, enzymes, organic acids, or secondary metabolites (Panday et al., 2008).

Another alternative manufacturing process that has been highly investigated in recent years is production of lutein via microalgae. Certain microalgae naturally produce high levels of lutein, and production per square meter can be hundreds of times greater than that of marigold (Fernandez-Sevilla et al., 2010). Microalgae produce free, nonesterified lutein, so the saponification and crystallization described above is not necessary (Del Campo et al., 2007). Pilot scale experiments have shown that *Muriellopsis* spp. accumulate high levels (between 0.4 and 0.6% by weight) of lutein when grown photoautotrophically and in closed outdoor systems. Year-round yield can reach about 180 mg/m^2/day (1.8 kg/hectare/day) in a closed system (Del Campo et al., 2007). Similarly, *Scenedesmus almeriensis,* cultured in a tubular photobioreactor inside a greenhouse, has been shown to produce 290 mg/m^2/day (2.9 kg/hectare/day). As of 2007, commercial systems for lutein production using microalgae did not exist, despite strong results using pilot scale systems (Del Campo et al., 2007). No information was found to indicate this had changed in recent years.

The petitioner states that its method of lutein extraction starts with nonorganically-produced marigold flowers and claims that the processing steps would not classify the final product as synthetic. The process involves extraction of oleoresin using hexane as described above and a simple de-esterification process, the details of which are confidential business information (Kemin Health, L.C., 2011). No information was found to indicate that the lutein manufacture process used in the United States varies from the predominant manufacture process used internationally, described above.

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)).

Lutein extracted from marigold oleoresin is synthetic. The lutein in marigold oleoresin is in the form of lutein esters and a chemical change is used to produce a final product of free lutein. The extraction of marigold oleoresin using hexane, as described in Evaluation Question #1, does not involve any chemical synthesis (JECFA, 2006; EFSA, 2008). Saponification of free lutein from the lutein esters found in marigold oleoresin is a chemical process through which the fatty acids are cleaved from the xanthophyll esters (EFSA, 2008).

The petitioner states that its method of lutein extraction is non-synthetic. While the details of the de-esterification process used by the petitioner are confidential, esterification is typically deemed a chemical process. The petitioner cites the NOSB's classification of pectin, which the petitioner claims is derived from a similar process, as evidence that lutein should be classified as nonsynthetic (Kemin Health, L.C., 2011).

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

Marigold flowers are a natural, agricultural source of xanthophylls containing high concentrations of lutein (Navarrete-Bolanos et al., 2004). However, lutein is present in marigold flowers in the form of lutein esters (Attokaran, 2011) and must be chemically altered to produce commercial formulations of free lutein as described in the response to Evaluation Question # 1 and # 2. Lutein is naturally present in free form in other plants, most notably in green vegetables like spinach, broccoli, kale, and green peas (Roodenburg et al., 2000), and potential extraction would not require chemical de-esterification. It is unclear whether other synthetic methods would be necessary for extraction. No information was found to indicate that commercial-scale extraction from these sources occurs.

No information was found to indicate whether organically-grown marigolds are available for commercial-scale lutein extraction.

Evaluation Question #4: 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?

The technical function of lutein is as a coloring agent and nutrient supplement (JECFA, 2006). The petitioned substance is lutein derived from marigold (*Tagetes erecta*), and meeting the "Lutein" monograph established by the U. S. Pharmacopeia (USP). FDA has responded to several GRAS notices submitted by manufacturers of lutein products, such as lutein esters, defined as a mixture of carotenoid xanthophylls esters including both esters of lutein and zeaxanthin (U.S. FDA, 2003), crystalline lutein, defined as a mixture of lutein and zeaxanthin (U.S. FDA, 2004), and suspended lutein, defined as a mixture of lutein and zeaxanthin in safflower oil (U.S. FDA, 2007). The GRAS notification for suspended lutein specifies its use in infant formulas at a maximum level of 250 micrograms per liter (U.S. FDA, 2007). FDA had no questions regarding the manufacturers' conclusions that lutein is GRAS under the intended uses; however, it has not made its own determinations regarding the GRAS status of these subject uses. GRAS notifications have been submitted to FDA recently by manufacturers of two lutein products: lutein and zeaxanthin preparation and FloraGLO ® Lutein 20% in Safflower Oil, which contains 20% lutein and 1% zeaxanthin by weight (Soni & Associates, Inc., 2011; Abbott Nutrition, 2011). FDA has not responded to these notifications. Lutein is not listed as GRAS under 21 CFR 182, 184, or 186.

As a result of the nonobjection responses from FDA (U.S. FDA, 2003; U.S. FDA, 2004; U.S. FDA, 2007), the petitioner refers to lutein as GRAS. This GRAS designation is recognized by the European Food Safety Authority, although they note that FDA has not changed its regulations (EFSA, 2008).

Evaluation Question #5: 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 technical function of lutein is a coloring agent and nutrient supplement (JECFA, 2006). It is not added to foods as a preservative.

Evaluation Question #6: 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)).

Lutein is an antioxidant that is found naturally in many foods and can be used to provide supplemental nutritional benefits and to enhance food color. However, no information was found to suggest that lutein is used to recreate or improve flavors, colors, textures, or nutritive values that are lost in processing.

Evaluation Question #7: 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)).

Lutein is an antioxidant. It is added to processed foods such as cornmeal, cereals, baked goods, and milk products (including infant formulas) as a nutritional supplement (JECFA, 2006).

Lutein is naturally present at high levels in many unprocessed green vegetables and at lower levels in other foods such as fruits and some processed foods (e.g., those made with corn and eggs) (Perry et al., 2009; Thorne Research, Inc., 2005; Krinsky et al., 2003). Additionally, lutein is added as a coloring agent and nutrient supplement to foods such as baked goods or baking mixes, beverages, cereals, gum, candy, dairy-based desserts or dessert mixes, sauces, gravies, soups, and packaged mixes for sauces, gravies, and soups (JECFA, 2006). Foods with lutein are often marketed for their nutritional benefits in promoting healthy eyes and skin, improving cardiovascular health, and reducing the risk of breast cancer (Lutein Information Bureau, 2006). See response to Evaluation Question #10 for further information.

Lutein is present at variable levels in human breast milk, along with other carotenoids such as zeaxanthin; however, its role in infant growth and development is currently not established (EFSA, 2008). See response to Evaluation Question #10 for more information.

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)).

No information regarding residues of heavy metals or other contaminants in lutein has been identified. No substances listed on FDA's Action Levels for Poisonous or Deleterious Substances in Human Food have been reported as contaminants of concern in lutein. The requirements for lutein in the 7th edition of the "Food Chemicals Codex" specify that it contain no less than 74% lutein and no more than 8.5% zeaxanthin in the purified fraction (U.S. Pharmacopeia, 2010a). At least 80% of the total carotenoids must be lutein. It cannot contain more than 1 microgram of lead per gram and 5 micrograms total heavy metals per gram (U.S. Pharmacopeia, 2010a).

Makers of dietary supplements can voluntarily apply for verification by U.S. Pharmacopeia (USP), which has a strict set of requirements for purity, potency, and quality of dietary supplements (U.S. Pharmacopeia, 2012). A dietary supplement marked with a "USP Verified" label reportedly "does not contain harmful levels of specified contaminant" including heavy metals (e.g., lead and mercury), pesticides, bacteria, molds, toxins, or other contaminants (U.S. Pharmacopeia, 2012). USP dietary supplements cannot contain more than 10 microgram of lead, 15 microgram of arsenic or total mercury, 2 microgram of methyl mercury (as Hg), or 5 microgram of cadmium (U.S. Pharmacopeia, 2010b), suggesting that any lutein supplement that is USP verified would not contain metals at levels above these limits.

Hexane, propylene glycol, and methanol are possible contaminants in commercially produced lutein, a result of the oleoresin preparation and saponification steps of lutein manufacture (JEFCA, 2006). When marigolds are harvested, they are often left unprotected outside for days or weeks before they are moved to the stages of drying and pelletizing in preparation for lutein extraction; mold growth is possible during this time (Bosma et al., 2003). No information was found to indicate whether pesticides used during marigold farming are potential contaminants of lutein.

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)).**

Growth of marigolds for commercial lutein production requires a large amount of land and resources such as water and fertilizers. Alternative production methods using microalgae are known to be less land-intensive (Fernandez-Sevilla et al., 2010), however, no information could be found regarding the amount of resources consumed in production of microalgae. The petitioner is requesting lutein be added to 7 CFR 205.606 (nonorganically produced agricultural products that may be used as ingredients in or on processed products labeled as "organic"). Nonorganic ingredients listed on 205.606 are permitted only when the product is not commercially available in organic form. Nonorganic marigolds can be grown using conventional inputs or other nonorganic production methods. Marigold crops are susceptible to insects such as spider mites, corn earworms, and blister beetles as well as diseases such as Alternaria leaf spot. Application of various chemicals can control pests and disease (Bosma et al., 2003), but may also pose risks to the environment.

Production of oleoresin from dried marigold flowers uses large volumes of organic solvents; saponification and subsequent purification also involve the use of solvents (Sujith et al., 2010) (see Evaluation Question #1). These solvents have the potential to enter the environment through waste streams. Storage tanks of solvent chemicals can rupture and/or leak, releasing these chemicals into the environment. Impact of a solvent released to the environment will depend on factors such as its quantity (amount released), toxicity, mobility, and persistence in the environment.

No other information was identified regarding the environmental impact of commercial marigold production or lutein production processes.

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

In humans, it has been shown that lutein and other carotenoid pigments accumulate in the macular region of the retina. There, lutein absorbs blue light and works as an antioxidant to protect macular cells from oxidative stress (Krinsky et al., 2003; Bowen et al., 2002). Macular accumulation of lutein is inversely related to age-related macular degeneration (AMD or ARMD), a leading cause of blindness in older individuals (Bowen et al., 2002). Studies have suggested that adequate lutein intake can prevent or ameliorate age-related macular degeneration, cataract development, and skin problems through its antioxidant action and filtering of blue light (Fernandez-Sevilla et al., 2010; Perry et al., 2009; Krinsky et al., 2003; Bowen et al., 2002). Lutein has also been studied for its role in cancer prevention (potentially because it protects against cell damage through oxidation and auto-oxidation of cellular lipids) and immune function enhancement (Navarrete-Bolanos et al., 2004).

Some sources such as online forums, blogs, and websites have suggested that lutein may play a role in autism and attention deficit hyperactivity disorder (ADHD) and that a lutein-free diet (or "Sara's Diet") can help decrease the extent of symptoms for these conditions in children (World Community Autism Program, 2012; Livestrong, 2011; Pauli, 2009). No peer-reviewed, scientific publications were found to support these claims.

In 2008, the European Food Safety Authority released a Scientific Opinion on the safety, bioavailability, and suitability of lutein as a nutrient for infants and young children (EFSA, 2008). The Opinion stated that, "a search in biomedical databases did not reveal any epidemiological or experimental study on beneficial effects of lutein intake on eye function and development in infants and young children." Further, no clinical data was identified on long-term effects of early lutein consumption. The Panel concluded that there is no information available to raise concerns regarding the safety of lutein added to infant formula up to 250 micrograms per liter (EFSA, 2008).

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

Lutein is petitioned to section 205.606 of the National List as an agricultural product. Nonorganic ingredients listed on section 205.606 of the National List are permitted only when the product is not commercially available in organic form.

No organic agricultural products were identified as viable alternatives for lutein as a nutritional supplement. Consumption of organic foods that naturally contain lutein, such as green leafy vegetables like spinach and kale, could be considered an alternative to the use of foods supplements containing lutein extracted from marigold flowers.

The petitioner investigated an alternative for lutein supplementation that uses lutein-containing organic vegetables, such as dried or dehydrated powdered spinach, instead of lutein purified from marigold oleoresin. The petitioner considered the mass of purified lutein currently added to different nonorganic processed foods and determined the mass of raw pureed spinach or dehydrated spinach powder that was required to reach the same lutein content in the final product. The petitioner concluded that the amount of spinach necessary to achieve that same lutein content would be so high that it would significantly change the characteristics of the food and result in visual changes that would render the product undesirable (Kemin Health, L.C., 2011).

As mentioned in "Specific Uses of the Substance," lutein is a natural component of human breast milk present at around 21–25 micrograms per liter (Bettler et al., 2010; Capeding et al., 2010). An alternative to lutein-supplemented infant formula might be organic cow milk-based formula, as ingredients such as skim milk powder and whey protein contain measurable amounts of lutein that are comparable to the amounts in human breast milk (Bettler et al., 2010). However, the serum lutein levels in infants fed unfortified milk-based formulas are substantially lower than the lutein levels in infants fed human breast milk, indicating a different bioavailability of the lutein in formulas and a need to fortify formulas with supplemental lutein in order to achieve the same lutein intake (Bettler et al., 2010). Further, adverse reactions to cow's milk are common in infants (Kvenshagen et al., 2008), so suitable alternative nutrition sources such as fortified soy-based formulas must be available.

Lutein is a carotenoid related to other carotenoids, such as beta-carotene and zeaxanthin, as described in "Composition of the Substance." Currently, beta-carotene is included on the National List under 21 CFR 205.606(d), titled "colors derived from agricultural products," and may function as a substitute for lutein when the desired function is as a coloring agent.

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