β-Carotene

Handling/Processing

Identification of Petitioned Substance

Chemical Name:
β-Carotene; Β-Carotene

CAS Number:
7235-40-7

Other Names:
β,β-Carotene; Carotaben; Provatene; Solatene; all-*trans*-β-Carotene

Other Codes:
EINECS No. 230-636-6
INS[1] No. 160a(ii)

Characterization of Petitioned Substance

Composition of the Substance:

Carotenoids are natural pigments, which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. They act as photosynthesis aids and for the photo protection of their hosts (Isler 1971; Britton and others 1995). There are several dozen carotenoids in the foods that we eat, and most of these carotenoids have antioxidant activity. β-Carotene is the most common carotenoid consisting of a highly branched, unsaturated chain containing identical substituted ring structures at each end; see Figure 1.

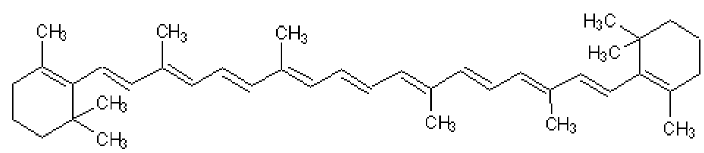

Figure 1. β-Carotene Chemical Structure

It is made of two molecules of retinol[2] (an alcohol), Figure 2 (see below), and possesses maximal provitamin A activity (Ball, 1996). Retinol can only be found in animal sources and can be converted by the body into retinal (an aldehyde) and retinoic acid (a carboxylic acid), other active forms of vitamin A.

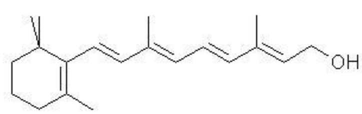

Figure 2. Retinol Chemical Structure

Carotene was first isolated from carrots by Wackenroder in 1831 (Davies, 1976). It is a general term describing certain polyene hydrocarbons containing 40 carbon atoms. Three of these, α-, β- , and γ-carotene, as well as some closely related oxygen-containing carotenoids, exhibit provitamin A activity (SCOGS Report No. 111). The petitioned substance has the molecular formula $C_{40}H_{56}$ and is comprised of 89.49% C and 10.51% H (Merck Index, 2006).

In plants, β-carotene occurs almost always together with chlorophyll (Merck Index, 2006). It is the major coloring principle in carrot and as well palm oil seed extracts. In addition, β-carotene is found in cantaloupe, apricots, sweet potatoes, pumpkin, winter squash, mangos, collard greens, spinach, kale, broccoli, and other orange, red, and dark green fruits and vegetables.

[1] International numbering system.
[2] Retinol is a form of vitamin A, also called preformed vitamin A.

Properties of the Substance:

The petitioned substance occurs as red crystals or crystalline powder (FCC, 2010-2011). The absorption spectrum of β-carotene shows between 400-500 nm, which is the green/blue part of the spectrum (Isler, 1971). Therefore, the molecule of β-carotene absorbs green/blue lights and gives off red/yellow colors.

The petitioned substance is insoluble in water, acids and alkalies, but is soluble in carbon disulfide and chloroform. β-Carotene is practically insoluble in methanol and ethanol, and is sparingly soluble in ether, hexane, and oils (FCC, 2010-2011). The diluted solution is yellow. It absorbs oxygen from the air giving rise to inactive, colorless oxidation products (Merck Index, 2006). In other words, β-carotene changes in color from a fairly deep reddish-orange to the oxidized product, which is a light, yellowish gray (Furia, 1972). β-Carotene melts between 176° C and 182° C, with decomposition. Its molecular weight is 536.87 g/mol (Merck Index, 2006; FCC, 2010-2011).

The best characterized natural functions of carotenoids (including β-carotene) are to serve as light-absorbing pigments during photosynthesis and protection of cells against photosensitization (SCF, 2000). In plants, carotenoids have the important antioxidant function of quenching (deactivating) singlet oxygen, an oxidant formed during photosynthesis (Halliwell and Gutteridge, 1999). Although important for plants, the relevance of singlet oxygen quenching to human health is less clear (LPI, 2009).

Specific Uses of the Substance:

β-Carotene is a direct human food ingredient which functions as a color additive and a nutrient supplement. This substance is used in dairy products, fats and oils, and processed fruits and fruit juices; it may be used in infant formula as a source of vitamin A in accordance with 21 CFR §184.1254. In order to be traded, the petitioned substance must be formulated in hydrophilic (juices and drinks) or lipophilic (butter, margarine, and cheese) matrices for food industry application (Ribeiro and others, 2011).

The petitioner stated that β-carotene would be used to color food and beverage products including, but not limited to, yogurts, dairy beverages, ice cream, pudding, confectionery, bakery products, and condiments.

According to FDA, the color additive β-carotene may be safely used in coloring drugs and cosmetics. It can be applied to an array of animal foods designed for pets, including dogs, cats, fish, and birds (Dufosse and others, 2005).

Approved Legal Uses of the Substance:

FDA— In 21 CFR §184.1245, it is stated "β-carotene (CAS Reg. No. 7235-40-7) has the molecular formula $C_{40}H_{56}$. It is synthesized by saponification of vitamin A acetate." Furthermore, in Section §73.95, it is stated "The color additive is β-carotene prepared synthetically or obtained from natural sources." Uses of β-carotene are listed in Table 1, see below.

Table 1. FDA Regulations, 21 CFR

Regulatory Citations		Status	Use Limits
SUBCHAPTER B—FOOD FOR HUMAN CONSUMPTION	Part 184—Direct Food Substances Affirmed As Generally Recognized As Safe Subpart E—Listing of Specific Substances Affirmed as GRAS		

	§184.1245 B-carotene	(1) As a nutrient supplement. (2) As an ingredient in dairy products, fats and oils, processed fruits and fruit juices; and in infant formula as a source of vitamin A.	No limitation other than current good manufacturing practice (GMP).
SUBCHAPTER A—GENERAL	Part 73—Listing of Color Additives Exempt from Certification		
	Subpart A—Food §73.95 β-Carotene	Color additive mixtures for food use.	The mixtures may contain only diluents that are suitable and that are listed in this Subpart as safe in color additive mixtures for coloring foods.
	Subpart B—Drugs §73.1095 β-Carotene	This color additive may be safely used in coloring drugs generally, including those intended for use in the area of the eye, in amounts consistent with GMP.	The diluents in color additive mixtures are limited to those listed in this subpart as safe and suitable in color additive mixtures for coloring ingested drugs.
	Subpart C—Cosmetics §73.2095 β-Carotene	This color additive may be safely used in coloring cosmetics generally, including cosmetics intended for use in the area of the eye, in amounts consistent with GMP.	

Action of the Substance:

The petitioned substance occurs naturally as its isomers, namely, all-*trans*, 9-cis, 13-*cis* and 15-*cis* forms (Wang and others, 1994) and functions as an accessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photo damage in all green plants including algae (Ben-Amotz and others, 1987).

β-Carotene can be used as a color additive (as a food colorant) and/or a nutrient supplement (as a source of vitamin A). Its actions in different applications are as follows:

- Use as a food colorant — the petitioned substance used to impart, preserve, or enhance the color or shading of a food. It is used to add or restore color in a food in order to enhance its visual appeal and to match consumer expectations.

- Use as a source of vitamin A[3] — β-carotene is a vitamin A precursor (or called a provitamin A carotenoid[4]) meaning it can be converted by the body to retinol[5] and be subsequently made into retinal

[3] Vitamin A is a general term for a group of compounds that includes provitamin A carotenoids and preformed vitamin A.

and retinoic acid (other forms of vitamin A). [Note: Common provitamin A carotenoids are α-carotene, β-carotene, and β-cryptoxanthin. Among these, β-carotene is most efficiently made into retinol; α-carotene and β-cryptoxanthin are also converted to vitamin A, but only half as efficiently as β-carotene (IMO, 2001).] Retinol and retinal can be reversibly oxidized and reduced; but retinoic acid cannot be converted back to retinal after it has been formed.

Absorbed β-carotene is principally converted to vitamin A by the enzyme β-carotene-15,15′-dioxygenase within intestinal absorptive cells (IOM, 2001). The central cleavage of β-carotene by this enzyme will, in theory, result in two molecules of retinal (also called retinaldehyde). β-Carotene can also be cleaved eccentrically to yield β-apocarotenals that can be further degraded to retinal or retinoic acid (Krinsky and others, 1993). The retinal form is required by the eye for the transduction of light into neural signals necessary for vision (Saari, 1994); the retinoic acid form is required to maintain normal differentiation of the cornea and conjunctival membranes, thus preventing xerophthalmia, as well as for the photoreceptor rod and cone cells of the retina (IOM, 2001). In addition, vitamin A plays an important role in bone growth, reproduction, immunity, cell development, and skin health (NIH, 2006).

Vitamin A in foods that come from animals can be well absorbed and used efficiently by the body. However, vitamin A in foods that come from plants cannot be as well absorbed as animal sources of Vitamin A.

Status

Domestic:

EPA — Neither List 4A (*Minimal Risk Inert Ingredients – By Chemical Name)* nor List 4B (*Other ingredients for which EPA has sufficient information to reasonably conclude that the current used pattern in pesticide products will not adversely affect public health or the environment – By Chemical Name*) contains β-carotene. Lists 4A and 4B were updated by August 2004. However, ".beta.,.beta.-Carotene; CAS No. 7235-40-7" is listed in EPA Substance Registry Services, updated on June 16, 2011.

FDA — β-Carotene is affirmed as GRAS, see Table 1 in the Approved Legal Uses of the Substance section, in 1979. The petitioned substance may be used as a nutrient supplement or a color additive. β-Carotene may be the subject of an antioxidant nutrient content claim on food labeling (21 CFR §101.54(g)(3)). According to 21 CFR §73.95 (e), certification of this color additive is not necessary for the protection of the public health and therefore batches thereof are exempt from the certification requirements.

International:

Codex —In the food additive groups listed on Table One (*Additives Permitted for Use Under Specified Conditions in Certain Food Categories or Individual Food Items*) of Codex General Standard for Food Additives: "INS 160a(ii) β-Carotenes (vegetable)" is under "CAROTENES, B-(VEGETABLE)"; "INS 160a(i) β-Carotenes (synthetic)" and "INS 160a(iii) β-Carotenes (*Blakeslea trispora*)" are under "CAROTENOIDS". They are classified as color. β-Carotene can be used in dairy, fruit and vegetable, fish and processed meat, baked, and confectionery products. This standard was revised in 2010.

European Union — "E 160a(ii) B-CAROTENE" is listed under ANNEX of *COMMISSION DIRECTIVE 2004/47/EC of 16 April 2004 amending Directive 94/45/EC as regards mixed carotenes (E 160a (i)) and β-carotene (E 160a (ii))*. Function as colors for use in foodstuffs (Directive 94/36/EC). [Note: "E 160a(i) MIXED

[4] Provitamin A carotenoids are found in foods that come from plants including oily fruits and red palm oil.
[5] Retinol, also called preformed vitamin A, is found in foods that come from animals, including beef liver, whole eggs, whole milk, margarine, and some fortified food products such as breakfast cereals.

CAROTENES" include plant and algal carotenes; "E 160a(ii) B-CAROTENE" include β-carotene and β-carotene from *Blakeslea trispora*.]

Canada — "β-Carotene" is included in Natural Health Products Ingredients Database. Purposes: color additive. "Carotene" is also under *Food Additives Permitted for Use in Canada*. On March 25, 2011, Canadian Food Inspection Agency proposed amendments to the Feeds Regulations. "β-carotene" is listed on Class 7 (*Vitamin Products and Yeast Products*) of Schedule IV (Part II) of the proposed updated version.

Japan — "β-Carotene (72)" is listed on Table 1 related to Articles 12 and 21 of the Food Sanitation Law Enforcement Regulations by Japan Ministry of Health, Labor, and Welfare (MHLW). Last amendment as of July 26, 2005. In addition, "β-Carotene" and "Carrot carotene, a substance composed mainly of carotene obtained from carrot roots" are appeared in *List of Designated Additives* and *List of Existing Food Additives*[6], respectively, under MHLW Food Additives.

IFOAM — Not listed.

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: 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)).

The petitioned substance can be manufactured by chemical synthesis; biological synthesis using microorganisms or algae; or through extraction from plant sources.

(A) Chemical Synthesis

Synthetic β-carotene has been produced by Roche since 1954 and BASF since 1960. Each company uses a different method for its production; however, both companies utilize the same precursor, β-ionone, which was originally obtained by the condensation of acetone with citral (Russell and Kenyon, 1943). The sources of citral, a C-10 unsaturated aldehyde, were lemon grass oil or turpentine from pine, which are natural products. [Note: Natural lemon grass oil varies widely in purity, availability, and price.] However, β-ionone is now produced from acetone or butadiene (Isler 1971; Britton et al. 1996).

The Roche production method of β-carotene is the first industrial synthesis (based on enol-ether condensation, i.e. the Grignard reaction), followed the C_{19}+C_2+C_{19} synthesis principle. The chain lengthening proceeds in three steps: (1) acetal formation, (2) Lewis acid-catalyzed insertion of the enol-ether, (3) hydrolysis of the acetal and elimination of alcohol (Britton and others, 1996).

The BASF production method of β-carotene is based on the Wittig condensation (Wittig reaction), followed the C_{20}+C_{20} synthesis principle. It starts with phosphonium salts reacting with an aldehyde, generating a double bond and enlarging the polyenic chain. During the reaction, vitamin A acetate is formed which can be used as a starting material for the preparation of carotenoids.

The synthesis process of β-carotene from Roche presents a yield of 60%, while the process used by BASF presents a yield of 85%. However, the BASF method, based on the Wittig reaction, requires triphenylphosphine oxide recycling, due to its low biodegradability (Isler 1971; Britton and others, 1996).

BASF can also produce 99.9% pure, crystalline β-carotene, but it does not sell it in this form (US Court of International Trade Reports, 2005). For example: In the production of Lucaroltin® 1% (a food colorant), it takes the synthetic β-carotene crystals and disperses them in vegetable oil with heat, making it into a

[6] The substances that were already marketed or used on the date of the amendment of the Food Sanitation Law and appear in the List of Existing Food Additives

solution. This solution mixes with another solution containing sugars and dextrin, then vitamin emulsifiers in the ester form and ascorbyl palmitate are added (US Court of International Trade Reports, 2005).

(B) Biological Production Methods

- β-Carotene from microorganisms (fungi, yeasts, or bacteria)

β-Carotene can be produced by filamentous fungi, such as *Blakeslea trispora* and *Phycomyces blakesleeanus*, which also generate ubiquinone, ergosterol, organic acids, and others carotenoids like lycopene, γ-carotene, and phytoene (Ribeiro and others, 2011). According to JECFA specification (2007), β-carotene is produced by a fermentation process using the two sexual mating types (+) and (–) of the fungus *Blakeslea trispora*. β-Carotene is then isolated from the biomass by solvent extraction and crystallized. The coloring principle consists predominantly of *trans*-β-carotene together with variable amounts of *cis* isomers of β-carotene. The solvents used in the extraction and purification are ethanol, isopropanol, ethyl acetate, and isobutyl acetate.

Some yeast species (such as *Rhodotorula glutinis*, *R. minuta*, *R. mucilaginosa*, and *R. graminis*) can also be used for the production of carotenoids. *R. glutinis* is able to grow in various agricultural raw materials (such as sugar cane juice, peat extract, whey, grape must, beet molasses, hydrolyzed mung bean waste flour, soybean and corn flour extracts and sugar cane molasses) for carotenoid production. Depending on the growing conditions, such as carbon and nitrogen sources, *R. glutinis* may produce carotenoid mixtures with profiles quite variable, but in general β-carotene is the main product (Ribeiro and others, 2011).

Among bacteria, some carotenogenic species can produce β-carotene as the main carotenoid. They must have the central metabolism inhibited by inorganic salts and urea, as in the case of *Flavobacterium multivorum* (Ribeiro and others, 2011).

- β-Carotene from algae

Algae are a group of non-vascular plants which are autotrophic and are able to harness solar energy. They account for the largest quantities of biomass accumulation through the photosynthesis mechanism (Dufosse and others, 2005). The genus *Dunaliella* is one of the most reported for the production of carotenoids and belongs to the group of halotolerant unicellular microalgae. Species from this genus can accumulate large amounts of β-carotene in chloroplasts when high luminous intensity is obtained (Ribeiro and others, 2011). Commonly cultivated species are *D. salina* and *D. bardawil* (Dufosse and others, 2005).

Carotenes are obtained by solvent extraction of the dried *Dunaliella*. The solvents used for the extraction are carbon dioxide, acetone, methanol, propan-2-ol, hexane, ethanol, and vegetable oil. The main coloring principles are *trans* and *cis*-β-carotene together with minor amounts of other carotenoids such as α-carotene and xanthophyll. Besides the color pigments, carotenes may contain lipids, naturally occurring in the source material, food grade vegetable oil, and tocopherol added to retard oxidation of the pigment (JECFA specification, 2007).

According to the petition, β-carotene is produced from natural strains of the algae *D. salina*, an algae grown in large saline lakes located in Whyalla, South Australia. It is extracted from the algae using carbon dioxide, ethanol, or vegetable oil. No less than 96% total extracted coloring matters will be in the form of β-carotene.

(C) Extraction from Plant (Vegetable)

β-Carotene from vegetables is derived from solvent extraction of carrots, oil of palm fruit, sweet potato, and other edible plants with subsequent purification. The solvents used for the extraction include hexane, acetone, ethyl acetate, ethanol, and ethyl lactate (Ribeiro and others, 2011). The main coloring principles are α- and β-carotenes of which β-carotene accounts for the major part. Besides the color pigments, these substances may contain oils, fats and waxes naturally occurring in the source material (JECFA specification, 2006).

Although β-carotenes obtained from both synthetic chemicals and natural sources (such as fungi, algae, or plant) have the same molecular polyenic structure, the β-carotenes made from natural sources contain several other carotenoids in low concentrations (Ribeiro and others, 2011).

(D) Other Methodology

Nowadays, combinatorial genetic engineering is being addressed, based on an increasing number of known carotenogenic gene sequences (Mijts and others, 2005). According to the review reported by Dufosse and others (2005), it is stated "Research projects mixing molecular biology and pigments were investigated all over the world and it seems that current productions are not effective in terms of final yield."

<u>Evaluation Question #2:</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))

As described in Evaluation Question (EQ) #1, the petitioned substance can be made from synthetic chemicals, or made from natural sources using microorganisms or algae or is extracted from plants. The most prevalent processes are as follows:

(A) Chemical Synthesis

The majority of β-carotene commercialized in the world is by chemical synthesis from β-ionone (Raja and others, 2007; Ribeiro and others, 2011). β-ionone was originally synthesized from natural resources, such as lemon grass oil or turpentine from pine, but currently β-ionone is produced from acetone or butadiene. According to 21CFR 184.1245 (a), β-carotene is synthesized by saponification of vitamin A acetate. It stated "The resulting alcohol is either reacted to form vitamin A Wittig reagent or oxidized to vitamin A aldehyde. Vitamin A Wittig reagent and vitamin A aldehyde are reacted together to form β-carotene."

The synthetic product is predominantly all *trans* isomers of β-carotene together with minor amounts of other carotenoids; diluted and stabilized forms (including solutions or suspensions of β-carotene in edible fats or oils, emulsions and water dispersible powders) are prepared from β-carotene (JECFA specification, 2006).

(B) Biological Production Processes

According to Echavarri-Erasun and Johnson (2002), fungi and microalgae appear most promising for industrial production of carotenoids.

- β-Carotene from filamentous fungi (*Blakeslea trispora*)

  The source organism, the mold *Blakeslea trispora*, is a plant commensal of tropical plants, some strains of which produce high levels of β-carotene. The fungus exists in (+) and (–) mating type, of which the (+) type synthesizes trisporic acid, a precursor of β-carotene. Mating the two types in a specific ratio, the (–) type then produces large amounts of β-carotene. Glucose and corn steep liquor could be used as carbon and nitrogen sources. By-product of cheese manufacture, i.e. whey, has also received consideration, with strains acclimatized to lactose.

The production process proceeds essentially in two stages (Dufosse, 2006):

- The initial stage, fermentation process, seed cultures are produced from the original strain cultures and subsequently used in an aerobic submerged batch fermentation to produce a biomass rich in β-carotene.
- The second stage, the recovery process, the biomass is isolated and transformed into a form suitable for isolating β-carotene, which is extracted from the biomass with ethyl acetate, suitably purified and concentrated, and the β-carotene crystallized from the mother liquor.

The final product is either crystalline β-carotene (purity>96%) or it is formulated as a 30% micronized suspension in vegetable oil. The production process is controlled by good manufacturing practice procedures, adequate hygiene control, and adequate control of the raw materials (Dufosse, 2006).

➢ β-Carotene from microalgae (*Dunaliella salina*)

According to Browitzka's report (1998), the halophilic green flagellate, *Dunaliella salina*, is the best natural source of the carotenoid β-carotene. The processes of commercial production β-carotene by *D. salina* are as follows (Dufosse and others, 2005; Dufosse, 2006; Oren, 2010):

- Cultivation — It is carried out in either extensive cultures in large unstirred outdoor ponds (extensive culture system), or more intensively in paddlewheel stirred raceway ponds (intensive culture system). *D. salina* is a halotolerant organism which grows in high salt concentration. Essentially the algae require bicarbonate as a source of carbon and other nutrients such as nitrate, sulfate, and phosphate. It can be operated in two stages. First, initial growth phase requires in nitrate rich medium; magnesium salt is essential as it is required for chlorophyll production. In the second stage, nitrate limitation is induced to stimulate carotenogenesis. For the carotenogenesis phase, nitrate depletion along with salinity maintenance and light stress are essential.
- Harvesting — For the extensive culture system, flocculation and surface adsorption are used. Flocculants such as alum (aluminum sulfate), ferric chloride, ferric sulfate, lime, or polysaccharides are employed. For the intensive culture system, centrifuges are generally applied (centrifugation using continuous-flow and automatic discharge) to harvest the cells.
- Drying — Algal biomass after harvesting can be dehydrated by using freeze–drying, spray-drying, or drum drying.
- Extraction — β-Carotene can be isolated from algal biomass or dried powder by using hot edible oil extraction, supercritical carbon dioxide, or other solvents (such as hexane, ethanol, chloroform, and diethyl ether).

The extracted β-Carotene can be concentrated, crystallized, and a range of different formulations produced, depending on the final application.

(C) Extraction from Plant (Vegetable)

For producing β-carotene from plant sources, the classical method is solvent extraction. In a review article reported by Aberoumand (2011), it stated "Today, only one crystalline carotene preparation extracted from dehydrated carrots is still on the market."

<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)).

Natural sources of β-carotene, as so-called natural β-carotene, have been mentioned in EQs #1 & #2. According to Aberoumand's review report (2011), only one crystalline carotene extracted from dehydrated carrots is still on the market today. However, other vegetable sources have been pointed out as having great potential for the production of β-carotene, shown in Table 2 (Ribeiro and others, 2011).

Table 2. Potential Vegetable Resources Rich in β-Carotene

Vegetable Resources	Carotenoids (µg/g)	β-Carotene (%)
Carrot (*Daucus carota*)	85–174	49–65
Palm (oil) (*Elaeis guineensis*)	470–700	54.4
Sweet potato (*Ipomoea batatas*)	160–226	92–95
Buriti (fruit) (*Mauritia vinifera*)	513.9	72.5
Barbados cherry (*Malpighia glabra*)	8.8–18.8	69.8–90.6
Tucumã (*Astrocaryum aculeatum*)	62.6–96.6	75.6–89.3
Pajurá (*Couepia bracteosa*)	17.8	92.1
Piquiá (*Caryocar villosum*)	21	85.4
Umari (*Poraqueiba sericea*)	102.9	78.9

It is estimated that the worldwide market of carotenoids will grow 2.3% per year, reaching USD 920 million in 2015 (BCC Research, 2008; Ribeiro and others, 2011). β-Carotene accounts for 32% of this market, with a global market estimated to surpass USD 280 million in 2015. Only 2% of the total β-carotene produced worldwide is natural and is mainly used as a nutritional supplement (Dufosse and others, 2005; Ribeiro and others, 2011).

BCC Research's report on *the Global Market for Carotenoids* (2008) stated in part:

> β-Carotene is still the most prominent carotenoid used in foods and supplements, but due to a changing consumer perception, primarily in Europe, the product is suffering from natural replacements, specifically carrot juice, and market growth in the past few years was much lower than expected. In parallel, the number of producers of synthetic and algae derived β-carotene rose sharply, which added to the imbalance of supply and demand, driving prices down…

<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))

The petitioned substance (β-carotene, CAS Reg. No. 7235–40–7) is listed on 21 CFR §184.1245 of Subpart B (*Listing of Specific Substances Affirmed as GRAS*) of PART 184 (*DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE*). In accordance with FDA, the affirmation of β-carotene as GRAS as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use (§184.1245):

- The ingredient is used as a nutrient supplement as defined in §170.3(o)(20).
- The ingredient is used in the following foods at levels not to exceed current good manufacturing practice: dairy product analogs as defined in §170.3(n)(10); fats and oils as defined in §170.3(n)(12); and processed fruits and fruit juices as defined in §170.3(n)(35). B-carotene may be used in infant formula as a source of vitamin A.

The following are excerpts from 21 CFR Part 170 *Food Additives* §170.3 *Definitions*:

> "§170.3 (o)(20) Nutrient supplements : Substances which are necessary for the body's nutritional and metabolic processes.
> §170.3 (n)(10) Dairy product analogs, including nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products.
> §170.3 (n)(12) Fats and oils, including margarine, dressings for salads, butter, salad oils, shortenings and cooking oils.

§170.3 (n)(35) Processed fruits and fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom."

β-Carotene was evaluated by the Select Committee on GRAS Substances (SCOGS) in 1979. The SCOGS concluded that there was no evidence in the available information on β-carotene that demonstrated, or suggested reasonable grounds to suspect a hazard to the public when it was used at levels at that time or might reasonably be expected in the future (SCOGS Report No. 111).

In addition, β-carotene is listed under *Everything Added to Food in the United States* (EAFUS) in FDA/CFSAN's the Priority-based Assessment of Food Additives (PAFA) database. The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.

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))

No information sources reviewed specifically address the primary function/purpose of β-carotene 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))

The petitioned substance is under FDA Regulation PART 73 — *LISTING OF COLOR ADDITIVES EXEMPT CERTIFICATION*. The color additive is β-carotene prepared synthetically or obtained from natural sources (21 CFR §73.95(a)(1)); it may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color those foods for which standards of identity have been promulgated unless added color is authorized by such standards (21 CFR §73.95(c)). According to FDA the standard of identity for margarine, it stipulates "…provitamin A (β-carotene) shall be deemed to be a color additive" (21 CFR §166.110(b)(6)). β-Carotene imparts a yellow color to foods.

When β-Carotene is used as food colorant, its concentrations generally are between 2 and 50 parts per million (ppm) so that its color contribution to the foods is from yellow to orange (Ribeiro and others, 2011). As Dziezak (1987) notes, colorants are added to consumable products for the sole purpose of enhancing the visual appeal. The reasons for adding colors to foods include (Aberoumand, 2011):

- to replace color lost during processing,
- to enhance color already present,
- to minimize batch to batch variations, and
- to color otherwise uncolored food.

β-Carotene can also be used as a nutrient ingredient to replace vitamin A lost in processing, or as an added nutrient that may be lacking in the diet (FDA Website, Types of Food Ingredients, 2010). It may be added in flour, breads, cereals, rice, macaroni, margarine, salt, milk, fruit beverages, energy bars, and instant breakfast drinks.

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))

The petitioned substance is a precursor of vitamin A (also called a provitamin A carotenoid). According to the FDA regulations, the affirmation of β-carotene as GRAS is used as a nutrient supplement (§184.1245(c)(1)) and it may be used in infant formula as a source of vitamin A (§184.1245(c)(2)). Vitamin A is a fat-soluble vitamin that is essential for humans and other vertebrates. It is important for normal vision, gene expression, reproduction, embryonic development, growth, and immune function.

In the report on *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc* by Food and Nutrition Board of Institute of Medicine (IOM) (2001), it has indicated that current dietary patterns appear to provide sufficient vitamin A to prevent deficiency symptoms such as night blindness. The estimated average requirement is based on the assurance of adequate stores of vitamin A. The Recommended Dietary Allowance (RDA) for men and women is 900 and 700 µg retinol activity equivalents (RAE)/day or 3000 and 2310 International Units (IU)/day, respectively (IOM, 2001). [Note: 1 RAE = 3.3 IU] However, there is no RDA for β-carotene. The IOM (2001) stated that consuming 3 mg to 6 mg of β-carotene daily (equivalent to 833 IU to 1,667 IU vitamin A) will maintain blood levels of β-carotene in the range associated with a lower risk of chronic diseases.

At present, it is unclear whether the biological effects of carotenoids in humans are a result of their antioxidant activity or other non-antioxidant mechanisms (LPI, 2009). Some provitamin A carotenoids have been shown to function as antioxidants in laboratory studies; however, this role has not been consistently demonstrated in humans (IOM, 2001). Although, the FDA's food labeling regulation (21 CFR §101.54(g)(3)) indicates that β-carotene may be a subject of the claim when the level of vitamin A present as β-carotene in the food that bears the claim is sufficient to qualify for the claim. For example, for the claim "good source of antioxidant β-carotene," 10 percent or more of the Recommended Daily Intake for vitamin A must be present as β-carotene per reference amount customarily consumed.

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))

According to the specification of β-carotene in Food Chemical Codex (2010-2011), it stipulates the impurity acceptable criterion for a heavy metal is not more than 5 mg/kg (5 ppm) lead. Moreover, the specification of the color additive β-carotene, which may be safely used for coloring foods, in FDA regulation (21 CFR §73.95(b)) specifies that lead is not more the 10 ppm and arsenic is not more than 3 ppm.

No information sources can be identified to suggest that the petitioned substance contains residues of heavy metals or other contaminants in excess of FDA's Action Levels for Poisonous or Deleterious Substances in Human Food.

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

I. MANUFACTURE

The petitioned substance can be produced from synthetic chemicals or natural sources (such as fungi, algae, or plants):

(A) Synthetic chemicals

There are two commonly used methods (Grignard and Wittig reactions) of chemical synthesis of β-carotene, see EQ #1. The synthesis process from the Grignard reaction presents a yield of 60%, while the process used the Wittig reaction presents a yield of 85 %. Although the yield of the Wittig reaction method is higher than the Grignard reaction method, the Wittig reaction method has a drawback—low biodegradability of triphenylphosphine oxide, which is used as a catalyst during one chemical reaction step. According to Fisher's Material Safety Data Sheet (MSDS) (2008), triphenylphosphine oxide is harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. This chemical has to be recycled. The industrial recovery process comprises three phases: distillation, chlorination with phosgene, and dehalogenation with aluminum (Ribeiro and others, 2011).

(B) Natural sources — the production of β-carotenes are made from renewable sources

- β-Carotene made from filamentous fungi (*Blakeslea trispora*)

The fungus *Blakeslea trispora* lives in commensalism with tropical plants; some strains in nature are big producers of β-carotene and other carotenoids. It has been shown to be nonpathogenic and nontoxigenic (Dufosse, 2006). The fungi are grown in large-scale fermenters using food-grade raw materials, such as glucose, corn steep liquor, and cheese whey (Dufosse, 2006). As in the recovery process, β-carotene is obtained from the fungal biomass by solvent extraction and crystallized with high purity, see EQ #2. Ishida and Chapman (2009) reported that ethyl acetate is most commonly used for extracting carotenoids. Ethyl acetate is not considered to be environmentally friendly and is highly flammable (explosive). Although it can be produced by reaction of ethanol and acetic acid, its primary source is from petroleum (Ishida and Chapman, 2009).

- β-Carotene made from microalgae (*Dunaliella salina*)

Dunaliella species are commonly observed in salt lakes in all parts of the world from tropical to temperate to polar regions where they often impart an orange-red color to the water. As in commercial cultivation of the production, β-carotene is accumulated as droplets in the algal chloroplast stroma, especially under the environmental conditions in high temperature, high salinity, high irradiance, and nutrient limitation (low nitrogen). Then, β-carotene may be obtained from algal biomass or dried powder by using hot edible oil extraction and supercritical carbon dioxide, see EQ #2.

In addition, it is desirable to re-utilize the culture medium remains after harvesting (biomass removal). *Dunaliella* growth medium could be recycled biologically by treating the medium with bacteria that are naturally present in medium because of the high concentration of glycerol, amino acids, and other organic compounds (Ben-Amotz, 1995). In a review article conducted by Dufosse et al. (2005), they concluded that algal forms are the richest source of pigments and can be produced in a renewable manner, since they produce some unique pigments sustainably. The report also stated that the production of β-carotene from *Dunaliella* will surpass synthetic as well as other natural sources due to microalgae sustainability of production and their renewable nature.

- β-Carotene made from plant extraction

β-Carotene is extracted from plant material using a solvent, such as hexane, acetone, ethyl acetate, ethanol, and ethyl lactate. Among these solvents, ethyl lactate is an environmentally friendly solvent produced from the fermentation of carbohydrate feedstock available from the corn and soybean industries (Ishida and Chapman, 2009). Colorless ethyl lactate has a relatively high flashpoint, is environmentally benign, and can be completely biodegraded into CO_2 and water. In Ishida and Chapman's research (2009), they indicated that ethyl lactate is almost as efficient as ethyl acetate, which is most commonly used for extracting carotenoids to be used in food products, for the extraction of β-carotene.

II. USE

No Occupation Safety and Health Administration (OSHA) Vacated Permissible Exposure Limits (PELs) are listed for β-carotene.

According the MSDS in the petition, it stated "Natural Carotene WD 20 AP is the extract of natural carotenoids; rendered water soluble using a blend of maltodextrin modified starch, sugar and MCT oil. Dlα-tocopherol & ascorbic acid are added as anti-oxidants." It also showed that ingredient is β-carotene and its CAS No. is **33261-80-20**. [Note: 33261-80-20 is NOT the CAS No. for β-carotene. Moreover, the content of β-carotene is not specified.] For ecological information, it stated "Natural Carotene WD 20 AP is biodegradable. Do not allow to enter natural waterways." This product should be handled in accordance with good occupational hygiene and safety practices, and avoid contact with skin and eyes. The workers should wear appropriate protective eyeglasses (or chemical safety goggles), gloves, and clothing; in addition to use suitable dust mask or breathing apparatus where aerosols created.

<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))

As stated above, see EQ # 7, β-carotene is a vitamin A precursor or a provitamin A carotenoid. Absorbed β-carotene can be converted by the body to retinol and be subsequently made into retinal and retinoic acid (other forms of vitamin A) (IOM, 2001). Vitamin A is used by eyes to synthesize the light-sensitive retinal pigments. In addition, vitamin A plays an important role in bone growth, reproduction, cell division, and cell differentiation (in which a cell becomes part of the brain, muscle, lungs, blood, or other specialized tissue.); it helps regulate the immune system, which will prevent or fight off infections by making white blood cells that destroy harmful bacteria and viruses (IOM, 2001). Vitamin A also may help lymphocytes (a type of white blood cell) fight infections more effectively. In addition, vitamin A promotes healthy surface linings of the eyes and the respiratory, urinary, and intestinal tracts (Semba, 1998). When those linings break down, it becomes easier for bacteria to enter the body and cause infection. It also helps the skin and mucous membranes function as a barrier to bacteria and viruses (Ross, 1999; Harbige, 1996).

According to IOM's report released in 2001, it has indicated that although a large body of observational epidemiological evidence suggests that higher blood concentrations of β-carotenes and other carotenoids obtained from foods are associated with a lower risk of several chronic diseases, there is currently not sufficient evidence to support a recommendation that requires a certain percentage of dietary vitamin A to come from provitamin A carotenoids in meeting the vitamin A requirement. However, IOM Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids of Year 2000 recommended the increase of consumption of carotenoid-rich fruits and vegetables for their health-promoting benefits. In addition, the IOM 2001 report stated that "β-carotene supplements are not advisable for the general population," although they also state that this advice "does not pertain to the possible use of supplemental β-carotene as a provitamin A source for the prevention of vitamin A deficiency in populations with inadequate vitamin A".

Provitamin A carotenoids such as β-carotene are generally considered safe because they are not associated with specific adverse health effects (NIH, 2006). Their conversion to vitamin A decreases when body stores are full. A high intake of provitamin A carotenoids can turn the skin yellow, but this is not considered dangerous to health. According to the Select Committee on GRAS Substances (SCOGS) Report on "Carotene (β-carotene)" (1979), it concluded that "There is no evidence in the available information on carotene (β-carotene) that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future."

The Joint FAO/WHO[7] Expert Committee on Food Additives (JECFA) has evaluated several β-carotenes (which may be produced by chemical synthesis or obtained by extraction from a microorganism, algae, or vegetables) containing the same chemical entity as the functional component in relation to its food additive use but obtained from different source materials and/or different manufacturing processes. The Committee has reached various conclusions in its evaluations for an acceptable daily intake (ADI) for a man:

[7] Food and Agriculture Organization/World Health Organization

(A) A group ADI of 0-5 mg/kg body weight (bw) for β-carotene, synthetic and from *Blakeslea trispora*, established at the 57th JECFA in 2001. [Note: 0-5 mg as sum of the carotenoids including β-carotene, β-apo-8'-carotenal, β-carotenoic acid methyl ester, and β-carotenoic acid ethyl ester (WHO FAS 6, 1975).]

In the 57th report of JECFA on Safety Evaluation of Certain Food Additives and Contaminants— β-carotene derived from *blakeslea trispora* (WHO FAS 48, 2001), the Committee concluded that, on the basis of the source organisms, the production process, and its composition characteristics, β-carotene from *B. trispora* does not raise specific concerns and from a toxicological point of view should be considered equivalent to chemically synthesized β-carotene, for which an ADI of 0–5 mg/kg bw was established by the Committee at its 18th meeting (see below). This opinion was supported by the negative results in two tests for genotoxicity (mutagenesis and chromosomal aberration) considered at the 57th meeting. Therefore, the Committee established a group ADI of 0–5 mg/kg bw for synthetic β-carotene and β-carotene derived from *B. trispora*. This ADI applies to use of β-carotene as a coloring agent and not to its use as a food supplement (WHO FAS 48, 2001).

A β-Carotene toxicological evaluation was conducted at the 18th meeting of JECFA in 1974. In the report published the next year (WHO FAS 6, 1975), the Committee stated that β-carotene is a normal constituent of the human diet and is commonly ingested over the entire lifespan of man. Its biological importance rests on the provitamin A function. Concerning the known clinical syndrome of hypervitaminosis A in man, evidence from human experience indicates that in very exceptional circumstances excessive dietary intakes can occur. Such cases have been reported in the literature but do not relate to food additive use of this color. Despite poor absorption from the gastrointestinal tract cases of human hypervitaminosis have occurred. The results of short-term toxicity studies in rats and dogs have shown that over a wide range of doses toxic effects have not been produced. Similarly, multi-generation tests in rats using levels up to 1000 ppm have not revealed any adverse effects. In addition, the JECFA concluded that "In the light of the above comments it appears justifiable to apply a smaller safety factor to the no-effect level established in long-term studies." Furthermore, estimate of ADI, 0-5 mg/kg bw, was established at the 18th meeting (WHO FAS 6, 1975).

According to WHO Technical Report Series No. 557 (1974), it indicated that carotenes (natural) were reviewed at the 18th meeting by the Committee when it was concluded that further information was required before a specification could be developed. Therefore, no toxicological evaluation was prepared and no ADI was established for natural carotene at that time. ADI of 0-5 mg/kg bw was established for synthetic carotene.

(B) No ADI allocated for β-carotene from algae established at the 41st JECFA in 1993.

Carotenes from natural sources (algal and vegetable) are reviewed by the JECFA at the 41st meeting and reported on Toxicological Evaluation of Certain Food Additives and Contaminants of WHO Food Additives Series No. 32. The Committee considered the data inadequate to establish an ADI for the dehydrated algal carotene preparations or for the vegetable oil extracts of *Dunaliella salina*. [Note: There is no history of use of *Dunaliella* algae as food (WHO FAS 32, 1993).]

(C) ADI "acceptable" for β-carotene from vegetables, provided the level of use does not exceed the level normally found in vegetables, established at the 41st JECFA in 1993.

In the toxicological monograph (WHO FAS 32, 1993), the JECFA identified that no relevant toxicological data on vegetable extracts were available. However, the Committee concluded that there was no objection to the use of vegetable extracts as coloring agents, provided that the level of use did not exceed the level normally present in vegetables. The report stated that "implicit in this conclusion is that the extracts should not be made toxic by virtue of the concentration of toxic compounds (including toxicants naturally occurring in the vegetables) nor by the generation of reaction products or residues of a nature or in such amounts as to be toxicologically significant."

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

Currently, "beta-carotene extract color, derived from carrots (CAS # 1393–63–1)" is listed on NOP the National List of Allowed and Prohibited Substance under § 205.606 Nonorganically produced agricultural products allowed as ingredients in or on processed products labeled as "organic." (d) Colors derived from agricultural products (7 CFR §205.606 (d)(3)).

Organic annatto extract is an organically produced agricultural ingredient that could be substituted for the petitioned substance. According to 606organic.com, a website administered and maintained by the Accredited Certifiers Association, Inc., annatto extract color is commercially available in an organic form from D. D. Williamson & Co., Inc. [Note: D. D. Williamson & Co., Inc. is also the petitioner for this substance (beta-carotene extract color).]

In the FDA regulations, annatto extract is a food color additive and is exempted from certification listed in 21 CFR §72.30. Annatto extract may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to color those foods for which standards of identity have been promulgated unless added color is authorized by such standards (21 CFR §73.30(c)). Certification of this color additive is not necessary for the protection of the public health in accordance with 21 CFR §72.30 (e). The yellow to orange colors of annatto comes from the outer layer of seeds of the tropical tree *Bixa orellana*. The carotenoids (bixin and norbixin) are responsible for the appearance of the yellow to orange colors. The pH and solubility affect the color hue; the greater the solubility in oil, the brighter is the color. Annatto extract are available in water soluble, oil soluble, and oil/water dispersible forms. Since it precipitates at low pH, it is also available as an emulsion, an acid proof state. Annatto has been used for over two centuries as a food color especially in cheese and in various other food products (Gordon and others, 1982; Aberoumand, 2011).

Based on the database of NOP Certified Operations, as of 2010, following is a tabulated list for the names and addresses of companies producing or handling organic annatto (NOP Certified Operations, 2010):

COMPANY	ADDRESS
Fundación Chankuap	Vidal Rivadeneira y Hernando de Benavente, Macas, Morona Santiago, EC Ecuador
Productos SKS Farms Cía. Ltda.	Julio Zaldumbide 398 y Mira Valle, Quito, Pichincha, EC Ecuador
Whole Herb Co.	Sonoma, CA 95476
Fores Trade Europe	Wijnkoopsbaai 16 Capelle a/d Ijssel, 2904 BP, Netherland
Aryan International FZC	P.O. Box.- 5232, Fujairah, United Arab Emirates
PR 200 - APROAP	Caixa Postal 149, Umuarama - PR CEP: 87502-970, Brazil
BA 036 - Coop. dos Produtores Org. do Sul da Bahia - CABRUCA	Rua Jasmim, Nº 25 Nelson Costa, Ilhéus - BA CEP: 45656-140, Brazil
Superior Natural Foods	44 St. Croix Trail South, Lakeland, MN 55043

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