## Item A

This petition seeks inclusion of **Synthetic beta-CAROTENE** on the National List as a non-agricultural (non-organic) substance allowed in or on processed products labeled as "organic" or "made with organic (specified ingredients)," at §205.605(b).

## Item B

### 1. The substance's chemical or material common name.

β-Carotene is a naturally occurring carotenoid. β-Carotene is the most abundant carotenoid in carrots and is found in other yellow and green vegetables. β-Carotene gives carrots their orange color.

Other chemical names for β-carotene are:

All-trans-beta-carotene
beta,beta-carotene
beta-carotene, all-trans-
Cyclohexene, 1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis(2,6,6-trimethyl-, (all-E)-
Provitamin A

The name 'provitamin A' reflects the fact that β-carotene is the normal dietary precursor of vitamin A.

### 2a. The name and address of the manufacturer/producer of the substance.

Synthetic crystalline β-carotene is produced by:

BASF SE
Carl-Bosch-Straße 38
67056 Ludwigshafen/Germany

### 2b. The name, address and telephone number and other contact information of the petitioner.

International Formula Council
1100 Johnson Ferry Road NE, Suite 300
Atlanta, GA 30342
Contact: Mardi Mountford, Executive Vice President
Phone: (678) 303-3027
Email: mmountford@kellencompany.com

**<u>3. The intended or current use of the substance as a nonagricultural ingredient.</u>**

Synthetic β-carotene is used as a minor component of the edible oil blends in infant formulas to maintain the nutritional quality of the lipid component of the infant formula. Lipid provides half of the total energy value (calories) of infant formula, so maintaining its quality is important. β-Carotene acts as an antioxidant, preventing oxidation and rancidity. Rancid fat causes diarrhea in infants.

In conventional foods, synthetic β-carotene is GRAS, for use as a direct food ingredient to add color or to add vitamin A activity to a variety of foods.

**<u>4. A list of the handling activities for which the substance will be used. If used for handling (including processing), the substance's mode of action must be described.</u>**

β-Carotene has two actions in the body, as a precursor to vitamin A and as an antioxidant and free radical scavenger.

β-Carotene is defined as a provitamin, as it is chemically related to the biologically active form of vitamin A and is converted by the body into this vitamin. Vitamin A has four essential physiological functions in the body: vision, growth, cell differentiation, and reproduction.

β-Carotene is a known quencher of free radicals and singlet oxygen. Free radicals are highly reactive species that include hydroxyl, peroxy, hypochlorite, superoxide, and alkoxy radicals and others. These entities react with the unsaturated bonds found in the lipids in all cellular membranes, resulting in loss of membrane fluidity, receptor alignment, and potentially cellular lysis. Free radical damage to enzymes and other proteins leads to inactivation and denaturation. Nucleic acids can also be damaged by free radicals, causing DNA mutations which may lead to carcinogenesis.

The antioxidant properties of β-carotene in the body make it useful as an ingredient in infant formulas. Infant formulas commonly contain a variety of polyunsaturated chain fatty acids (PUFA) and other physiologically important carotenoids as part of the lipid component of the overall nutrient system. Examples of PUFA include linoleic acid, alpha-linolenic acid, eicosapentaenoic acid (EPA), arachidonic acid (ARA), docosahexaenoic acid (DHA), and others. The Infant Formula Act and FDA regulations require a minimum amount of linoleic acid. Arachidonic and docosahexaenoic acids have been shown to provide beneficial effects in preterm infants such as enhanced brain and vision development. Examples of other physiologically important carotenoids are lutein and lycopene.

PUFA and other carotenoids tend to be more sensitive to oxidation than many other ingredients commonly found in nutritional formulas. Due to their chemical structure, exposure to heat and atmospheric levels of oxygen can cause a series of chemical reactions resulting in free radical formation. These free radicals can continue to break down these lipophilic components in an auto-oxidative process. The result is the development of undesirable off-flavors and odors and the eventual degradation of the beneficial PUFA and carotenoids. PUFA and other more sensitive carotenoids are especially susceptible to oxidation during high-heat processing, spray drying processing, or even during relatively short storage periods after the formula has been sealed and packaged. Oxidative stability has become especially challenging with infant formulas that contain relatively high concentrations of arachidonic and docosahexaenoic acid for optimal eye and cognitive development.

One method of controlling the undesirable oxidation in powdered infant formulas is the addition of safe antioxidants soluble in edible oils, such as ascorbyl palmitate, β-carotene, mixed tocopherols, and others. β-Carotene has been found particularly useful as an antioxidant in infant formula oil blends at concentrations ranging from 6 to 12 ppm by weight of the total oil content of the infant formula.

As an antioxidant, however, β-carotene tends to discolor the otherwise white-appearing infant formula with a red-orange carotenoid hue. These colors can stain clothing and are often viewed as a negative by many consumers. Although off colors can be eliminated by simply removing the β-carotene from the formula, such removal is not generally desirable from a nutrition standpoint, and will also generally result in an unacceptable reduction in oxidation stability within the formula, with the subsequent development of rancid odors especially in those powder formulations containing ARA and DHA.

**5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.**

Synthetic β-carotene is produced by the Wittig condensation of synthetic intermediates commonly used in the production of other carotenoids used in food, according to U.S. Patent No.2,917,539, issued December 15, 1959. A copy of this patent is available in Appendix A.

**A detailed description of the production process of synthetic β-carotene, shown in Appendix B, is a ''Trade secret'' qualifying as Confidential Business Information (CBI).**

**6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.**

Synthetic β-carotene has been added as an antioxidant to infant formula edible oil blends since before 1960. Synthetic β-carotene has not been reviewed by State or private organic certification programs for its use as a general food additive in foods labeled as “organic”.

On January 17, 2007, “beta-carotene extract color derived from carrots” was petitioned as a food colorant for inclusion on the National List at §205.606. It ultimately was approved by the NOSB for this purpose. “Beta-carotene extract color derived from carrots” was added to the National List on June 27, 2007. On 20 July, 2009, NOP received a petition to amend this listing of “beta-carotene extract color derived from carrots" to read "beta-carotene extract color derived from carrots and algae."

On July 15, 2011, NOP published a Technical Evaluation Report on "Beta Carotene." Both synthetic and nonsynthetic beta-carotene are mentioned in this review.

The "Approved Legal Uses of the Substance" section of this document does not make a clear differentiation of critical distinction between the use of sundry sources of beta-carotene as a colorant subject to the FDA regulation at 21 CFR 73.95 and the use of beta-carotene as a GRAS food additive for use in infant formula and other foods permitted at 21 CFR 184.1245. 21 CFR 73.95 reads as follows:

> §73.95 [beta]-Carotene.
>
> (a) Identity. (1) The color additive is [beta]-carotene prepared synthetically or obtained from natural sources.
>
> (2) Color additive mixtures for food use made with [beta]-carotene may contain only diluents that are suitable and that are listed in this subpart as safe in color additive mixtures for coloring foods.
>
> (b) Specifications. [beta]-carotene shall conform to the following specifications:
>
> > Physical state, solid.
> >
> > 1 percent solution in chloroform, clear.
> >
> > Loss of weight on drying, not more than 0.2 percent.
> >
> > Residue on ignition, not more than 0.2 percent.
> >
> > Lead (as Pb), not more than 10 parts per million.
> >
> > Arsenic (as As), not more than 3 parts per million.
> >
> > Assay (spectrophotometric), 96-101 percent.
>
> (c) Uses and restrictions. The color additive [beta]-carotene 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 under section 401 of the act unless added color is authorized by such standards.
>
> (d) Labeling. The label of the color additive and any mixtures prepared therefrom and intended solely or in part for coloring purposes shall conform to the requirements of Sec. 70.25 of this chapter.
>
> (e) Exemption from certification. 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 of section 721(c) of the act.

Infant formulas do not contain added color, which would require labeling as "artificial color"! The ingredients in an infant formula must comply with the food additive regulations of FDA. In this case, the regulation at 21 CFR 184.1245 applies. 21 CFR 184.1245 reads as follows:

§184.1245 Beta-carotene.

(a) Beta-carotene (CAS Reg. No. 7235-40-7) has the molecular formula $C_{40}H_{56}$. It is synthesized by saponification of vitamin A acetate. 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 beta-carotene.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), p. 73, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal--register/code--of--federal--regulations/ibr--locations.html.

(c) In accordance with Sec. 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as a nutrient supplement as defined in Sec. 170.3(o)(20) of this chapter.

(2) The ingredient is used in the following foods at levels not to exceed current good manufacturing practice: dairy product analogs as defined in Sec. 170.3(n)(10) of this chapter; fats and oils as defined in Sec. 170.3(n)(12) of this chapter; and processed fruits and fruit juices as defined in Sec. 170.3(n)(35) of this chapter. Beta-carotene may be used in infant formula as a source of vitamin A in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act or with regulations promulgated under section 412(g) of the act.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

The evaluation of beta-carotene extract color derived from carrots prepared by the NOSB Handling Committee in February 2007 properly made this distinction when it wrote: “Color additives, in general, cannot, by definition qualify for GRAS status, as GRAS only applies to food additives.” Consequently, it would violate FDA regulation to use the colorant “beta-carotene extract color derived from carrots (or algae)” in an infant formula. An additional consideration is that the allergenic principles in carrots (or algae) may be present in an extract and as such make these sources undesirable in the diet of newborn infants.

The July 15, 2011, Technical Evaluation Report rightly points out that FDA at 21 CFR 101.54(g)(3) recognizes the antioxidant properties of beta-carotene: “Beta-carotene may be a subject of the claim when the level of vitamin A present as beta-carotene in the food that bears the claim is sufficient to qualify for the claim. For example, for the claim ``good source of antioxidant beta-carotene," 10 percent or more of the RDI for vitamin A must be present as beta-carotene per reference amount customarily consumed.”

## 7. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) specification for synthetic β-carotene is included in Appendix C.

β-Carotene is regulated by FDA. Certification of this color additive when used as a food is not necessary for the protection of the public health and therefore batches thereof are exempt from the requirements of section 706(c) of the Federal Food, Drug, and Cosmetic Act: 21CFR 73.96. Certification of this color additive when used as a drug is not necessary for the protection of the public health and therefore batches thereof are exempt from the requirements of section 706(c) of the Federal Food, Drug, and Cosmetic Act: 21CFR73.1095. β-Carotene added directly to human food is affirmed as generally recognized as safe (GRAS): 21 CFR 184.1245. A copy of this regulation is included in Appendix C.

## 8a. The Chemical Abstracts Service (CAS) Registry Number for β-carotene is 7235-40-7.

## 8b. Labels of infant formulas containing synthetic β-carotene.

See Appendix D

## 9a. The substance's physical properties

β-Carotene forms deep purple, hexagonal prisms. It is sparingly soluble in methanol and ethanol and moderately soluble in oils, ether, and petroleum ether. β-Carotene is insoluble in water

β-Carotene absorbs oxygen from the air giving rise to inactive, colorless oxidation products. The crystalline material is stored under inert gas (nitrogen) or suspended in edible oils or in an aqueous solution containing antioxidants and protected from light.

The empirical formula of β-carotene is $C_{40}H_{56}$. Its molecular weight is 536.87.

## 9b. Chemical mode of action

(a) **Chemical interactions**: β-Carotene interacts with PUFA and other fat-soluble carotenoids, such as lycopene and lutein, to enhance the oxidative stability of edible oils.

(b) **Toxicity and environmental persistence**: β-Carotene, as such or as a natural component of carrots, melons, and sweet potatoes, is compostable and does not persist in the environment. The mammalian body converts β-carotene to vitamin A only if the body requires vitamin A. Eating pureed carrots and other highly yellow vegetables can lead to the harmless and reversible condition of carotenemia in infants and adults. See point (d).

(c) **Environmental impacts from its use and/or manufacture**: Synthetic β-carotene is produced in Ludwigshafen, Germany, according to an ISO 14000 Environmental Management System. The ISO 14000 Environmental Management System certificate for Ludwigshafen is available in Appendix E. The production process conforms to the strict environmental requirements of Germany and the European Union.

(d) **Effects on human health**: β-Carotene has well-established physiological effects. The obvious but harmless cosmetic effect of excessive intake of food or dietary supplements high in β-carotene is the condition of "carotenemia," a reversible condition associated with a deep orange discoloration of the skin. This is not a worry in this application, since the amounts of β-carotene used in infant formula are in the micro range. The most well-known effect of β-carotene is its vitamin A activity. β-Carotene is a precursor to vitamin A, which is essential for normal function of the retina; in the form of retinal, it combines with opsin (red pigment in the retina) to form rhodopsin (visual purple), which is necessary for visual adaptation to darkness. It is also necessary for growth of bone, testicular and ovarian function, and embryonic development, and for regulation of growth and differentiation of epithelial tissues. β-Carotene also functions physiologically as an antioxidant and free radical scavenger.

(e) **Effects on soil organisms, crops, or livestock**: Sources of β-carotene in the diets of dairy cows are responsible for the yellow color of butter. Forage carrots are fed in Europe to livestock with no ill effects.

## 10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.

The MSDS and product specification of **Lucarotin 30 SUN,** the material used to add β-carotene to the edible oils of infant formula products, are available in Appendix E. This material is a 30% dispersion of crystalline β-carotene in sunflower oil.

The Food and Drug Administration has affirmed the GRAS status of β-carotene at 21 CFR 184.1245 (see Appendix C). The Food Chemicals Codex specification for β-carotene is available in Appendix E.

There is no substance report from NIEHS. However, the Hazardous Substance DataBank information for β-carotene available on the National Library of Medicine and created by the National Toxicology Program is attached as Appendix F.

**11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies.**

A comprehensive review entitled "Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans," was recently published in 2009 in the journal Molecular Nutrition and Food Research[1]. This review is available in Appendix G.

**12. A ''Petition Justification Statement'' which provides justification for inclusion of β-carotene on the National List, §205.605(b)**

Human colostrum contains very high concentrations of β-carotene (2130 ±1660 micrograms/L).[2] Carotenoids in colostrum are up to five times higher than in mature human milk,[3] so that the human milk-fed, term infant attains serum levels of both vitamin E and β-carotene comparable to those in the adult within 4 to 6 days of breast-feeding. When lactation is fully established, human milk β-carotene falls to 380 from 490 mcg/L.[4] Providing additional β-carotene in the lactating woman's diet can increase her β-carotene levels by as much as three-fold.[5]

Unless an infant formula is deliberately fortified with carotenoids such as β-carotene, it will contain very small amounts of these physiologically active substances. Sommerburg et al.[6] measured carotenoids in eight infant formula preparations and could not detect any β-carotene in four of them. Formula-fed infants had different plasma carotenoid profiles compared to human milkfed infants. β-Carotene was significantly lower in formula-fed infants [14 (0-32) mcg/L, median and interquartile ranges] than in infants soon after birth [24 (19-310) mcg/L, $P<0.05$] and in human milk-fed infants [32 (22-63) mcg/L, $P<0.05$].

The amount of β-carotene deliberately added to infant formula ranges from 125 to 450 mcg/L of reconstituted formula.

In the United States, 'baby foods' are introduced into the diet of infants at four to six months of age. The most popular vegetable baby foods are the yellow vegetables carrots, winter squash, and sweet potatoes. Carrots, from which the name "carotene" originates, contain about 100 ppm

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[1] Mol. Nutr. Food Res. 2009, 53, S194 –S218.
[2] Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and beta-carotene in the newborn infant. Ostrea EM Jr, Balun JE, Winkler R, Porter T. Am J Obstet Gynecol. 1986 May;154(5):1014-7.
[3] Carotenoid supply in breast-fed and formula-fed neonates. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Eur J Pediatr. 2000 Jan-Feb;159(1-2):86-90.
[4] Beta-carotene isomers in human serum, breast milk and buccal mucosa cells after continuous oral doses of all-trans and 9-cis beta-carotene. Johnson EJ, Qin J, Krinsky NI, Russell RM. J. Nutr. 127:1993-1999, 1997.
[5] Comparison of the effects of supplemental red palm oil and sunflower oil on maternal vitamin A status. Lietz G, Henry CJ, Mulokozi G, Mugyabuso JK, Ballart A, Ndossi GD, Lorri W, Tomkins A. Am J Clin Nutr. 2001 Oct;74(4):501-9.
[6] Carotenoid supply in breast-fed and formula-fed neonates. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Eur J Pediatr. 2000 Jan-Feb;159(1-2):86-90.

of β-carotene, or 3,000 mcg per ounce of carrots. A four-ounce serving of baby carrots can easily provide 10,000 mcg of β-carotene!

β-Carotene functions as an excellent antioxidant in infant formula when added at levels found in human milk. It is important to protect the fats and oils used in infant formulas from oxidation. Fat oxidation is a real danger in powdered infant formula, since there are dried by high temperature spray-drying in an oxygen-containing atmosphere.

Only synthetic β-carotene is a GRAS substance according to the FDA regulation. Beta-carotene extract color is approved as a colorant but it is not approved as a GRAS food additive, so it cannot be used as a source of β-carotene in infant formula.

## **13. Confidential Business Information Statement**

A detailed description of the production process of synthetic β-carotene, shown in Appendix B, is a ‘‘Trade secret’’ qualifying as Confidential Business Information (CBI). This description is a ‘‘Trade secret’’ that is (1) commercially valuable, (2) used in the applicant’s business, and (3) maintained in secrecy.

# Appendices

**Petition for addition to the National List of the Synthetic beta-CAROTENE on the National List of Substances Allowed as Ingredients in or on Processed Products Labeled as "organic" or "made with organic (specified ingredients or food group(s))."**

United States Patent Office 2,917,539
Patented Dec. 15, 1959

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2,917,539

# PROCESS FOR THE MANUFACTURE OF CAROTENOIDS

Otto Isler, Marc Montavon, and Rudolf Ruegg, Basel, and Paul Zeiller, Neuallschwil, Basel Land, Switzerland, assignors to Hoffmann-La Roche Inc., Nutley, N.J., a corporation of New Jersey

No Drawing. Application December 20, 1956
Serial No. 629,457

Claims priority, application Switzerland
December 29, 1955

18 Claims. (Cl. 260—488)

The present invention relates to a new process for the manufacture of carotenoids.

The process of this invention comprises condensing by means of a metal-organic reaction 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne (hereinafter called $C_{12}$-hydrocarbon) at both ends with a compound selected from the group consisting of 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, 4-(2,6,6-trimethyl-4-R-1-cyclohexen-1-yl)- and 4-(2,6,6 - trimethyl - 4 - R-1-cyclohexylidene)-2-methyl-2-buten-1-als wherein R represents a member selected from the group consisting of hydrogen, hydroxy and acyloxy (hereinafter called, respectively, $\beta$-dehydro-$C_{14}$-aldehyde, and $\beta$-$C_{14}$-aldehyde and iso-$C_{14}$-aldehyde when R represents hydrogen), and hydrolyzing the resulting condensation product to the corresponding diol compound (hereinafter called $C_{40}$-diol), subjecting the diol compound to a treatment causing elimination of two molecules of water and allyl rearrangement, partially hydrogenating the triple bonds in the resulting carotenoid compound, and isomerizing the resulting di-cis compound to the all-trans compound.

The carotenoids obtained by the present process are useful as dyestuffs for foods, e.g. for dyeing margarine, oils, butter, fats, ice-cream powder and the like, in order to give them orange to red shades. Some of these carotenoids have also a vitamin A-like activity.

The starting compounds required for carrying out the process of this invention may be prepared, for example, in the manner described hereinafter. In this specification temperatures are given in degrees centigrade.

*$C_{12}$-hydrocarbon.—3,8-dimethyl-3,5,7-decatrien-1,9-di-yne*

0.1 mole of ethereal phenyl-lithium solution was added, at 0°, to a suspension of 0.1 mole of triphenyl-(3-methyl-2-penten-4-yn-1-yl)-phosphonium bromide (M.P. 152–155°; obtained by condensing triphenylphosphine with 1-bromo-3-methyl-2-penten-4-yne in glacial acetic acid) in 150 ml. of absolute ether, and the mixture was stirred under nitrogen for 3 hours. The resulting dark-red solution contained triphenyl - (3 - methyl - 2-penten-4-yn-1-ylidene)-phosphine. To this solution was added an ethereal solution of 0.1 mole of 3-methyl-2-penten-4-yn-1-al, and the reaction mixture was heated at 40° for 1 hour. The reaction mixture was then filtered, the filtrate was washed neutral with water, then dried and concentrated in vacuo. The residue was dissolved in a small quantity of ethanol or methanol, and the solution was cooled to —40° whereupon the $C_{12}$-hydrocarbon crystallized. By sublimation in a high vacuum there was obtained a product melting at 91–93° and having absorption maxima in the ultra-violet spectrum at 304, 318 and 334 m$\mu$

($E^{1\%}_{1\,cm.}$=2310, 3495 and 3240)

2

*Iso-$C_{14}$-aldehyde.—4-(2,6,6-trimethyl-1-cyclohexylidene)-2-methyl-2-buten-1-al*

The ethoxy-acetylene carbinol obtained by condensing ethoxy-acetylene with 2,6,6-trimethyl-1-cyclohexanone was partially hydrogenated at the triple bond in a manner known per se, the resulting ethoxy-ethylene carbinol was hydrolysed with acid, the resulting (2,6,6-trimethyl-1-cyclohexylidene)acetaldehyde was acetalised, the acetal was condensed in the presence of an acidic condensing agent with a propenyl ether, and the resulting condensation product was treated with acid. The resulting aldehyde shows an U.V. absorption maximum at 288 m$\mu$ (in petroleum ether); the phenylsemicarbazone melts at 158–159°.

*4 - acetoxy - iso-$C_{14}$-aldehyde.—4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexylidene)-2-methyl-2-buten-1-al*

138 g. of 2,6,6-trimethyl-1-cyclohexen-4-one [which can be made, for example, from isophorone by known procedures; compare Kharasch, Journal of the American Chemical Society, 63, 2308 (1941)] in 50 ml. of glacial acetic acid were stirred for two hours at 0–10° with 160 ml. of peracetic acid (containing 530 mg. of peracetic acid per ml.) and the mixture was allowed to stand overnight 20°. Then, while adding ice, the reaction mixture was made weakly alkaline (pH about 8) by adding 30% aqueous NaOH solution, and the reaction mixture was shaken for one hour at 20°. Then the mixture was extracted twice, each time with 800 ml. of diethyl ether, and the ether solutions were washed once with 200 ml. of saturated ammonium chloride solution. The ether solutions were combined and dried over sodium sulfate, the solvent was driven off, and the residue was distilled in a high vacuum. A forerun passed over between 70 and 80°, and then 2,6,6-trimethyl-2-cyclo-hexen-1-ol-4-one was obtained as an almost colorless oil having B.P. 110–112°/0.1 mm. Hg, $n_D^{20}$=1.501, U.V. absorption maximum at 226 m$\mu$ ($E_1^1$=1110 in petroleum ether solution), after standing for some time. The phenylsemicarbazone had M.P. 189–190°, U.V. absorption maxima at 240.5 m$\mu$ and 285 m$\mu$ ($E_1^1$=807 and 778 in ethanol).

To 154 g. of 2,6,6-trimethyl-2-cyclohexen-1-ol-4-one in 200 ml. of glacial acetic acid and 500 ml. of water were quickly added dropwise 70 g. of chromic anhydride in 200 ml. of water, while stirring and cooling, so that the temperature did not rise above 30°. The mixture was then stirred overnight at 20°. Then the reaction mixture was saturated with ammonium chloride and was extracted with 1000 ml. of petroleum ether (boiling range 30–60°). The aqueous layer was again extracted in a second separatory funnel with 500 ml. of petroleum ether. The petroleum ether solutions were washed with saturated ammonium chloride solution to which a little ammonia had been added, and then with pure saturated ammonium chloride solution. The washed extracts were dried over sodium sulfate and the solvent was driven off. The product, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, was distilled under a water pump vacuum; B.P. 92–94°/11 mm. Hg yellow oil which solidified to crystalline form in the refrigerator, $n_D^{21}$=1.490, U.V. absorption maximum at 238 m$\mu$ ($E_1^1$=942 in petroleum ether). The phenylsemicarbazone had M.P. 190°, then resolidified and melted again at 230°, U.V. absorption maxima at 242.5 m$\mu$ and 325.5 m$\mu$ ($E_1^1$=875 and 580 in ethanol).

65 g. of 2,6,6-trimethyl-2-cyclohexene-1,4-dione in 250 ml. of glacial acetic acid were slowly reacted with 130 g. of zinc dust, while stirring, so that the temperature did not rise above 50°. Then the reaction mixture was stirred for an additional period of one hour. The reaction mixture was filtered, diluted with 1000 ml. of water and then saturated with ammonium chloride. The mixture was

extracted twice, each time with 800 ml. of petroleum ether (boiling range 30–60°). The petroleum ether solutions were washed with 300 ml. of saturated ammonium chloride solution to which some ammonia was added, and then were washed with pure saturated ammonium chloride solution. (In case a portion of the product crystallizes from the petroleum ether solution, it is filtered off, the crystalline material is dissolved in diethyl ether, then the diethyl ether solution is washed as indicated above, dried over sodium sulfate and then combined with the petroleum ether solution.) The solvent was driven off until the product 2,6,6-trimethyl-1,4-cyclohexanedione started to crystallize out; colorless needles, M.P. 63–65°, having no absorption maximum in the ultraviolet spectrum between 220 and 280 m$\mu$. The phenyl-semicarbazone had M.P. 218–220°, U.V. absorption maximum at 250 m$\mu$ ($E_1^1$=1030 in ethanol).

34.6 g. of 2,6,6-trimethyl-1,4-cyclohexandione, 100 ml. of benzene, 19 g. of ethylene glycol and 0.2 g. of p-toluene-sulfonic acid were refluxed for seven hours while separating the water which was formed. After cooling, the reaction mixture was poured into 300 ml. of 5% sodium bicarbonate solution, and the 2,6,6-trimethyl-4-ethylenedioxy-1-cyclohexanone product was obtained by extraction with diethyl ether and distillation of the extract. The product was obtained as a colorless oil, having B.P. 70°/0.02 mm. Hg, $n_D^{21}$=1.469.

To a lithium amide suspension prepared by dissolving 6.7 g. of lithium in 2000 ml. of liquid ammonia were added slowly, while stirring, 52 g. of 1-methoxy-2-methyl-3-butyn-2-ol. The mixture was stirred for one hour and then 79 g. of 2,6,6-trimethyl-4-ethylenedioxy-1-cyclohexanone were added, and the reaction mixture was stirred overnight at the boiling temperature of the ammonia. 60 g. of ammonium chloride were added and then the ammonia was driven off. The residue was taken up in diethyl ether, and insoluble material was filtered off; the ether solution was washed with a saturated solution of ammonium chloride, then was dried over sodium sulfate, and the ether was driven off. The residue was suspended in 450 ml. of petroleum ether and was extracted four times, each time with 300 ml. of 70% methanol. The methanol extracts were washed three times, each time with 150 ml. of petroleum ether, then were diluted with saturated ammonium chloride solution and the precipitated material was taken up in diethyl ether. The ether solution was washed with water, dried over sodium sulfate, and the ether was driven off. There were thus obtained 92 g. of 4-(2,6,6-trimethyl-4-ethylenedioxy-1-hydroxy - 1 - cyclohexyl)-2-methyl-1-methoxy-3-butyn-2-ol as a yellow viscous oil.

92 g. of the latter were dissolved in 3000 ml. of dry diethyl ether, were mixed while stirring at 0–5° with a solution of 22.5 g. of lithium aluminum hydride in 300 ml. of dry diethyl ether, and the reaction mixture was refluxed for four hours. Then the reaction mixture was cooled with ice, 250 ml. of methanol were added slowly while stirring at 0–5°, and the clear solution was poured into a mixture of 100 g. of ice and 600 ml. of saturated ammonium chloride solution. The precipitated aluminum hydroxide was filtered off, the precipitate was washed with diethyl ether, and the washings were added to the filtrate. The combined liquors were washed with water, dried over sodium sulfate and the solvents were driven off. The residue was partitioned between petroleum ether and 70% methanol, in the manner indicated above, and from the methanol extracts there were obtained 70 g. of 4-(2,6,6-trimethyl - 4 - ethylenedioxy -1-hydroxyl-1-cyclohexyl)-2-methyl-1-methoxy-3-buten-2-ol as a light-yellow, viscous oil.

70 g. of the latter were mixed with 140 ml. of formic acid and the mixture was heated for 25 minutes at 100°. The reaction mixture was poured onto ice and extracted with di-ethyl ether, the ethereal solution was washed with water and with dilute sodium bicarbonate solution,



dried over sodium sulfate, and the ether was driven off. The residue was dissolved in 200 ml. of glacial acetic acid, 26 ml. of water and 32 g. of sodium acetate were added, and the mixture was heated at 95° for two hours. Then it was diluted with ice water, and was extracted with diethyl ether, the ether extract was washed with water and with dilute sodium bicarbonate solution, dried over sodium sulfate and the ether was driven off. The residue was distilled in vacuum, thereby yielding 4-(2,6,6-trimethyl-4-oxo-1-cyclohexylidene)-2-methyl-2-buten-1-al as a yellow oil having B.P. ca. 110°/0.02 mm. Hg, $n_D^{21}$=1.555 (U.V. absorption maximum at 284 m$\mu$ in petroleum ether).

A solution of 31 g. of 4-(2,6,6-trimethyl-4-oxo-1-cyclohexylidene)-2-methyl-2-buten-1-al in 34 ml. of orthoformic acid ethyl ester and 7 ml. of absolute ethanol was mixed with 0.65 ml. of orthophosphoric acid and 0.05 g. of p-toluenesulfonic acid, and the mixture was allowed to stand for 24 hours at room temperature. 7 ml. of pyridine were added and then the mixture was poured upon ice and dilute sodium bicarbonate solution, the resulting mixture was extracted with petroleum ether, the petroleum ether extract was washed with water, dried over sodium sulfate, the solvent was driven off and the residue was dried in vacuo at 60°. There were thus obtained 40 g. of 4-(2,6,6-trimethyl-4-oxo-1-cyclohexylidene)-2-methyl-1,1-diethoxy-2-butene (U.V. absorption maximum at 248 m$\mu$ in petroleum ether).

40 g. of the latter product were dissolved in 600 ml. of dry diethyl ether and were mixed slowly, while stirring at 0–5°, with a solution of 2.8 g. of lithium aluminum hydride in 40 ml. of diethyl ether. The reaction mixture was stirred for one hour at room temperature, then was cooled to 0–5°; 20 ml. of methanol were added slowly, and the reaction mixture was poured upon ice and saturated ammonium chloride solution. The precipitated aluminum hydroxide was filtered off and washed with diethyl ether, the ether was added to the filtrate, the combined liquors were dried over sodium sulfate and the solvent material was driven off. There was obtained 39.5 g. of 4-(2,6,6-trimethyl-4-hydroxyl-1-cyclohexylidene)-2-methyl-1,1-diethoxy-2-butene.

39.5 g. of the latter were acetylated by mixing it with 40 ml. of pyridine and 20 ml. of acetic anhydride and permitting the mixture to stand for 20 hours. The reaction mixture was poured into ice water, extracted with petroleum ether, the organic layer was washed with cold sodium bicarbonate solution, dried over sodium sulfate and the solvent was driven off, yielding 42 g. of 4-(2,6,6-trimethyl-4-(acetoxy-1-cyclohexylidene)-2-methyl-1,1-diethoxy-2-butene.

42 g. of the latter were mixed with 400 ml. of glacial acetic acid, 50 ml. of water and 65 g. of sodium acetate and heated at 95° for three hours. Then the reaction mixture was diluted with ice water and was extracted with diethyl ether. The ethereal solution was washed several times with water, dried over sodium sulfate and the ether was driven off. There were thus obtained 31 g. of 4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexylidene)-2-methyl-2-buten-1-al (U.V. absorption maximum at 284 m$\mu$ in petroleum ether).

*4-acetoxy-β-$C_{14}$-aldehyde.—4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexen-1-yl)-2-methyl-2-buten-1-al*

31 g. of 4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexylidene)-2-methyl-2-buten-1-al were dissolved in 40 ml. of toluene, mixed with 16 g. of isopropenyl acetate and 0.2 g. of p-toluenesulfonic acid and the mixture was heated at 120–140° while continuously removing the acetone which was formed. After approximately two hours, the reaction mixture was cooled down, poured into ice water and extracted with petroleum ether. The petroleum ether solution was washed with cold sodium bicarbonate solution and then with water, dried over sodium sulfate

and the solvent was distilled off. There were thus obtained 34 g. of 4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexen-1-yl)-2-methyl-1-acetoxy-1,3-butadiene (U.V. absorption maximum at 262 mμ in petroleum ether).

34 g. of the latter were dissolved in 750 ml. of methanol, mixed with 75 ml. of water and 27 g. of sodium bicarbonate and the mixture was refluxed for six hours while stirring. Then the reaction mixture was diluted with ice water, extracted with diethyl ether, the ether solution was washed with water, dried over sodium sulfate and the ether was driven off. In order to achieve acetylation of the hydroxy group, attached to the ring, the residue, presumably containing at least some 4-(2,6,6-trimethyl-4-hydroxy-1-cyclohexen-1-yl)-2-methyl-2-buten-1-al, was mixed with 60 ml. of pyridine and 30 ml. of acetic anhydride and the mixture was allowed to stand for 20 hours at room temperature. 100 ml. of ice water were added and the mixture was then extracted with diethyl ether. The ethereal solution was washed with cold sodium bicarbonate solution and then with water, dried over sodium sulfate and the solvent was driven off. The 4-(2,6,6-trimethyl-4-acetoxy-1-cyclohexen-1-yl)-2-methyl-2-buten-1-al thus obtained is a yellowish oil and shows U.V. absorption maximum at 234 mμ (petroleum ether). Its phenylsemicarbazone melts at 190–192°; U.V. absorption maxima at 238 and 276 mμ (petroleum ether).

*Dehydro-β-$C_{14}$-aldehyde. — 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-methyl-2-buten-1-al*

136 parts by weight of 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-2-methyl-2-buten-1-al together with 97 parts by volume of isopropenyl acetate and 0.7 part by weight of p-toluene-sulphonic acid were heated at 100–140° for 3–4 hours, while passing a slow current of nitrogen through the mixture, the acetone formed during the reaction being continuously removed from the reaction mixture by distillation. After cooling, the thus obtained crude 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-methyl-1-acetoxy-1,3-butadiene was directly hydrolysed. For this purpose, 650 parts by volume of methyl alcohol, 65 parts by volume of water and 46 parts by weight of sodium bicarbonate were added, and the mixture was refluxed for 5–6 hours, while stirring. The reaction mixture was then poured into 2000 parts by volume of ice water and weakly acidified with dilute sulphuric acid. The reaction product was taken up in ether, and the ethereal solution was washed with sodium bicarbonate solution and dried over sodium sulphate. After removal of the solvent by distillation, the residue was distilled in a high vacuum. There were thus obtained 98 parts by weight of 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-methyl-2-buten-1-al; B.P. 80°/0.05 mm. Hg; $n_D^{22}=1.530$; U.V. absorption maxima at 224 and 268 mμ.

$$E^{1\%}_{1\,\text{cm.}}=795 \text{ and } 345$$

respectively (in petroleum ether). Phenylsemicarbazone: melting point 184–185°.

In the first step of the process according to this invention a metal-organic derivative of the $C_{12}$-hydrocarbon is reacted with β-dehydro-$C_{14}$-aldehyde, or with a β-$C_{14}$- or iso-$C_{14}$-aldehyde in which the oxygen of an oxygen-containing group which does not take place in the reaction may be attached to the 4-position of the nucleus. Metal-organic compounds which are particularly suitable for this reaction include the alkali metal derivatives and the magnesium-organic derivatives of the $C_{12}$-hydrocarbon. These metal-organic derivatives can be obtained in a known manner by reacting the $C_{12}$-hydrocarbon with the corresponding metals or derivatives thereof, such as sodium and lithium amide, alkyl- or aryl-lithium compounds, and alkyl- or aryl-magnesium halides, e.g. phenyl-lithium, phenyl-magnesium bromide and ethyl-magnesium bromide. The reaction is best carried out in an inert solvent, such as ether, benzene and the like, or in liquid ammonia. It is advantageous to use the same reaction medium and the same reaction vessel as used for the preparation of the metal-organic derivative. The condensation takes place already at room temperature and can be accelerated and completed by heating. In order to avoid secondary reactions, it is advantageous to effect the condensation in an inert atmosphere, e.g. under nitrogen. After completion of the reaction, the resulting metal-organic reaction product is hydrolyzed in a known manner, e.g. by means of water, ammonium acetate solution, etc. The obtained $C_{40}$-diol need not be purified before being further reacted. It may be identified by the Zerewitinoff test and the U.V. absorption spectrum. This diol consists of a mixture of stereoisomeric forms.

In the second step of the present invention the $C_{40}$-diol is subjected, if desired after esterification, to a treatment causing splitting off of two molecules of water or acid and allyl rearrangement. Rearrangement and splitting off of water or acid may also be brought about by the action of aqueous or anhydrous hydrohalic acid. Conveniently, the $C_{40}$-diol is esterified, e.g. acetylated, prior to the cleavage and rearrangement. A suitable mode of carrying out this step consists in treating a solution of the $C_{40}$-diol or of an ester thereof in an inert solvent, such as ether, methylene chloride, dioxane and the like, with anhydrous hydrohalic acid. Only a small quantity of acid need be used if the reaction is accelerated by heating. It is advantageous to effect the reaction in ethyl ether and to use an excess of alcoholic hydrochloric acid. According to another mode of executing this step, the $C_{40}$-diol or an ester thereof is treated in a halogenated hydrocarbon having a high dipole moment with aqueous hydrohalic acid at a temperature below 0°, and subsequently hydrogen halide is split off from the resulting halogenated compound by means of water or a basic compound. Solvents which may be used for this purpose include methylene chloride and chloroform, and concentrated aqueous hydrobromic acid may be used as the aqueous hydrohalic acid. The elimination of water or acid may also be performed by means of phosphorus oxychloride in pyridine. There are thus obtained carotenoids which still contain triple bonds in the positions 11,12 and 11',12' instead of double bonds and which have a characteristic U.V. absorption spectrum.

The third step of the process of this invention consists in partially hydrogenating the triple bonds. This hydrogenation may be carried out in suspension. e.g. in petroleum ether, toluene and the like, preferably by means of a deactivated palladium catalyst and in the presence of quinoline. The carotenoids obtained by partial hydrogenation have the cis-configuration at the resulting double bonds and possess an U.V. absorption spectrum which is typical of this type of compounds.

In the fourth step of the present process the cis-carotenoids are isomerized to the corresponding all-trans compounds. The isomerization is carried out in a known manner, for example by the action of iodine or light in solution, but preferably by heating in petroleum ether.

The present invention will now be illustrated by the following examples, however without being limited thereto.

EXAMPLE 1

*β-Carotene*

3.6 g. (0.023 mole) of 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne were dissolved in 50 ml. of absolute ether, and to the solution was added 0.05 mole of ethereal phenyl-lithium solution. The mixture was refluxed for 30 minutes. Then a solution of 11 g. (0.05 mole) of 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-methyl-2-buten-1-al in 100 ml. of ether were added dropwise, and the reaction mixture was boiled for 2 hours. The reaction mixture was then hydrolyzed with aqueous ammonium acetate solution, and the ethereal layer was separated, dried and concentrated. The residue, i.e. 1,18-di(2,6,6-trimethyl-1-cyclohexen-1-yl) - 3,7,12,16 - tetramethyl-4,15-dihydroxy-2,7,9,11,16 - octadecapentaen - 5, 13-di-yne, was a resinous product (having 1.9 active hydrogen atoms and absorption maxima in the ultra-violet spectrum at 326 and 341 mμ) which was used for the next step without any further purification. The resin was dissolved in 200 ml. of methylene chloride, 10 ml. of glacial acetic acid were added to the solution, and the mixture was cooled to −40° in a carbon dioxide atmosphere, while stirring. Then, 9 ml. of aqueous hydrobromic acid (60%) were added in one portion, the mixture was stirred at −35° for 1½ minutes, and subsequently 200 ml. of ice water were run into the mixture. After further stirring the mixture for 2 hours at 0°, the methylene chloride layer was separated, washed with water and sodium bicarbonate solution, dried with $Na_2SO_4$ and concentrated in vacuo. The residue, i.e. 11,12-11′,12′-bis-dehydro-β-carotene, was a tough resin or a foamy solid (having no active hydrogen atoms and possessing absorption maxima in the ultra-violet spectrum at 334 and 408 mμ). This product can be purified by chromatography. The crude product can also be used for the next step without any preliminary purification.

11.4 g. of 11,12-11′,12′-bis-dehydro-β-carotene were dissolved in 100 ml. of petroleum ether (boiling range 80–100°), and the solution was hydrogenated under normal conditions after the addition of 0.5 ml. of quinoline and 5 g. of a lead-poisoned palladium catalyst. After the calculated amount of hydrogen had been absorbed, the catalyst was removed by filtration and the filtrate was extracted with dilute sulfuric acid to remove the quinoline. By concentrating the solution in the usual manner there was obtained 11,12-11′,12′-di-cis-carotene. The product was purified by recrystallisation from benzene-alcohol. The purified product melts at 154°; absorption maxima in the ultra-violet spectrum at 276, 334, 338, 401 and 405 mμ. The isomerisation was effected by heating the product for 10 hours at 90–100° in high-boiling petroleum ether in a carbon dioxide atmosphere. The resulting β-carotene melted at 180°; U.V. absorption maxima at 452 and 480 mμ.

EXAMPLE 2

*Bis-dehydro-β-carotene*

1.4 g. of 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne were dissolved in 100 ml. of absolute ether, and to the solution were added 20 ml. of N ethereal phenyl-lithium solution. After refluxing the mixture for 30 minutes a solution of 3.5 g. of 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-methyl-2-buten-1-al in 100 ml. of absolute ether was added, and the reaction mixture was then boiled for one hour. After hydrolysing the reaction mixture with ammonium chloride solution, separating the ethereal layer, drying and concentrating, there was obtained 1,18-di(2,6,6-trimethyl-1,3-cyclohexadien - 1 - yl) - 3,7,12,16-tetramethyl-4,15-dihydroxy-2,7,9,11,16-octadecapentaen-5, 13-di-yne in the form of a tough resin; absorption maxima in the ultra-violet spectrum at 266, 330 and 346 mμ. The crude product was dehydrated in the manner described in Example 1 to obtain 3,4-3′,4′-11,12-11′,12′-tetradehydro-β-carotene; U.V. absorption maxima at 335, 382 and 403 mμ. By partially hydrogenating and isomerizing this compound in the manner described in Example 1 there was obtained 3,4-3′,4′-bis-dehydro-β-carotene of M.P. 190–191°; ultra-violet spectrum with a broad absorption maximum at 471 mμ.

EXAMPLE 3

*Zeaxanthene*

0.8 g. of 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne were dissolved in 50 ml. of absolute ether, and to the resulting solution there were added dropwise 10 ml. of N ethereal phenyl-lithium solution. After boiling for one hour, a solution of 2.5 g. of 4-(2,6,6-trimethyl-4-acetoxy-cyclohexylidene)-2-methyl-2-buten-1-al in 50 ml. of absolute ether was added to the light-yellow suspension whereupon a thick white precipitate formed. After refluxing for one hour, the reaction mixture was hydrolyzed by means of ammonium acetate solution. The ethereal layer was separated, washed with water, dried over sodium sulfate and concentrated in vacuo. There remained a foamy solid consisting of 1,18-di-(2,6,6-trimethyl-4-acetoxy - cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15-dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13, - di-yne; yield: 3–4 g. This product was used for the next step without any preliminary purification. It was dissolved in 80 ml. of benzene, and 2 ml. of pyridine were added to the resulting solution. To the solution was added in a nitrogen atmosphere, while stirring, a mxiture of 0.7 ml. of phosphorus oxychloride, 0.7 ml. of pyridine and 20 ml. of benzene. The reaction mixture was heated for 1 hour at 70–80° and then poured into ice water. The separated benzene layer was washed neutral, dried and concentrated. The residue, i.e. 3,3′-di-acetoxy-11,12-11′,12′-bis-dehydro-β-carotene, which consisted of a tough resin, was purified by chromatography on alumina by means of petroleum ether-benzene, and the purified product was saponified by heating in a nitrogen atmosphere with 10% methanolic potassium hydroxide solution in ethereal solution. The resulting 3,3′-dihydroxy-11,12-11′,12′-bis-dehydro-β-carotene showed absorption maxima in the ultra-violet spectrum at 334 and 408 mμ. The partial hydrogenation and the isomerization were carried out in the manner described in Example 1. In the ultra-violet spectrum, the thus obtained synthetic zeaxanthene showed the same absorption maxima at 452 and 480 mμ as β-carotene.

We claim:

1. A process which comprises condensing 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne bilaterally with a compound selected from the group consisting of 4-(2,6,6-trimethyl)-1,3-cyclohexadien-1-yl)-2-methyl-2-buten-1-al, 4-(2,6,6-trimethyl - 4 - R - 1 - cyclohexen - 1 - yl) - 2 - methyl - 2-buten-1-al and 4-(2,6,6-trimethyl-4-R-1-cyclohexylidene)-2-methyl-2-buten-1-al, wherein R represents a member selected from the group consisting of hydrogen, hydroxy and lower alkanoyloxy, in an inert solvent by means of a metal organic reaction, hydrolyzing the reaction product in aqueous solution to obtain, respectively, a diol of the group consisting of 1,18-di-(2,6,6-trimethyl-1,3-cyclohexadien - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy-2,7,9,11,16-octadecapentaen-5,13-di-yne, 1,18-di-(2,6,6 - trimethyl - 4 - R - 1 - cyclohexen - 1 - yl) - 3,7,-12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne and 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1 - cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen-5,13-di-yne, wherein R has the same significance as above, treating said diol with acid whereupon two molecules of water are eliminated and allyl rearrangement simultaneously occurs, thereby producing respectively, a carotenoid selected from the group consisting of 1,18-di-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl) - 3,7,12,16-tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen - 5,13 - di-yne, 1,18-di-(2,6,6-trimethyl-4-R-1-cyclohexen-1-yl)-3,7,-12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen-5,13-di-yne and 1,18-di-(2,6,6-trimethyl-4-R-1-cyclohexylidene) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen-5,13-di-yne, wherein R has the same significance as above, and selectively catalytically hydrogenating the triple bonds in said last named carotenoids to double bonds.

2. A process as in claim 1 wherein 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne di-lithium derivative is used in said initial condensation.

3. A process as in claim 1 wherein 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne di-sodium derivative is used in said initial condensation.

4. A process as in claim 1 wherein 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne di-alkylmagnesium derivative is used in said initial condensation.

5. A process as in claim 1 wherein the end products are heated thereby isomerizing di-cis carotenoid to all-trans carotenoid.

6. A process which comprises condensing 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne bilaterally with a compound selected from the group consisting of 4-(2,6,6-trimethyl-1,3 - cyclohexadien - 1 - yl) - 2 - methyl - 2 - buten-1-al, 4-(2,6,6-trimethyl-4-R-1-cyclohexen-1-yl)-2-methyl-2 - buten - 1 - al and 4 - (2,6,6 - trimethyl - 4 - R - 1-cyclohexylidene) 2-methyl-2-buten-1-al, wherein R represents a member selected from the group consisting of hydrogen, hydroxy and lower alkanoyloxy, in an inert solvent by means of a metal organic reaction, hydrolyzing the reaction product in aqueous solution to obtain, respectively, a dihydroxy diol of the group consisting of 1,18-di-(2,6,6-trimethyl - 1,3 - cyclohexadien - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen-5,13 - di - yne, 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1-cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne and 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1 - cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy-2,7,9,11,16 - octadecapentaen - 5,13 - di - yne, wherein R has the same significance as above, esterifying the two central hydroxy groups of said diol with a lower fatty acid, treating the resulting ester with acid whereupon two molecules of acid are eliminated and allyl rearrangement simultaneously occurs, thereby producing, respectively, a carotenoid selected from the group consisting of 1,18 - di - (2,6,6 - trimethyl - 1,3 - cyclohexadien-1 - yl) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen - 5,13 - di - yne, 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1 - cyclohexen - 1 - yl) - 3,7,12,16-tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen - 5,13-di - yne and 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1-cyclohexylidene) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen - 5,13 - di - yne, wherein R has the same significance as above, and selectively catalytically hydrogenating the triple bonds in said last named carotenoids to double bonds.

7. A process which comprises condensing a metal organic derivative of 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne in an inert solvent bilaterally with a compound selected from the group consisting of 4-(2,6,6-trimethyl-1,3-cyclohexadien - 1 - yl) - 2 - methyl - 2 - buten - 1 - al, 4 - (2,6,6 - trimethyl - 4 - R - 1 - cyclohexen - 1 - yl) - 2-methyl - 2 - buten - 1 - al and 4 - (2,6,6 - trimethyl - 4-R - 1 - cyclohexylidene) - 2 - methyl - 2 - buten - 1 - al, wherein R represents a member selected from the group consisting of hydrogen, hydroxy and lower alkanoyloxy, and hydrolyzing the reaction product in aqueous solution to obtain, respectively, a diol of the group consisting of 1,18 - di - (2,6,6 - trimethyl - 1,3 - cyclohexadien - 1 - yl)-3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16-octadecapentaen - 5,13 - di - yne, 1,18 - di - (2,6,6 - trimethyl - 4 - R - 1 - cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen-5,13 - di - yne and 1,18 - di - (2,6,6 - trimethyl - 4 - R-1 - cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne, wherein R has the same significance as above.



8. A process which comprises condensing 3,8-dimethyl-3,5,7 - decatrien - 1,9 - di - yne bilaterally with 4 - (2,6,6 - trimethyl - 1 - cyclohexen - 1 - yl) - 2 - methyl - 2-buten - 1 - al in an inert solvent by means of a metal organic reaction, hydrolyzing the reaction product in aqueous solution to obtain 1,18-di-(2,6,6-trimethyl-1-cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne, treating the last named compound with acid whereupon two molecules of water are eliminated and allyl rearrangement simultaneously occurs, thereby producing 1,18-di - (2,6,6 - trimethyl - 1 - cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen - 5,13-di-yne, selectively catalytically hydrogenating the triple bonds in the last named compound to double bonds to obtain di-cis-β-carotene and heating the di-cis-β-carotene to obtain all-trans-β-carotene.

9. 1,18 - di - (2,6,6 - trimethyl - 1,3 - cyclohexadien-1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne.

10. 1,18 - di - (2,6,6 - trimethyl - 4 - hydroxy - 1 - cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy - 2,7,9,11,16 - octadecapentaen - 5,13 - di - yne.

11. 1,18 - di - (2,6,6 - trimethyl - 4 - hydroxy - 1 - cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy-2,7,9,11,16-octadecapentaen-5,13-di-yne.

12. 1,18 - di - (2,6,6 - trimethyl - 4 - lower alkanoyloxy - 1 - cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl-4,15-dihydroxy-2,7,9,11,16-octadecapentaen-5,13-di-yne.

13. 1,18-di-(2,6,6-trimethyl-4-lower alkanoyloxy-1-cyclohexylidene) - 3,7,12,16 - tetramethyl - 4,15 - dihydroxy-2,7,9,11,16-octadecapentaen-5,13-di-yne.

14. 1,18 - di - (2,6,6 - trimethyl - 1,3 - cyclohexadien-1 - yl) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17 - octadecaheptaen-5,13-di-yne.

15. 1,18 - di - (2,6,6 - trimethyl - 4 - hydroxy - 1 - cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17-octadecaheptaen-5,13-di-yne.

16. 1,18-di-(2,6,6-trimethyl-4-lower alkanoyloxy-1-cyclohexen - 1 - yl) - 3,7,12,16 - tetramethyl - 1,3,7,9,11,15,17-octadecaheptaen-5,13-di-yne.

17. 3,8-dimethyl-3,5,7-decatrien-1,9-di-yne.

18. 11,12-11′,12′-di-cis-zeaxanthene.


**References Cited in the file of this patent**

UNITED STATES PATENTS

2,609,396 Inhoffen et al. ---------- Sept. 2, 1952

OTHER REFERENCES

Inhoffen et al.: Annalen der Chemie, vol. 573, pgs. 1–16 (1951).

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Zechmeister et al.: Jour. Amer. Chem. Soc., vol. 75 (1953), pg. 4493.

Zechmeister et al.: Jour. Amer. Chem. Soc., vol. 75 (1953), pg. 5341.

Inhoffen et al.: Annalen der Chemie, vol. 585, pgs. 126–131 (1954).

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**CONFIDENTIAL BUSINESS INFORMATION DELETED**

complete dehydration of gypsum, below 300 °C, in an electric oven.

(b) The ingredient meets the specifications of the "Food Chemicals Codex," 3d Ed. (1981), p. 66, which is incorporated by reference. Copies may be obtained from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.*

(c) The ingredient is used as an anticaking agent as defined in §170.3(o)(1) of this chapter, color and coloring adjunct as defined in §170.3(o)(4) of this chapter, dough strengthener as defined in §170.3(o)(6) of this chapter, drying agent as defined in §170.3(o)(7) of this chapter, firming agent as defined in §170.3(o)(10) of this chapter, flour treating agent as defined in §170.3(o)(13) of this chapter, formulation aid as defined in §170.3(o)(14) of this chapter, leavening agent as defined in §170.3(o)(17) of this chapter, nutrient supplement as defined in §170.3(o)(20) of this chapter, pH control agent as defined in §170.3(o)(23) of this chapter, processing aid as defined in §170.3(o)(24) of this chapter, stabilizer and thickener as defined in §170.3(o)(28) of this chapter, synergist as defined in §170.3(o)(31) of this chapter, and texturizer as defined in §170.3(o)(32) of this chapter.

(d) The ingredient is used in food at levels not to exceed good manufacturing practice in accordance with §184.1(b)(1). Current good manufacturing practice results in a maximum level, as served, of 1.3 percent for baked goods as defined in §170.3(n)(1) of this chapter, 3.0 percent for confections and frostings as defined in §170.3(n)(9) of this chapter, 0.5 percent for frozen dairy desserts and mixes as defined in §170.3(n)(20) of this chapter, 0.4 percent for gelatins and puddings as defined in §170.3(n)(22) of this chapter, 0.5 percent for grain products and pastas as defined in §170.3(n)(23) of this chapter, 0.35 percent for processed vegetables as defined in §170.3(n)(36) of this chapter, and 0.07 percent or less for all other food categories.

(e) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

[45 FR 6086, Jan. 25, 1980; 45 FR 26319, Apr. 18, 1980, as amended at 49 FR 5611, Feb. 14, 1984]

### § 184.1240 Carbon dioxide.

(a) Carbon dioxide (empirical formula $CO_2$, CAS Reg. No. 124–38–9) occurs as a colorless, odorless, noncombustible gas at normal temperatures and pressures. The solid form, dry ice, sublimes under atmospheric pressure at a temperature of −78.5 °C. Carbon dioxide is prepared as a byproduct of the manufacture of lime during the "burning" of limestone, from the combustion of carbonaceous material, from fermentation processes, and from gases found in certain natural springs and wells.

(b) The ingredient must be of a purity suitable for its intended use.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitations other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as a leavening agent as defined in §170.3(o)(17) of this chapter; a processing aid as defined in §170.3(o)(24) of this chapter; and a propellant, aerating agent, and gas as defined in §170.3(o)(25) of this chapter.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

[48 FR 57270, Dec. 29, 1983, as amended at 73 FR 8607, Feb. 14, 2008]

### § 184.1245 Beta-carotene.

(a) *Beta*-carotene (CAS Reg. No. 7235–40–7) has the molecular formula $C_{40}H_{56}$. It is synthesized by saponification of vitamin A acetate. 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 *beta*-carotene.

(b) The ingredient meets the specifications of the Food Chemicals Codex, 3d Ed. (1981), p. 73, which is incorporated by reference. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used as a nutrient supplement as defined in §170.3(o)(20) of this chapter.

(2) 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) of this chapter; fats and oils as defined in §170.3(n)(12) of this chapter; and processed fruits and fruit juices as defined in §170.3(n)(35) of this chapter. *Beta*-carotene may be used in infant formula as a source of vitamin A in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act or with regulations promulgated under section 412(g) of the act.

(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

[52 FR 25211, July 6, 1987]

### § 184.1250 Cellulase enzyme preparation derived from Trichoderma longibrachiatum.

(a) Cellulase enzyme preparation is derived from a nonpathogenic, nontoxicogenic strain of *Trichoderma longibrachiatum* (formerly *T. reesei*). The enzyme, cellulase, catalyzes the endohydrolysis of 1,4-beta-glycosidic linkages in cellulose. It is obtained from the culture filtrate resulting from a pure culture fermentation process.

(b) The ingredient meets the general and additional requirements for enzyme preparations in the monograph specifications on enzyme preparations in the ‘‘Food Chemicals Codex,’’ 4th ed. (1996), pp. 129 to 134, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Box 285, Washington, DC 20055 (Internet *http://www.nap.edu*), or may be examined at the Center for Food Safety and Applied Nutrition's Library, 5100 Paint Branch Pkwy., College Park, MD 20740, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*.

(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:

(1) The ingredient is used in food as an enzyme as defined in §170.3(o)(9) of this chapter for the breakdown of cellulose.

(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.

[64 FR 28361, May 26, 1999]

### § 184.1257 Clove and its derivatives.

(a) Cloves are the dried unopened flower buds and calyx tubes, harvested before the flowers have opened, of the clove tree *Eugenia caryophyllata* Thunberg, native to tropical Asia. Their derivatives include essential oils (cloves, CAS Reg. No. 8000–34–8; buds; leaves, CAS Reg. No. 8015–97–2; stems, CAS Reg. No. 8015–98–3; and eugenol, CAS Reg. No. 97–53–0), oleoresins, and natural extractives obtained from clove buds, leaves, and stems.

# ß-CAROTENES, synthetic

*Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI of 0-5 mg/kg bw for beta carotene, synthetic and from Blakeslea trispora, was established at the 57th JECFA (2001).*

**SYNONYMS** CI Food Orange 5, INS No. 160a(i); CI (1975) No. 40800

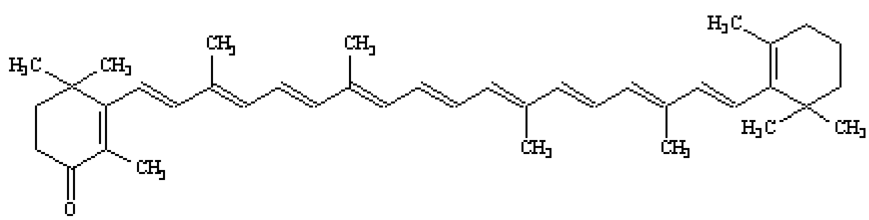
**DEFINITION** These specifications apply to predominantly all trans (Z) isomer of ß-carotene together with minor amounts of other carotenoids; diluted and stabilized forms are prepared from ß-carotene meeting these specifications and include solutions or suspensions of ß-carotene in edible fats or oils, emulsions and water dispersible powders; these preparations may have different cis/trans isomer ratios; the analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms (appropriate methods should be available from the manufacturer).

C.A.S. number 7235-40-7

Chemical names ß-Carotene, ß,ß-carotene

Chemical formula $C_{40}H_{56}$

Structural formula




Formula weight 536.88

Assay Not less than 96% total colouring matters, expressed as ß-carotene

**DESCRIPTION** Red to brownish-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas

**FUNCTIONAL USES** Colour

**CHARACTERISTICS**

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils; soluble in chloroform

Spectrophotometry

Determine the absorbance of the sample solution C (See Method of Assay) at 455 nm and 483 nm. The ratio $A_{455}/A_{483}$ is between 1.14 and 1.19.
Determine the absorbance of the sample solution C at 455 nm and that of sample Solution B (See Method of Assay) at 340 nm. The ratio $A_{455}/A_{340}$ is not lower than 15.

Test for carotenoid

The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1 N sulfuric acid.

Carr-Price reaction

A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS

PURITY

Sulfated ash (Vol. 4)

Not more than 0.1%
Test 2 g of the sample (Method I)

Subsidiary colouring matters

Carotenoids other than ß-carotene: Not more than 3% of total colouring matters.
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

## TESTS

PURITY TESTS

Subsidiary colouring matters

Carotenoids other than ß-carotene
Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 µl of this solution as a streak 2 cm from the bottom of a TLC-plate (Silicagel 0.25 mm). Immediately develop the chromatogram with a solvent mixture of 95 parts dichloromethane and 5 parts diethyl ether in a saturated chamber, suitably protected from light, until the solvent front has moved 15 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silicagel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (solution 1).

Remove the silicagel adsorbent that contains the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (solution 2).

Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (solution 3). Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum about 464 nm, using chloroform as blank.

Calculation
Calculate the percentage of carotenoids other than ß-carotene (%) =

$$\frac{A_2 \times 100}{10\,A_3 + A_2}$$

where
$A_2$ = absorbance of Solution 2
$A_3$ = absorbance of Solution 3

**METHOD OF ASSAY**

Proceed as directed under *Total Content by Spectrophotometry* in Volume 4 using the following conditions:

W = 0,08 g
$V_1 = V_2 = V_3$ = 100 ml
$v_1 = v_2$ = 5 ml
$A^{1\%}_{1\,cm}$ = 2500
$lambda_{max}$ = about 455 nm

## Similac® formulas for every baby's nutritional needs

Use as directed by your baby's doctor.

| Advance | Sensitive | Expert Care |
|---|---|---|
| Complete nutrition for the 1st year | For babies with fussiness, gas, & spit-up | For babies needing extra TLC |
| Advance® | Sensitive | Alimentum® |
| Organic | Spit-Up | NeoSure® |
| | Soy | Diarrhea |

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SAMPLE UPC

0 70074 59884 0

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**Have product-related questions?**
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8:30 am–5:00 pm,
Eastern time, weekdays.
www.Similac.com

U.S. Patent Nos. 5,700,590; 6,136,858; 6,596,767; 7,090,879; D416,801 and D419,455



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Abbott

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DO NOT ADD WATER

DHA/ARA
BIRTH TO 12 MONTHS
MILK-BASED

BRAND FED #1 IN HOSPITALS

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## ADVANCE®

IMMUNE SUPPORT
Early Shield®
BRAIN & EYE
STRONG BONES

LUTEIN & DHA

USDA ORGANIC

## Organic
Complete Nutrition

For Your Baby's 1st Year

Infant Formula with Iron

HALAL

U-D 1 QT (946 mL)

**NUTRIENTS** PER 100 CALORIES (5 FL OZ)

| | | | | | |
|---|---|---|---|---|---|
| PROTEIN | 2.07 | G | WATER | 133 | G |
| FAT | 5.40 | G | LINOLEIC ACID | 860 | MG |
| CARBOHYDRATE | 10.9 | G | | | |

**VITAMINS**

| | | | | | |
|---|---|---|---|---|---|
| VITAMIN A | 300 | IU | NIACIN | 1050 | MCG |
| VITAMIN D | 60 | IU | FOLIC ACID (FOLACIN) | 15 | MCG |
| VITAMIN E | 1.5 | IU | PANTOTHENIC ACID | 450 | MCG |
| VITAMIN K | 8 | MCG | BIOTIN | 4.4 | MCG |
| THIAMIN (VIT. $B_1$) | 100 | MCG | VITAMIN C (ASCORBIC ACID) | 9 | MG |
| RIBOFLAVIN (VIT. $B_2$) | 150 | MCG | CHOLINE | 16 | MG |
| VITAMIN $B_6$ | 60 | MCG | INOSITOL | 4.7 | MG |
| VITAMIN $B_{12}$ | 0.25 | MCG | | | |

**MINERALS**

| | | | | | |
|---|---|---|---|---|---|
| CALCIUM | 78 | MG | COPPER | 90 | MCG |
| PHOSPHORUS | 42 | MG | IODINE | 6 | MCG |
| MAGNESIUM | 6 | MG | SELENIUM | 1.8 | MCG |
| IRON | 1.8 | MG | SODIUM | 24 | MG |
| ZINC | 0.75 | MG | POTASSIUM | 105 | MG |
| MANGANESE | 5 | MCG | CHLORIDE | 65 | MG |

**INGREDIENTS:** WATER, **ORGANIC** NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 0.5% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, CARRAGEENAN, MAGNESIUM CHLORIDE, SALT, FERROUS SULFATE, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, d-ALPHA-TOCOPHERYL ACETATE, L-CARNITINE, ZINC SULFATE, NIACINAMIDE, CALCIUM PANTOTHENATE, RIBOFLAVIN, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, POTASSIUM IODIDE, SODIUM SELENATE, VITAMIN $D_3$, CYANOCOBALAMIN, POTASSIUM HYDROXIDE AND NUCLEOTIDES (ADENOSINE 5'-MONOPHOSPHATE, CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE).

**CONTAINS MILK AND SOY INGREDIENTS.**

*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)



## USE AS DIRECTED BY A DOCTOR
### Directions for Preparation and Use

**USE BY DATE ON BOTTLE • DO NOT ADD WATER**

**DO NOT USE IF BAND AROUND CAP OR INNER FOIL SEAL IS DAMAGED.**

Your baby's health depends on carefully following these directions. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to boil (sterilize) bottles, nipples and rings before use.

**Use**

1. Shake very well before each use. Remove protective band; twist off and clean cap
2. Invert cap; press down to pierce foil, then turn cap a half turn. Remove foil
3. Pour formula into bottle; attach nipple. Once feeding begins, use within 1 hour or discard

**Prepare and feed all formula in this container within 3 days after opening.**

**Storage** Once opened, store quart bottle in refrigerator. Store prepared bottles in refrigerator and **feed to baby within 48 hours.** Store unopened containers at room temperature; avoid extreme temperatures. Do not reuse container.

**Warning** Do not use this container to warm formula. **Never use a microwave to warm formula.** Serious burns can result.

**Abbott Nutrition**, Abbott Laboratories, Columbus, Ohio 43219-3034 USA

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USDA ORGANIC

IMMUNE SUPPORT
Early Shield®
BRAIN & EYE
STRONG BONES

LUTEIN & DHA

For Your Baby's 1st Year

Infant Formula with Iron

HALAL U-D

NET WT. 1.45 LB (658 g)

## NUTRIENTS PER 100 CALORIES (5 FL OZ, PREPARED AS DIRECTED)

| | | | | | |
|---|---|---|---|---|---|
| PROTEIN | 2.07 | G | WATER | 133 | G |
| FAT | 5.63 | G | LINOLEIC ACID | 860 | MG |
| CARBOHYDRATE | 10.4 | G | | | |

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| VITAMIN A | 300 | IU | NIACIN | 1050 | MCG |
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## MINERALS

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| IRON | 1.8 | MG | SODIUM | 24 | MG |
| ZINC | 0.75 | MG | POTASSIUM | 105 | MG |
| MANGANESE | 5 | MCG | CHLORIDE | 65 | MG |

**INGREDIENTS: ORGANIC** NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 2% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, ASCORBYL PALMITATE, FERROUS SULFATE, SALT, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, MAGNESIUM CHLORIDE, ZINC SULFATE, MIXED TOCOPHEROLS, d-ALPHA-TOCOPHERYL ACETATE, NIACINAMIDE, CALCIUM PANTOTHENATE, L-CARNITINE, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, SODIUM SELENATE, VITAMIN $D_3$, CYANOCOBALAMIN, POTASSIUM IODIDE, POTASSIUM HYDROXIDE AND NUCLEOTIDES (CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE, ADENOSINE 5'-MONOPHOSPHATE).
**CONTAINS MILK AND SOY INGREDIENTS.**

*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)

**Abbott Nutrition**, Abbott Laboratories
Columbus, Ohio 43219-3034 USA

CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL



**USE BY DATE ON CONTAINER • USE AS DIRECTED BY A DOCTOR**
**Directions for Preparation and Use**

Your baby's health depends on carefully following these directions. Proper hygiene, handling and storage are important when preparing infant formula. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to use cooled, boiled water for mixing and if you need to boil (sterilize) bottles, nipples and rings before use.

1. Wash your hands, surfaces and utensils. Pour water into clean bottle (see mixing guide)

2. Add 1 unpacked level scoop (8.6 g) to each 2 fl oz of water. Return dry scoop to holder in lid

3. Cap bottle; shake well; attach nipple. Once feeding begins, use within 1 hour or discard

**Storage:** Once mixed, store bottles in refrigerator and **feed to baby within 24 hours.** Store unopened or opened container at room temperature; avoid extreme temperatures. **Use opened container contents within 1 month.** Do not reuse container.

**Warning:** Powdered infant formulas are not sterile and should not be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor. **Never use a microwave to warm formula.** Serious burns can result.

**DO NOT USE IF OUTER QUALITY SEAL OR INNER FOIL SEAL IS DAMAGED.**

U.S. Patent Nos. 5,700,590; 6,136,858; 6,596,767; 7,090,879; D576,035 and D578,401

POSITION ONLY
0 70074 50822 1



**MIXING GUIDE**
GUÍA DE MEZCLA

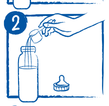
PEEL HERE
Desprenda aquí

IMPORTANT NOTICE: BREAST MILK IS BEST FOR BABIES. Before using an infant formula, ask the advice of your health-care professional. If you choose to supplement breastfeeding with formula or to formula feed exclusively, there is no better formula than Vermont Organics™ ORGANIC Infant Formula DHA and ARA. **Powdered infant formulas are NOT sterile and should NOT be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor.**

## DIRECTIONS FOR PREPARATION AND USE

Your baby's health depends on carefully following these preparation, use and storage instructions; changes could affect your baby's nutrition and safety. Before preparing the infant formula, make sure to always wash your hands. Clean can top before opening. **Ask your baby's doctor about the need to boil or sterilize water for formula and the proper preparation of bottle and feeding utensils.**

1. Pour desired amount of warm water (approx. 100°F/40°C) into bottle. (See Feeding Chart below.)



2. Add powder. Always add powder to water.

3. Cap bottle and shake well until powder is dissolved. Feed immediately. Discard any remaining formula in bottle after 1 hour from start of feeding.

Use this Feeding Chart for proper amount of water and powder.

| To Make | Water | Powder (Use scoop enclosed to measure) |
|---|---|---|
| 2 fl oz bottle | 2 fl oz | **1** unpacked level **scoop** (8.7 g) |
| 4 fl oz bottle | 4 fl oz | **2** unpacked level **scoops** (17.4 g) |
| 6 fl oz bottle | 6 fl oz | **3** unpacked level **scoops** (26.1 g) |
| 8 fl oz bottle | 8 fl oz | **4** unpacked level **scoops** (34.8 g) |
| 1 quart | 29 fl oz | **1** unpacked level standard measuring **cup &** **2** unpacked level **scoops** (123 g) |

**Storage:** Store unused prepared bottled formula in the refrigerator at 35-40°F (2-4°C); **use within 24 hours.** DO NOT FREEZE. Warm infant formula to room temperature and shake well before feeding. Prepared formula should not be without refrigeration more than 2 hours. Store open and unopened cans in a dry area at room temperature. Cover the opened can tightly with plastic cap; use contents within 1 month. Avoid any extreme temperatures.

**Warning: Do not use microwave to prepare or warm formula. Serious burns may occur.**

Filled by weight, not by volume. Contents may settle during shipment.

Contents yield approximately 190 fl oz of formula

Las instrucciones en Español se encuentran al dorso de esta etiqueta.

Easy to Digest
Powder • Add Water

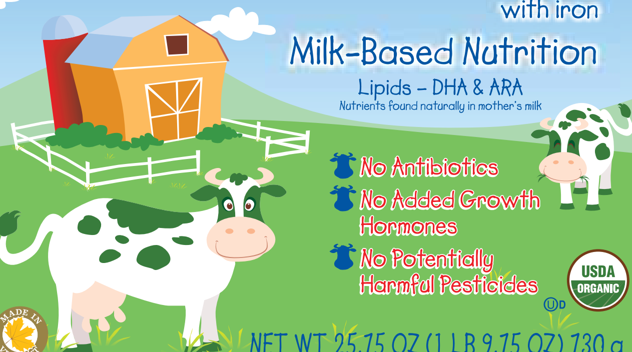
Vermont Organics™

For Babies
0-12 Months

# organic infant formula with iron

## Milk-Based Nutrition

Lipids – DHA & ARA
Nutrients found naturally in mother's milk



- No Antibiotics
- No Added Growth Hormones
- No Potentially Harmful Pesticides

USDA ORGANIC

MADE IN VERMONT

NET WT 25.75 OZ (1 LB 9.75 OZ) 730 g

INGREDIENTS: **ORGANIC** REDUCED MINERALS WHEY, **ORGANIC** VEGETABLE OILS (PALM OR PALM OLEIN, HIGH OLEIC (SAFFLOWER OR SUNFLOWER), COCONUT, SOY), **ORGANIC** NONFAT MILK, **ORGANIC** LACTOSE, **AND LESS THAN 1%:** MORTIERELLA ALPINA OIL*, CRYPTHECODINIUM COHNII OIL**, VITAMIN A PALMITATE, BETA-CAROTENE, VITAMIN D (CHOLECALCIFEROL), VITAMIN E (dl-ALPHA TOCOPHERYL ACETATE), MIXED TOCOPHEROL CONCENTRATE, VITAMIN K (PHYTONADIONE), ASCORBYL PALMITATE, THIAMINE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, CYANOCOBALAMIN, NIACINAMIDE, FOLIC ACID, CALCIUM PANTOTHENATE, BIOTIN, ASCORBIC ACID, CHOLINE CHLORIDE, INOSITOL, CALCIUM CHLORIDE, CALCIUM HYDROXIDE, FERROUS SULFATE, ZINC SULFATE, MANGANESE SULFATE, CUPRIC SULFATE, POTASSIUM BICARBONATE, POTASSIUM CHLORIDE, POTASSIUM IODIDE, POTASSIUM HYDROXIDE, SODIUM SELENITE, SODIUM CITRATE, TAURINE, SOY LECITHIN, NUCLEOTIDES (ADENOSINE-5'-MONOPHOSPHATE, CYTIDINE-5'-MONOPHOSPHATE, DISODIUM GUANOSINE-5'- MONOPHOSPHATE, DISODIUM INOSINE-5'-MONOPHOSPHATE, DISODIUM URIDINE-5'MONOPHOSPHATE).

**CONTAINS MILK AND SOY INGREDIENTS.**

Diluted: Each 5 fl oz (150 mL) contains 100 Calories

| NUTRIENTS: | PER 100 CALORIES: |
|---|---|
| PROTEIN g | 2.2 |
| FAT g | 5.3 |
| CARBOHYDRATE g | 10.6 |
| WATER g | 134 |
| LINOLEIC ACID mg | 750 |
| **VITAMINS:** | |
| VITAMIN A IU | 300 |
| VITAMIN D IU | 60 |
| VITAMIN E IU | 2 |
| VITAMIN K mcg | 8 |
| THIAMINE (VITAMIN $B_1$) mcg | 100 |
| RIBOFLAVIN (VITAMIN $B_2$) mcg | 150 |
| VITAMIN $B_6$ mcg | 63 |
| VITAMIN $B_{12}$ mcg | 0.2 |
| NIACIN mcg | 750 |
| FOLIC ACID (FOLACIN) mcg | 7.5 |
| PANTOTHENIC ACID mcg | 315 |

| VITAMINS: | PER 100 CALORIES: |
|---|---|
| BIOTIN mcg | 2.3 |
| VITAMIN C (ASCORBIC ACID) mg | 9 |
| CHOLINE mg | 15 |
| INOSITOL mg | 4.1 |
| **MINERALS:** | |
| CALCIUM mg | 63 |
| PHOSPHORUS mg | 42 |
| MAGNESIUM mg | 7 |
| IRON mg | 1.8 |
| ZINC mg | 0.75 |
| MANGANESE mcg | 15 |
| COPPER mcg | 71 |
| IODINE mcg | 9 |
| SELENIUM mcg | 2.1 |
| SODIUM mg | 23 |
| POTASSIUM mg | 84 |
| CHLORIDE mg | 56 |

**DISTRIBUTED BY:**
VERMONT ORGANICS INFANT FORMULA
147 IND. PARK RD, GEORGIA, VT 05468

**CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL 02FTP**

* A SOURCE OF ARACHIDONIC ACID (ARA)
** A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)

life's DHA+ARA™

*life'sDHA+ARA™* is a trademark of Martek Biosciences Corporation.

Questions or Comments:
1-800-538-7615
9:00 a.m. to 5:00 p.m. EST
Monday – Friday

Produced under an ISO-9001 Quality System.

All infant formulas sold in the U.S. are required to be manufactured in accordance with, and meet the nutritional requirements of, the Federal Food, Drug and Cosmetic Act for infant formula under the regulation of the U.S. Food and Drug Administration.

6 83744 13001 3

USE BEFORE DATE ON BOTTOM OF CAN

Breast milk is best. But if you decide to supplement breastfeeding with formula or formula-feed exclusively, Vermont Organics™ ORGANIC Infant Formula is a wholesome choice for your baby.

Made in the heart of the Green Mountains, Vermont Organics™ ORGANIC meets all USDA organic certification requirements. In addition to containing sources of organic fat, protein and carbohydrate, Vermont Organics™ ORGANIC is produced without the use of antibiotics, added growth hormones or potentially harmful pesticides.

L199-0730-0351

IMPORTANT NOTICE: BREAST MILK IS BEST FOR BABIES. Before using an infant formula, ask the advice of your health-care professional. This organic soy infant formula powder is free of lactose and cow's milk protein. It is intended to meet the nutritional needs of term infants and babies who are sensitive to lactose or those who do not consume milk protein or milk products. **Powdered infant formulas are NOT sterile and should NOT be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor.**

## DIRECTIONS FOR PREPARATION AND USE

Your baby's health depends on carefully following these preparation, use and storage instructions; changes could affect your baby's nutrition and safety. Before preparing the infant formula, make sure to always wash your hands. Clean can top before opening. **Ask your baby's doctor about the need to boil or sterilize water for formula and the proper preparation of bottle and feeding utensils.**


1. Pour desired amount of warm water (approx. 100°F/40°C) into bottle. (See Feeding Chart below.)
2. Add powder. Always add powder to water.
3. Cap bottle and shake well until powder is dissolved. Feed immediately. Discard any remaining formula in bottle after 1 hour from start of feeding.

Use this Feeding Chart for proper amount of water and powder.

| To Make | Water | Powder (Use scoop enclosed to measure) |
|---|---|---|
| 2 fl oz bottle | 2 fl oz | 1 unpacked level **scoop** (8.8 g) |
| 4 fl oz bottle | 4 fl oz | 2 unpacked level **scoops** (17.6 g) |
| 6 fl oz bottle | 6 fl oz | 3 unpacked level **scoops** (26.4 g) |
| 8 fl oz bottle | 8 fl oz | 4 unpacked level **scoops** (35.2 g) |
| 1 quart | 29 fl oz | 1 unpacked level standard measuring **cup &** 2 unpacked level **scoops** (125 g) |

**Storage:** Store unused prepared bottled formula in the refrigerator at 35-40°F (2-4°C); **use within 24 hours.** DO NOT FREEZE. Warm infant formula to room temperature and shake well before feeding. Prepared formula should not be without refrigeration more than 2 hours. Store open and unopened cans in a dry area at room temperature. Cover the opened can tightly with plastic cap; use contents within 1 month. Avoid any extreme temperatures.

**Warning: Do not use microwave to prepare or warm formula. Serious burns may occur.**

Filled by weight, not by volume. Contents may settle during shipment.

Contents yield approximately 187 fl oz of formula

Martek DHA

Las instrucciones en Español se encuentran al dorso de esta etiqueta.

Easy to Digest
Powder • Add Water

Vermont Organics™

For Babies
0-12 Months

# soy organic infant formula with iron

## Soy-Based Nutrition

Lipids – DHA & ARA

Nutrients found naturally in mother's milk


- Milk-Free
- Lactose-Free
- No Potentially Harmful Pesticides

MADE IN VERMONT

USDA ORGANIC

Ⓤ Pareve

NET WT 25.75 OZ (1 LB 9.75 OZ) 730 g

INGREDIENTS: **ORGANIC** CORN SYRUP SOLIDS, **ORGANIC** SOY PROTEIN, **ORGANIC** PALM OLEIN OR PALM OIL, **ORGANIC** COCONUT OIL, **ORGANIC** HIGH OLEIC (SAFFLOWER OR SUNFLOWER) OIL, **ORGANIC** SOY OIL, **AND LESS THAN 1%:** MORTIERELLA ALPINA OIL*, CRYPTHECODINIUM COHNII OIL**, ASCORBYL PALMITATE, L-CARNITINE, L-METHIONINE, MIXED TOCOPHEROL CONCENTRATE, SOY LECITHIN, TAURINE, CALCIUM CHLORIDE, CALCIUM PHOSPHATE, CUPRIC SULFATE, FERROUS SULFATE, MAGNESIUM CHLORIDE, MANGANESE SULFATE, POTASSIUM BICARBONATE, POTASSIUM CHLORIDE, POTASSIUM CITRATE, POTASSIUM HYDROXIDE, POTASSIUM IODIDE, SODIUM CITRATE, SODIUM SELENITE, ZINC SULFATE, ASCORBIC ACID, BETA-CAROTENE, BIOTIN, CALCIUM PANTOTHENATE, CHOLINE BITARTRATE, CYANOCOBALAMIN, FOLIC ACID, INOSITOL, NIACINAMIDE, PYRIDOXINE HYDROCHLORIDE, RIBOFLAVIN, THIAMINE HYDROCHLORIDE, VITAMIN A PALMITATE, VITAMIN D (CHOLECALCIFEROL), VITAMIN E (dl-ALPHA TOCOPHERYL ACETATE), VITAMIN K (PHYTONADIONE).
**CONTAINS SOY INGREDIENTS.**

Diluted: Each 5 fl oz (150 mL) contains 100 Calories

| NUTRIENTS: | PER 100 CALORIES: |
|---|---|
| PROTEIN g | 2.5 |
| FAT g | 5.3 |
| CARBOHYDRATE g | 10.6 |
| WATER g | 134 |
| LINOLEIC ACID mg | 750 |
| **VITAMINS:** | |
| VITAMIN A IU | 300 |
| VITAMIN D IU | 60 |
| VITAMIN E IU | 2 |
| VITAMIN K mcg | 8 |
| THIAMINE (VITAMIN $B_1$) mcg | 80 |
| RIBOFLAVIN (VITAMIN $B_2$) mcg | 90 |
| VITAMIN $B_6$ mcg | 60 |
| VITAMIN $B_{12}$ mcg | 0.3 |
| NIACIN mcg | 1000 |
| FOLIC ACID (FOLACIN) mcg | 16 |
| PANTOTHENIC ACID mcg | 500 |

| VITAMINS: | PER 100 CALORIES: |
|---|---|
| BIOTIN mcg | 3 |
| VITAMIN C (ASCORBIC ACID) mg | 12 |
| CHOLINE mg | 24 |
| INOSITOL mg | 6 |
| **MINERALS:** | |
| CALCIUM mg | 105 |
| PHOSPHORUS mg | 69 |
| MAGNESIUM mg | 11 |
| IRON mg | 1.8 |
| ZINC mg | 1.2 |
| MANGANESE mcg | 25 |
| COPPER mcg | 75 |
| IODINE mcg | 15 |
| SELENIUM mcg | 2.8 |
| SODIUM mg | 36 |
| POTASSIUM mg | 120 |
| CHLORIDE mg | 80 |

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147 IND. PARK RD, GEORGIA, VT 05468

**CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL 02FTP**

* A SOURCE OF ARACHIDONIC ACID (ARA) ** A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)
Ⓤ PAREVE Contains no dairy ingredients. Manufactured on dairy equipment.

Questions or Comments:
1-800-538-7615
9:00 a.m. to 5:00 p.m. EST
Monday – Friday

Produced under an ISO-9001 Quality System.

All infant formulas sold in the U.S. are required to be manufactured in accordance with, and meet the nutritional requirements of, the Federal Food, Drug and Cosmetic Act for infant formula under the regulation of the U.S. Food and Drug Administration.

6 83744 13002 0

USE BEFORE DATE ON BOTTOM OF CAN

Breast milk is best. But if you decide to supplement breastfeeding with formula or formula-feed exclusively, and lactose-sensitivity is a concern, Vermont Organics™ Soy ORGANIC Infant Formula is a wholesome choice for your baby.

Made in the heart of the Green Mountains, Vermont Organics™ Soy ORGANIC meets all USDA organic certification requirements. A milk-free, lactose-free baby formula containing organic soy protein.

L188-0730-0351

# Safety data sheet
## Lucarotin® 30 M

Revision date : 2006/05/08 Page: 1/6
Version: 1.0 (30041157/MDS_GEN_US/EN)

## 1. Substance/preparation and company identification

Company
BASF CORPORATION
100 Campus Drive
Florham Park, NJ 07932

24 Hour Emergency Response Information
CHEMTREC: 1-800-424-9300
BASF HOTLINE: 1-800-832-HELP

Synonyms: Suspension of beta-Carotene in Corn Oil; 30041157

## 2. Composition/information on ingredients

| CAS Number | Content (W/W) | Chemical name |
|---|---|---|
| 8001-30-7 | 60.0 - 80.0 % | Corn oil |
| 7235-40-7 | 20.0 - 40.0 % | beta-Carotene |
| 10191-41-0 | 0.5 - 1.5 % | D,L-alpha-Tocopherol |

## 3. Hazard identification

**Emergency overview**

CAUTION: MAY CAUSE EYE, SKIN AND RESPIRATORY TRACT IRRITATION.
INGESTION MAY CAUSE GASTRIC DISTURBANCES.
Avoid contact with the skin, eyes and clothing.
Avoid inhalation of mists/vapours.
Use with local exhaust ventilation.
Wear a NIOSH-certified (or equivalent) organic vapour/particulate respirator.
Wear NIOSH-certified chemical goggles.
Wear chemical resistant protective gloves.
Wear protective clothing.
Eye wash fountains and safety showers must be easily accessible.

**Potential health effects**

**Primary routes of exposure**
Routes of entry for solids and liquids include eye and skin contact, ingestion and inhalation. Routes of entry for gases include inhalation and eye contact. Skin contact may be a route of entry for liquified gases.

**Acute toxicity:**
Ingestion may cause gastrointestinal disturbances.
*Information on: beta-Carotene*
*Virtually nontoxic after a single ingestion.*

**Irritation:**
Irritating to respiratory system. Irritating to eyes and skin.

*Information on: beta-Carotene*
*Not irritating to the skin. Not irritating to the eyes.*

## 4. First-aid measures

**General advice:**
Remove contaminated clothing.

**If inhaled:**
Keep patient calm, remove to fresh air.

**If on skin:**
Wash thoroughly with soap and water.

**If in eyes:**
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

If irritation develops, seek immediate medical attention.

**If swallowed:**
Rinse mouth and then drink plenty of water.

Seek medical attention.

## 5. Fire-fighting measures

| | |
|---|---|
| Flash point: | 147 °C |
| Autoignition: | 330 °C |

**Suitable extinguishing media:**
water fog, foam, dry extinguishing media

**Hazards during fire-fighting:**

No particular hazards known.

**Protective equipment for fire-fighting:**
Firefighters should be equipped with self-contained breathing apparatus and turn-out gear.

**Further information:**
Dispose of fire debris and contaminated extinguishing water in accordance with official regulations.

**NFPA Hazard codes:**
Health : 1 Fire: 1 Reactivity: 0 Special:

## 6. Accidental release measures

**Personal precautions:**
No special precautions necessary.

**Environmental precautions:**
Do not discharge into drains/surface waters/groundwater.

# Safety data sheet
## Lucarotin® 30 M



## 7. Handling and storage

### Handling

**General advice:**
Mix thoroughly before use.

**Protection against fire and explosion:**
Risk of self-ignition when a large surface area is produced due to fine dispersion. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

### Storage

**General advice:**
Keep container tightly closed and dry; store in a cool place. Protect from the effects of light. Keep under inert gas.
Store at ambient temperature. Keep container tightly closed. Store in a light-impervious container.

**Temperature tolerance**
Protect from temperatures above: 50 °C
The packed product must be protected against exceeding the indicated temperature.

## 8. Exposure controls and personal protection

**Advice on system design:**
Provide local exhaust ventilation to control vapours/mists.

### Personal protective equipment

**Respiratory protection:**
Wear a NIOSH-certified (or equivalent) organic vapour/particulate respirator.

**Hand protection:**
Wear chemical resistant protective gloves., Consult with glove manufacturer for testing data.

**Eye protection:**
Tightly fitting safety goggles (chemical goggles).

**Body protection:**
Body protection must be chosen based on level of activity and exposure.

**General safety and hygiene measures:**
Handle in accordance with good industrial hygiene and safety practice. Wash soiled clothing immediately.

## 9. Physical and chemical properties

| | | |
|---|---|---|
| Form: | oily, dispersion | |
| Odour: | odourless | |
| Colour: | red | |
| solidification temperature: | -6 °C | |
| Vapour pressure: | < 0.00001 hPa | ( 50 °C) |
| Density: | approx. 0.935 g/cm3 | ( 20 °C) |
| Viscosity, dynamic: | 354 mPa.s | ( 20 °C) |
| Solubility in water: | | sparingly soluble |

## 10. Stability and reactivity

# Safety data sheet
## Lucarotin® 30 M



**Conditions to avoid:**
Avoid heat. Avoid light. Avoid moisture. Avoid excess pressure.

**Substances to avoid:**
No substances known that should be avoided.

**Hazardous reactions:**
No hazardous reactions if stored and handled as prescribed/indicated.

**Decomposition products:**
Hazardous decomposition products: No hazardous decomposition products if stored and handled as prescribed/indicated.

**Thermal decomposition:**
200 - 230 °C

**Corrosion to metals:**
No corrosive effect on metal.

## 11. Toxicological information

**Oral:**

*Information on: beta-Carotene*
*LD50/rat: > 5,000 mg/kg (BASF-Test)*

----------------------------------

**Skin irritation:**

*Information on: beta-Carotene*
*rabbit: non-irritant (BASF-Test)*

----------------------------------

**Eye irritation :**

*Information on: beta-Carotene*
*rabbit: non-irritant (OECD Guideline 405)*

----------------------------------
**Genetic toxicity:**
*Information on: beta-Carotene*
*Results from a number of mutagenicity studies with microorganisms, mammalian cell culture and mammals are available. Taking into account all of the information, there is no indication that the substance is mutagenic.*
----------------------------------

**Reproductive toxicity:**
*Information on: beta-Carotene*
*The results of animal studies gave no indication of a fertility impairing effect.*
----------------------------------

**Developmental toxicity/teratogenicity:**
*Information on: beta-Carotene*
*No indications of a developmental toxic / teratogenic effect were seen in animal studies.*
----------------------------------

## 12. Ecological information

*Information on: beta-Carotene*

*No data available concerning bioaccumulation.*

*Information on: beta-Carotene*
*Acute and prolonged toxicity to fish:*
*DIN 38412 Part 15 static*
*golden orfe/LC50 (96 h): > 10,000 mg/l*
*Tested above maximum solubility. The details of the toxic effect relate to the nominal concentration.*
----------------------------------

*Information on: beta-Carotene*
*Toxicity to microorganisms:*
*DIN 38412 Part 27 (draft) static*
*bacterium/EC50 (0.5 h): > 10,000 mg/l*
*The product has low solubility in the test medium. An aqueous solution prepared with solubilizers has been tested. The details of the toxic effect relate to the nominal concentration.*
----------------------------------

## 13. Disposal considerations

**Waste disposal of substance:**
Dispose of in accordance with national, state and local regulations.

**Container disposal:**
Dispose of in a licensed facility. Recommend crushing, puncturing or other means to prevent unauthorized use of used containers.

## 14. Transport information

**Land transport**
*USDOT*

Not classified as a dangerous good under transport regulations

**Sea transport**
*IMDG*

Not classified as a dangerous good under transport regulations

**Air transport**
*IATA/ICAO*

Not classified as a dangerous good under transport regulations

## 15. Regulatory information

**Federal Regulations**

**Registration status:**

TSCA, US released / listed

**OSHA hazard category:** Not hazardous

**SARA hazard categories (EPCRA 311/312):** Not hazardous

**State regulations**

**State RTK**

| CAS Number | Chemical name | State RTK |
|---|---|---|
| 8001-30-7 | Corn oil | PA |

## 16. Other information

**HMIS III rating**
Health: 1 Flammability: 1 Physical hazard: 0

HMIS uses a numbering scale ranging from 0 to 4 to indicate the degree of hazard. A value of zero means that the substance possesses essentially no hazard; a rating of four indicates high hazard.

**Local contact information**
prod_reg@basf.com

Lucarotin is a registered trademark of BASF Corporation or BASF AG
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END OF DATA SHEET

# Lucarotin® Dispersions

BASF
The Chemical Company

## Chemical names of active ingredients
Beta-carotene, provitamin A

| | |
|---|---|
| **CAS-No.** | 7235-40-7 |
| **EINECS-No.** | 230-636-6 |

| PRD-Nos. | Articles | |
|---|---|---|
| 30041157* | Lucarotin 30 M | |
| | 50082735 | 4 x 5 kg aluminium bottle |
| 30085626* | Lucarotin 30 SUN | |
| | 51988215 | 4 x 5 kg aluminium bottle |

* The product is kosher.

## Country of origin
Germany

## Description
Brick-red, oily dispersions with a neutral flavor containing beta-carotene in microcrystalline form in vegetable oils.

## Composition

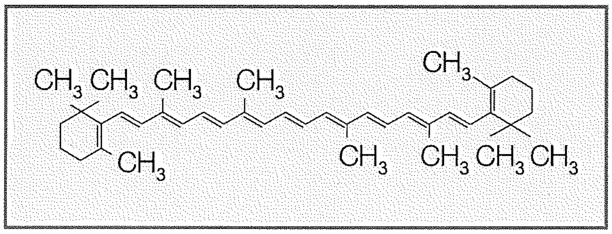
| | |
|---|---|
| Lucarotin 30 M | corn oil |
| Lucarotin 30 SUN | sunflower oil |

## Specifications

| | | |
|---|---|---|
| Assay | Lucarotin 30 M | min. 30% |
| | Lucarotin 30 SUN | min. 30% |

Further information see separate document: "Standard Specification (not for regulatory purposes)" available via BASF's WorldAccount: https://worldaccount.basf.com (registered access).

## Monographs
The included active ingredient complies with the current "Betacarotene" Ph. Eur. and "ß-Carotene" FCC monograph as well as the purity requirements of Directive 2008/128/EC (E 160 a).




$C_{40}H_{56}$ **Molar mass 536.9 g/mol**

## Regulations
Beta-carotene is approved for use as a food colorant and as provitamin A source in most countries. However, specific regulations on the ingredients used in the respective countries and for the intended use have to be observed.

## Stability
The Lucarotin dispersions do not contain a stabilizer. Stored in the unopened original packaging at room temperature (max. 25 °C), the products are stable for at least 36 months. As beta-carotene may sink to the bottom of the container, the dispersions should always be stirred prior to use.

## Storage/Handling
The products are sensitive to atmospheric oxygen, light, heat and moisture. Lucarotin dispersions should therefore be stored under nitrogen in the tightly sealed, lightproof packaging in a cool place. Once opened, it is recommended to use the remaining contents as quickly as possible.

## Applications
*Dietary supplements:*
The Lucarotin dispersions are used in soft capsules as provitamin A, as an active ingredient and as a colorant.

*Food products:*
Used as both yellow-orange colorant and provitamin A. Even at low concentrations, beta-carotene dispersions have a high tinctorial strength. They are suitable for coloring as well as for standardizing the color of oils, fats, margarine, butter, processed

cheese, cheese spreads, milk replacement products, ice cream, soups, sauces, fillings of baked goods and egg products. They are added to the oily phase.

**Important: Beta-carotene dispersions should be stirred briefly prior to use.**

The Lucarotin dispersions are usually processed as stock solution in a suitable quantity of oil, prepared by careful heating to 40 °C. This stock solution is then added to the food product.

The table below provides approximate of 100% beta-carotene, which are added to 1 kg of various food products. The quantity is dependent on the desired shade and should be determined in small scale tests.

| | |
|---|---|
| Butter[1] | 14 – 16 mg/kg |
| Cream fillings[1] | 1 – 10 mg/kg |
| Egg products[1] | 2 – 5 mg/kg |
| Fats, oils | 7 – 10 mg/kg |
| Replacement products based on vegetables oils[1] | 2 – 5 mg/kg |
| Cheese preparations[1] | 1 – 2 mg/kg |
| Margarine | 6 – 12 mg/kg |
| Salad dressings[1] | 3 – 9 mg/kg |
| Processed cheese[1] | 10 – 25 mg/kg |
| Sauces[1] | 4 – 20 mg/kg |
| Ice cream[1] | 2 – 6 mg/kg |
| Soups[1] | 0.2 – 1 mg/kg |

[1] The Lucarotin dry powders can also be used to color these food products.

- **Butter:**
  the stock solution is heated to 45 °C and added to the cream
- **Pasta products containing egg:**
  a stock solution containing about 0.5% beta-carotene in oil is evenly mixed with a defined quantity of flour; the colored premix is added to the flour prior to production
- **Imitation cheese:**
  the beta-carotene stock solution in vegetable oil is heated to 50 – 60 °C and added during production
- **Margarine:**
  the beta-carotene dispersion is completely dissolved in the oily phase prior to emulsification
- **Salad dressings:**
  the vegetable oil is heated to 45 – 50 °C before adding the beta-carotene dispersion
- **Processed cheese:**
  a beta-carotene stock solution is prepared in melted butter and added to the cheese mixture prior to the melting process
- **Ice cream:**
  the required quantity of beta-carotene is stirred into fat or oil until it is completely dissolved; the temperature of the oil should be at least 20 °C, preferably 37 – 50 °C
- **Soups:**
  the oil for the soup is heated and the beta-carotene dispersion dissolved in it

**Note**
The Lucarotin Dispersions must be handled in accordance with the Safety Data Sheet.



November 2009

# CERTIFICATE

Management Systems Solutions

## DQS GmbH

**Deutsche Gesellschaft zur Zertifizierung von Managementsystemen**

hereby certifies that the company

## BASF SE
### Operating Division Care Chemicals

67056 Ludwigshafen
Germany

has implemented and maintains an **Environmental Management System**.

Scope:
Development, manufacturing and marketing of ingredients and additives for food and feed, cosmetic ingredients, aroma chemicals, superabsorbents and performance chemicals for detergents and formulators

Through an audit, documented in a report, it was verified that the management system fulfills the requirements of the following standard:

## ISO 14001 : 2004

| | |
|---|---|
| Certificate registration no. | 467055 UM |
| Excerpt from Certificate Registration No. | 019089 UM |
| Date of certification | 2009-12-02 |
| Valid until | 2012-12-01 |

TGA-ZM-02-90

Michael Drechsel
Managing Director

Jan Böge
Managing Director

August-Schanz-Straße 21, 60433 Frankfurt am Main



Member of IQNet

# Annex to Certificate
# Registration No. 467055 UM

## BASF SE
### Operating Division Care Chemicals

67056 Ludwigshafen
Germany

| Location | Scope |
|---|---|
| 092373<br>**BASF SE**<br>**Global Business Unit**<br>**Global Hygiene, Home & Personal Care Businesses**<br>67056 Ludwigshafen<br>Germany | Development, manufacturing and marketing of superabsorbents |
| 002854<br>**BASF SE**<br>**Global Business Unit**<br>**Nutrition Ingredients**<br>67056 Ludwigshafen<br>Germany | Development, manufacturing and marketing of ingredients and additives for food and feed |
| 467339<br>**BASF SE**<br>**Global Business Unit**<br>**Citral & Aroma Chemicals**<br>67056 Ludwigshafen<br>Germany | Manufacturing and marketing of Aroma Chemicals |
| 467054<br>**BASF SE**<br>**Regional Business Unit**<br>**Care Chemicals and Formulators Europe**<br>67056 Ludwigshafen<br>Germany | Development, manufacturing and marketing of cosmetic ingredients and performance chemicals for detergents and formulators |

This annex (edition: 2009-12-02) is only valid in connection with the above-mentioned certificate.

determine the absorbance values of the *Sample solutions* at the potassium emission line at 766.7 nm. Plot the absorbance values of the *Sample solutions* versus their contents of potassium, in µg/mL; draw the straight line best fitting the three points and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in µg, of potassium in each mL of *Sample solution A.* Calculate the percent potassium in the portion of *Sample* taken by multiplying the concentration, in µg/mL, of potassium found in *Sample solution A* by 0.2.

**Acceptance criteria:** NMT 0.2%

- **SODIUM**

[NOTE—The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the spectrophotometer.]

**Standard stock solution:** 10.0 mg/mL sodium, made by transferring 6.355 g of sodium chloride, previously dried at 105° for 2 h, into a 250-mL volumetric flask, dilute to volume with water, and mix.

**Standard solution:** 250 µg/mL sodium: from *Standard stock solution*

**Sample:** 4 g

**Sample stock solution:** Transfer the *Sample* into a 100-mL volumetric flask, dissolve in and dilute to volume with water, and mix.

**Sample solutions:** Add 0, 2.0, and 4.0 mL of the *Standard solution* to three separate 25-mL volumetric flasks. Add 20.0 mL of the *Sample stock solution* to each flask, dilute to volume with water, and mix. These solutions contain 0 (*Sample solution A*), 20.0 (*Sample solution B*), and 40.0 (*Sample solution C*) µg/mL of sodium.

**Analysis:** Using a suitable atomic absorption spectrophotometer equipped with an air-acetylene flame and using water as the blank, concomitantly determine the absorbance values of the *Sample solutions* at the sodium emission line at 589.0 nm. Plot the absorbance values of the *Sample solutions* versus their contents of sodium, in µg/mL; draw the straight line best fitting the three points and extrapolate the line until it intersects with the concentration axis. From the intercept, determine the amount, in µg, of sodium in each mL of *Sample solution A.* Calculate the percent sodium in the portion of *Sample* taken by multiplying the concentration, in µg/mL, of sodium found in *Sample solution A* by 0.003125.

**Acceptance criteria:** NMT 0.1%

## SPECIFIC TESTS

- **OPTICAL (SPECIFIC) ROTATION,** Appendix IIB

**Sample solution:** 100 mg/mL (using a previously dried sample)

**Acceptance criteria:** $[\alpha]_D^{20}$ between −29.0° and −32.0°, calculated on the anhydrous basis

- **PH,** *pH Determination,* Appendix IIB

**Sample solution:** 50 mg/mL

**Acceptance criteria:** Between 5.5 and 9.5

- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC

**Sample:** 2 g

**Acceptance criteria:** NMT 0.5%

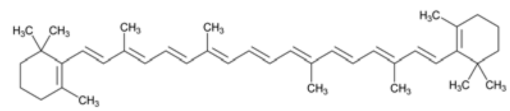
- **WATER,** *Water Determination,* Appendix IIB

**Acceptance criteria:** NMT 4.0%

# β-Carotene

**First Published:** Prior to FCC 6

Carotene




$C_{40}H_{56}$ Formula wt 536.88

INS: 160a(ii) CAS: [7235-40-7]

## DESCRIPTION

β-Carotene occurs as red crystals or as crystalline powder. It is insoluble in water and in acids and alkalies, but is soluble in carbon disulfide and in chloroform. It is sparingly soluble in ether, in solvent hexane, and in vegetable oils, and is practically insoluble in methanol and in ethanol. It melts between 176° and 182°, with decomposition.

**Function:** Nutrient; color

**Packaging and Storage:** Store in a cool place in tight, light-resistant containers under inert gas.

[NOTE—Carry out all work in low-actinic glassware and in subdued light]

## IDENTIFICATION

- **VISIBLE ABSORPTION SPECTRUM**

**Sample solution:** Use *Sample solution B* prepared for the *Assay* (below).

**Analysis:** Using a suitable spectrophotometer, determine the absorbance of *Sample solution B* at 455 nm and at 483 nm.

**Acceptance criteria:** The ratio of absorbance values obtained, $A_{455}/A_{483}$, is between 1.14 and 1.18.

- **VISIBLE ABSORPTION SPECTRUM**

**Sample solutions:** Use *Sample solution A* and *Sample solution B* prepared for the *Assay* (below).

**Analysis:** Using a suitable spectrophotometer, determine the absorbance of *Sample solution B* at 455 nm and that of *Sample solution A* at 340 nm.

**Acceptance criteria:** The ratio of absorbance values obtained, $A_{455}/A_{340}$, is NLT 1.5.

## ASSAY

- **PROCEDURE**

**Sample stock solution:** Transfer 50 mg of sample into a 100-mL volumetric flask, dissolve it in 10 mL of acid-free chloroform, immediately dilute to volume with cyclohexane, and mix.

**Sample solution A:** 5 mL of *Sample stock solution* diluted to 100 mL with cyclohexane
**Sample solution B:** 5 mL of *Sample solution A* diluted to 50 ml with cyclohexane
**Analysis:** Determine the absorbance of *Sample solution B* using a suitable atomic absorption spectrophotometer with a 1-cm cell, set to the wavelength of maximum absorption at about 455 nm, using cyclohexane as the blank. Calculate the quantity, in mg, of $C_{40}H_{56}$ in the sample taken by the formula:

$$\text{Result} = 20{,}000\ A/250$$

A = absorbance of the solution
250 = absorptivity of pure $\beta$-carotene

**Acceptance criteria:** NLT 96.0% and NMT 101.0% of $C_{40}H_{56}$, calculated on the dried basis

## IMPURITIES

### Inorganic Impurities

- **Lead,** *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  **Sample:** 5 g
  **Acceptance criteria:** NMT 5 mg/kg

## SPECIFIC TESTS

- **Loss on Drying,** Appendix IIC**:** In a vacuum over phosphorus pentoxide at 40° for 4 h
  **Acceptance criteria:** NMT 0.2%
- **Residue on Ignition (Sulfated Ash),** Appendix IIC
  **Sample:** 2 g
  **Acceptance criteria:** NMT 0.2%

# Carrageenan

**First Published:** First Supplement, FCC 6

Irish moss (from *Chondrus spp.*)
Eucheuman (from *Eucheuma spp.*)
Iridophycan (from *Iridaea spp.*)
Hypnean (from *Hypnea spp.*)
Processed Eucheuma Seaweed, PES, PNG-carrageenan, and Semi-refined carrageenan (from *E. spinosum* or *E. cottonii*)

INS: 407 CAS: [9000-07-1]

## DESCRIPTION

Carrageenan occurs as a yellow or tan to white, coarse to fine powder. It is obtained from certain members of the class Rhodophyceae (red seaweeds). The principal commercial sources of carrageenans are the following families and genera of the class Rhodophyceae[1] :

Furcellariaceae such as *Furcellaria;*
Gigartinaceae such as *Chondrus, Gigartina, Iridaea;*
Hypnaeceae such as *Hypnea;*
Phyllophoraceae such as *Phyllophora, Gynmogongrus, Ahnfeltia;*
Solieriaceae such as *Eucheuma, Anatheca, Meristotheca.*

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium, and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked $\alpha$-(1→3) and $\beta$-(1→4) in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as *kappa-, iota-,* and *lambda*-carrageenan. *Kappa*-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; *iota*-carrageenan is similar except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between *kappa*-carrageenan and *iota*-carrageenan, there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In *lambda*-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1→3-linked) and D-galactose-2,6-disulfate (1→4-linked).

Carrageenan may be obtained from any of the cited seaweeds by extraction into water or aqueous dilute alkali. It may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. Additionally, carrageenan may be obtained by extracting the cleaned seaweed with alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying and milling to a powder. Carrageenan obtained by this method contains a higher percentage of algal cellulose. The alcohols used during recovery and purification of carrageenan are restricted to methanol, ethanol, and isopropanol.

Carrageenan is insoluble in ethanol but it is soluble in water at 80°, forming a viscous clear or cloudy and slightly opalescent solution that flows readily. Some samples form a cloudy viscous suspension in water. Carrageenan disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum-drying processes.

**Function:** Thickener, gelling agent, stabilizer, emulsifier
**Packaging and Storage:** Store in well-closed containers.
[Note—Carrageenan must be well dispersed in water in many of the following tests so dispersion technique must be kept in mind throughout this monograph. Carrageenan is best dispersed by slowly sprinkling the powder into cold water with continuous vigorous stirring. This allows the carrageenan particles to wet and hydrate effectively prior to dissolving. Adding carrageenan directly to hot water, or too rapidly to cold water, or not stirring vigorously will cause the carrageenan particles to form lumps which are very difficult to break down and solubilize. If appropriate, carrageenan disperses more readily in cold water if first

[1]In the United States, only the following seaweed species from the families Gigartinaceae and Solieriaceae are authorized as sources of carrageenan intended for use in foods (Title 21 US Code of Federal Regulations Part 172, section 620 (21 CFR 172.620)): *Chondrus crispus, C. ocellatus, Eucheuma cottonii, E. spinosum, Gigartina acicularis, G. pistillata, G. radula,* and *G. stellata.*

Monographs

**BETA-CAROTENE**
CASRN: 7235-40-7

## Human Health Effects:

**Human Toxicity Excerpts:**

/SIGNS AND SYMPTOMS/ Two large studies have found an increased incidence in lung cancers when beta-carotene supplements were given to individuals with a history of smoking and/or asbestos exposure. One study of 29,000 males with a history of smoking found an 18% increase in the incidence of lung cancer in the group receiving 20 mg of beta-carotene a day for 5 to 8 years as compared with those receiving placebo . Another study of 18,000 individuals found 28% more lung cancers in individuals with a history of smoking and/or asbestos exposure who took 30 mg of beta-carotene in addition to 25,000 Units of retinol a day for 4 years as compared with those receiving placebo . However, one study of 22,000 male physicians, some of them smokers and former smokers, found no increased risk of lung cancer at doses of 50 mg of beta-carotene every other day for 12 years .
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

/EPIDEMIOLOGY STUDIES/ Epidemiological studies have suggested a protective effect of vegetables and fruits on urinary tract cancer but the possible protective nutrients are unknown. We studied the effect of alpha-tocopherol (a form of vitamin E) and beta-carotene supplementation on urinary tract cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. A total of 29,133 male smokers aged 50-69 years from southwestern Finland were randomly assigned to receive alpha-tocopherol (50 mg), beta-carotene (20 mg), both agents, or a placebo daily for 5-8 years (median 6.1 years). Incident urothelial cancers (bladder, ureter, and renal pelvis; n = 169) and renal cell cancers (n = 102) were identified through the nationwide cancer registry. The diagnoses were centrally confirmed by review of medical records and pathology specimens. The supplementation effects were estimated using a proportional hazards model. Neither alpha-tocopherol nor beta-carotene affected the incidence of urothelial cancer, relative risk 1.1 (95% confidence interval (CI) 0.8-1.5) and 1.0 (95% CI 0.7-1.3), respectively, or the incidence of renal cell cancer, relative risk 1.1 (95% CI 0.7-1.6) and 0.8 95% CI 0.6-1.3), respectively. Long-term supplementation with alpha-tocopherol and beta-carotene has no preventive effect on urinary tract cancers in middle-aged male smokers.
[Virtamo J et la; Cancer Causes Control 11 (10): 933-9 (2000)] **PEER REVIEWED** PubMed Abstract

/EPIDEMIOLOGY STUDIES/ The Beta-Carotene and Retinol Efficacy Trial (CARET) was terminated 21 months ahead of schedule due to an excess of lung cancers. Deaths from cardiovascular disease also increased (relative risk=1.26 (95% confidence interval (CI) 0.99-1.61)) in the group assigned to a combination of 30 mg beta-carotene and 25 000 IU retinyl palmitate (vitamin A) daily. The basis for increased cardiovascular mortality is unexplained. Data on serum lipids, available for 1474 CARET Vanguard participants who were enrolled in the two CARET pilot studies and transitioned to the Vanguard study /were analyzed/. Total cholesterol and triglycerides were measured 2 months prior to, 4 and 12 months following randomization, and annually thereafter for up to 7 y. In the asbestos-exposed pilot (N = 816), participants were assigned to beta-carotene and retinol or to placebo; in the smokers pilot (N = 1029), participants were assigned to beta-carotene, retinol, a combination, or placebo. Serum cholesterol showed a decline over time in both arms; serum triglycerides had a continuous decline over time in the placebo arm, but an initial increase that persisted in the active arm. Both serum cholesterol concentrations (P < 0.0003) and serum triglycerides (P < 0.0001) were significantly higher in the participants receiving vitamin A and/or a combination of vitamin A and beta-carotene (n = 863) as compared to the placebo group (n = 611). Those in this active intervention group had an average cholesterol concentration 5.3 mg/dl (0.137 mmol/l) higher than those in the placebo arm. /It was concluded/ the differences in cholesterol and triglyceride concentrations between the groups following randomization may account in part for the unexpected excess in cardiovascular deaths seen in the active intervention arm of CARET.
[Cartmel B et al; Eur J Clin Nutr 59 (10): 1173-80 (2005)] **PEER REVIEWED**
PubMed Abstract

/EPIDEMIOLOGY STUDIES/ The Physicians' Health Study (PHS) was a randomized trial of beta-carotene (50 mg, alternate days) and aspirin in primary prevention of cancer and cardiovascular disease among 22,071 US male physicians. This report updates results for beta-carotene and examines effect modification by baseline characteristics. Beta-carotene's effect on cancer over nearly 13 years was examined overall and within subgroups defined by baseline characteristics using proportional-hazards models. 2667 incident cancers were confirmed, with 1117 prostate, 267 colon, and 178 lung cancers. There were no significant differences with supplementation in total (relative risk (RR) = 1.0, 95% confidence interval (CI) = 0.9-1.0); prostate (RR = 1.0, 95% CI = 0.9-1.1); colon (RR = 0.9, 95% CI = 0.7-1.2); or lung (RR = 0.9, 95% CI = 0.7-1.2) cancer, and no differences over time. In subgroup analyses, total cancer was modestly reduced with supplementation among those aged 70+ years (RR = 0.8, 95% CI = 0.7-1.0), daily drinkers of alcohol (RR = 0.9, 95% CI = 0.8-1.0), and those in the highest BMI quartile (RR = 0.9, 95% CI = 0.7-1.0). Prostate cancer was reduced with supplementation among those in the highest BMI quartile (RR = 0.8, 95% CI = 0.6-1.0), and colon cancer was reduced among daily drinkers of alcohol (RR = 0.5, 95% CI = 0.3-0.8). The PHS found no overall effect of beta-carotene on total cancer, or the three most common site-specific cancers. The possibility of risk reduction within specific subgroups remains.
[Cook NR et al; Cancer Causes Control 11 (7): 617-26 (2000)] **PEER REVIEWED** PubMed Abstract

/EPIDEMIOLOGY STUDIES/ Antioxidants may retard atherogenesis and limit inflammatory processes involved in aneurysm formation. We evaluated effects of alpha-tocopherol and beta-carotene supplementation on incidence of large abdominal aortic aneurysm (AAA) in a

randomised, double-blind, placebo-controlled trial. Subjects (n=29133) were 50-69-years-old male smokers, participants in the Finnish alpha-Tocopherol, beta-Carotene Cancer Prevention (ATBC) Study. They were randomised to receive either 50 mg/day of alpha-tocopherol, or 20 mg/day of beta-carotene, or both, or placebo in a 2x2 design. Incidence of AAA was evaluated from mortality and hospital registers. During 5.8 years of follow-up, 181 men were diagnosed with either ruptured AAA (n=77) or nonruptured large AAA treated with aneurysmectomy (n=104). Relative risk (RR) for AAA was 0.83 (95% confidence interval [CI] 0.62-1.11) among men receiving alpha-tocopherol compared with those who did not, and 0.93 (95% CI 0.69-1.24) among men receiving beta-carotene compared with those who did not. A modest though nonsignificant decrease in risk for nonruptured AAA was observed among alpha-tocopherol supplemented men (RR 0.71, 95% CI 0.48-1.04) compared with men not receiving alpha-tocopherol. For beta-carotene, RR for nonruptured AAA was 0.86 (95% CI 0.59-1.27) compared with men not receiving beta-carotene. Neither antioxidant affected risk for ruptured AAA. In conclusion, long-term supplementation with alpha-tocopherol or beta-carotene had no preventive effect on large AAA among male smokers.
[Tornwall ME et al; Atherosclerosis 157 (1): 167-73 (2001)] **PEER REVIEWED**
PubMed Abstract

/EPIDEMIOLOGY STUDIES/ In the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, alpha-tocopherol supplementation decreased prostate cancer incidence, whereas beta-carotene increased the risk of lung cancer and total mortality. Postintervention follow-up provides information regarding duration of the intervention effects and may reveal potential late effects of these antioxidants. Postintervention follow-up assessment of cancer incidence and cause-specific mortality (6 years (May 1, 1993-April 30, 1999)) and total mortality (8 years (May 1, 1993-April 30, 2001)) of 25 563 men. In the ATBC Study, 29 133 male smokers aged 50 to 69 years received alpha-tocopherol (50 mg), beta-carotene (20 mg), both agents, or placebo daily for 5 to 8 years. End point information was obtained from the Finnish Cancer Registry and the Register of Causes of Death. Cancer cases were confirmed through medical record review.Site-specific cancer incidence and total and cause-specific mortality and calendar time-specific risk for lung cancer incidence and total mortality. Overall posttrial relative risk (RR) for lung cancer incidence (n = 1037) was 1.06 (95% confidence interval (CI), 0.94-1.20) among recipients of beta-carotene compared with nonrecipients. For prostate cancer incidence (n = 672), the RR was 0.88 (95% CI, 0.76-1.03) for participants receiving alpha-tocopherol compared with nonrecipients. No late preventive effects on other cancers were observed for either supplement. There were 7261 individuals who died by April 30, 2001, during the posttrial follow-up period; the RR was 1.01 (95% CI, 0.96-1.05) for alpha-tocopherol recipients vs nonrecipients and 1.07 (95% CI, 1.02-1.12) for beta-carotene recipients vs nonrecipients. Regarding duration of intervention effects and potential late effects, the excess risk for beta-carotene recipients was no longer evident 4 to 6 years after ending the intervention and was primarily due to cardiovascular diseases. The beneficial and adverse effects of supplemental alpha-tocopherol and beta-carotene disappeared during postintervention follow-up. The preventive effects of alpha-tocopherol on prostate cancer require confirmation in other trials. Smokers should avoid beta-carotene supplementation.
[Virtamo J et al; JAMA 290 (4): 476-85 (2003)] **PEER REVIEWED** PubMed Abstract

/EPIDEMIOLOGY STUDIES/ This study investigated the effects of alpha-tocopherol and beta-carotene supplementation on the incidence of gastric cancer. A total of 29,133 male smokers, aged 50-69 years, participated in a placebo-controlled prevention trial, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in southwestern Finland between 1985 and 1993. The men were randomly assigned to receive alpha-tocopherol (50 mg/day) or beta-carotene (20 mg/day) supplementation in a 2 x 2 factorial design. We identified 126 gastric cancer cases during the median follow-up of six years. Of these, 122 were adenocarcinomas: 75 of intestinal type, 30 of diffuse type, and 17 of mixed type. There was no significant effect for either supplementation on the overall incidence of gastric cancer: relative risk (RR) 1.21, 95% confidence interval (CI) 0.85-1.74 for alpha-tocopherol, and RR 1.26, 95% Cl 0.88-1.80 for beta-carotene. Subgroup analyses by histologic type suggested an increased risk for beta-carotene on intestinal type cancers, RR 1.59, 95% CI 0.99-2.56. There were no differences across anatomic locations (cardia/noncardia) in the effects of alpha-tocopherol or beta-carotene supplementation. Our study found no overall preventive effect of long-term supplementation with alpha-tocopherol or beta-carotene on gastric cancer in middle-aged male smokers.
[Malila N et al; Cancer Causes Control 13 (7): 617-23 (2002)] **PEER REVIEWED** PubMed Abstract

/EPIDEMIOLOGY STUDIES/ The Beta-Carotene and Retinol Efficacy Trial (CARET) tested the effect of daily beta-carotene (30 mg) and retinyl palmitate (25,000 IU) on the incidence of lung cancer, other cancers, and death in 18,314 participants who were at high risk for lung cancer because of a history of smoking or asbestos exposure. CARET was stopped ahead of schedule in January 1996 because participants who were randomly assigned to receive the active intervention were found to have a 28% increase in incidence of lung cancer, a 17% increase in incidence of death and a higher rate of cardiovascular disease mortality compared with participants in the placebo group. After the intervention ended, CARET participants returned the study vitamins to their study center and provided a final blood sample. They continue to be followed annually by telephone and mail self-report. Self-reported cancer endpoints were confirmed by review of pathology reports, and death endpoints were confirmed by review of death certificates. All statistical tests were two-sided. With follow-up through December 31, 2001, the post-intervention relative risks of lung cancer and all-cause mortality for the active intervention group compared with the placebo group were 1.12 (95% confidence interval [CI] = 0.97 to 1.31) and 1.08 (95% CI = 0.99 to 1.17), respectively. Smoothed relative risk curves for lung cancer incidence and all-cause mortality indicated that relative risks remained above 1.0 throughout the post-intervention follow-up. By contrast, the relative risk of cardiovascular disease mortality decreased rapidly to 1.0 after the intervention was stopped. During the post-intervention phase, females had larger relative risks of lung cancer mortality (1.33 versus 1.14; P = .36), cardiovascular disease mortality (1.44 versus 0.93; P = .03), and all-cause mortality (1.37 versus 0.98; P = .001) than males. The previously reported adverse effects of beta-carotene and retinyl palmitate on lung cancer incidence and all-cause mortality in cigarette smokers and individuals with occupational exposure to asbestos persisted after drug administration was stopped although they are no longer statistically significant. Planned subgroup analyses suggest that the excess risks of lung cancer were restricted primarily to females, and cardiovascular disease mortality primarily to females and to former smokers.
[Goodman GE et al; J Natl Cancer Inst 96 (23): 1743-50 (2004)] **PEER REVIEWED** PubMed Abstract

/EPIDEMIOLOGY STUDIES/ To study the association between dietary and serum antioxidant vitamins and carotenoids and risk for colorectal cancer in male smokers. A prospective cohort study within a randomised, double-blind, placebo-controlled trial testing supplementation with alpha-tocopherol (50 mg/day), beta-carotene (20 mg/day) or both in preventing cancer. Participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study with complete dietary data and serum samples available from baseline. These included 26,951 middle-aged male smokers among whom 184 colorectal cancer cases were diagnosed during 8 y of follow-up. Relative risks were calculated with Cox proportional hazards models adjusting for trial supplementation, age, body mass index, serum cholesterol, cigarettes smoked per day and physical activity. There was no significant association between dietary vitamin C or E, alpha-or gamma-tocopherol, retinol, alpha- or beta-carotene, lycopene or lutein+zeaxanthin and risk for colorectal cancer. Serum alpha-tocopherol, beta-carotene or retinol was also not associated with the risk, neither did the season when baseline blood was drawn modify the relationship between serum beta-carotene and colorectal cancer risk. Our data support the results from previous studies in which no association between dietary antioxidant vitamins and carotenoids and risk for colorectal cancer has been observed. Likewise, no association between baseline serum antioxidant concentrations and colorectal cancer risk was evident.
[Malila N et al; Eur J Clin Nutr 56 (7): 615-21(2002)] **PEER REVIEWED**
PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ Because COX-2 has been implicated as a causative factor in colon carcinogenesis, the present study was designed to investigate the relation between the growth-inhibitory effect of the carotenoid and COX-2 expression in colon cancer cells. The effects of beta-carotene on the growth of human colon adenocarcinoma cells overexpressing (LS-174, HT-29, WiDr) or not expressing (HCT116) COX-2 /were studied/. COX-2 expression induced by heregulin-alpha, apoptosis induction, reactive oxygen species (ROS) production, and extracellular signal-regulated kinase 1/2 (ERK1/2) activation /was also studied/. beta-Carotene (0.5-2.0 micromol/L) decreased COX-2 expression ($P < 0.05$) and prostaglandin E(2) (PGE(2)) production ($P < 0.05$) in colon cancer cells. This effect was not observed in cells treated with retinoic acid or retinol. The downregulation of COX-2 by the carotenoid occurred in both untreated and heregulin-treated cells. It was accompanied by an increased ability of cells to undergo apoptosis and by a decrease in intracellular ROS production and in the activation of ERK1/2. Moreover, cells not expressing COX-2 were insensitive to the growth-inhibitory and proapoptotic effects of the carotenoid. Here, we report that the suppression of COX-2 by beta-carotene may represent a molecular mechanism by which this compound acts as an antitumor agent in colon carcinogenesis.
[Palozza P et al; J Nutr 135 (1): 129-36 (2005)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ It was shown that high doses of beta-carotene (>30 uM) decrease proliferation of prostate cancer cells in vitro. However, it is rather doubtful whether such concentration of beta-carotene is really accessible at cellular level. The effect of 3 and 10 uM beta-carotene on proliferation and gene expression in LNCaP and PC-3 prostate cancer cell lines /was studied/. Beta-carotene-more efficiently absorbed from medium by androgen-sensitive LNCaP cells - increased proliferation of LNCaP cells whereas it had weaker effect on PC-3 cells. Initial global analysis of expression of genes in both cell lines treated with

10 uM beta-carotene (Affymetrix HG-U133A) showed remarkable differences in number of responsive genes. Their recognition allows for conclusion that differences between prostate cancer cell lines in response to beta-carotene treatment are due to various androgen sensitivities of LNCaP and PC-3 cells. Detailed analysis of expression of selected genes in beta-carotene treated LNCaP cells at the level of mRNA and protein indicated that the observed increase of proliferation could have been the result of slight induction of a few genes affecting proliferation (c-myc, c-jun) and apoptosis (bcl-2) with no significant effect on major cell cycle control genes (cdk2, RB, E2F-1).
[Dulinska J et al; Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1740 (2): 189-201 (2005)] **PEER REVIEWED**

/ALTERNATIVE and IN VITRO TESTS/ This study was conducted to investigate the altered gene expression of MCF-7 cell before and after the treatment with beta-carotene using cDNA microarray and to investigate the mechanism which beta-carotene induce breast cancer cell apoptosis. Two fluorescence cDNA probes were made using reverse transcriptional reaction from mRNA of beta-carotene untreated or treated MCF-7 cells (human estrogen receptor positive breast cancer cells), marked with two different fluorescence dyes (cy3 and cy5) respectively, hybridized with expressed cDNA microarray scanned and analyzed by computer system and finally the expressed gene was produced. A total of 21 genes related to cell apoptosis, cell signal transduction, protein translation and immunity were expressed differently after the treatment of beta-carotene, which 3/21 were up-regulated (AF040958, AK001555,g41894),18/21 were down-regulated(hshsp90r,U83857,AB014509,AF126028,AF053641,AF117386,AF050127,NM_012177,humtopi,AJ250915,U37547,U78798,NM_004849,NM_005346,AF004711,NM_006595,NM_001418,AB015051). /It was concluded that/ beta-carotene may inhibit the growth of breast cancer cells through inducing apoptosis,breaking signal transduction,and blocking protein translation.
[Li Z et al; Ai Zheng 22 (4): 380-4 (2003)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ The inhibitory effect of beta-carotene on the proliferation of hepatic cancer was studied. Cells from a hepatic cancer cell line SMMC-7721 were incubated in culture media with 20, 40 and 80 mumol/L beta-carotene for 12, 24 and 48 h respectively. MTT test, Trypan blue exclusion test and DNA gel electrophoresis were used. The results of MTT test revealed that beta-carotene (20-80 mumol/L) could inhibit the proliferation of SMMC-7721 cells in a dose-dependent manner. DNA gel electrophoresis showed that the apoptosis of hepatic cancer cells could be induced by beta-carotene (40 mumol/L). It is concluded that the proliferation of hepatic cancer cells inhibited by beta-carotene, probably through interfering DNA metabolism and inducing cell apoptosis.
[Luo W et al; Wei Sheng Yan Jiu 30 (4): 213-4 (2001)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ An alpha-tocopherol, beta-carotene supplementation trial (ATBC) and a chemoprevention trial with beta-carotene and retinoids (CARET trial) were conducted in the 1990s in populations at risk for the development of lung cancer. Both trials had to be discontinued due to significant increases in lung cancer and cardiovascular mortality. Clinical trials to test the cancer preventive effects of beta-carotene are still ongoing, and high concentrations of this provitamin are contained in numerous dietary supplements. Using a cell line derived from a human pulmonary adenocarcinoma (PAC) of Clara cell lineage and immortalized human small airway epithelial cells, our data show that low concentrations of beta-

carotene that can be realistically expected in human tissues after oral administration caused a significant increase in intracellular cAMP and activated PKA, as well as in phosphorylation of ERK1/2 and CREB. Furthermore, the proliferation of cells was significantly stimulated by identical concentrations of beta-carotene as monitored by MTT assays. Control experiments with retinol also showed stimulation of cell proliferation and activation of PKA in both cell lines. In light of the fact that PAC is the leading type of lung cancer, these findings suggest that the growth promoting effects of beta-carotene on this cancer type observed in our experiments may have contributed to the unfortunate outcome of the ATBC and CARET trials. This interpretation is supported by the fact that elevated levels of cAMP in the cardiovascular system play a major role in the genesis of cardiovascular disease, which was also greatly promoted in the CARET trial. Our data challenge the widely accepted view that beta-carotene may be useful as a cancer preventive agent.
[Al-Wadei HA et al; Int J Cancer 118 (6): 1370-80 (2006)] **PEER REVIEWED**
PubMed Abstract

/OTHER TOXICITY INFORMATION/ The effect of 14 wk of beta-carotene supplementation (20 mg/day) on the frequency of micronuclei in sputum was studied in 114 heavy smokers in a double blind trial. Micronuclei reflect DNA damage in exfoliated cells and may thus provide a marker of early stage carcinogenesis. Pretreatment blood levels of cotinine, beta-carotene, retinol and vitamins C and E were similar in the placebo group (n = 61) and the treatment group (n = 53). Plasma beta-carotene levels increased 13-fold in the treatment group during intervention. Initial micronuclei counts (per 3,000 cells) were higher in the treatment group than in the placebo group (5.0 vs 4.0, p< 0.05). During intervention, the treatment group showed a 47% decrease, whereas the placebo group showed a non-significant decrease (16%). After adjustment for the initial levels, the treatment group had 27% lower micronuclei counts than the placebo group at the end of the trial (95% confidence interval: 9-41%). These results indicate that beta-carotene may reduce lung cancer risk in man by preventing DNA damage in early stage carcinogenesis.
[van Poppel G et al; Br J Cancer 66 (6): 1164-8 (1992)] **PEER REVIEWED**
PubMed Abstract

**Drug Warnings:**
NOT EFFECTIVE AS SUNSCREEN IN NORMAL INDIVIDUALS & SHOULD NOT BE USED FOR THAT PURPOSE ... USED WITH CAUTION IN PT WITH IMPAIRED RENAL OR HEPATIC FUNCTION BECAUSE SAFE USE ... HAS NOT BEEN ESTABLISHED.
[American Society of Hospital Pharmacists. Data supplied on contract from American Hospital Formulary Service and other current ASHP sources., p. 1976] **PEER REVIEWED**

Beta carotene is well tolerated. Carotenodermia is usually the only adverse effect. Patients should be forewarned that carotenodermia will develop after 2-6 weeks of therapy, usually first noticed as yellowness of the palms of the hands or soles of the feet and to a lesser extent of the face. Some patients may experience loose stools during beta carotene therapy, but this is sporadic and may not require discontinuance of therapy. Ecchymoses and arthralgia have been reported rarely
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3555] **PEER REVIEWED**

Beta carotene should be used with caution in patients with impaired renal or hepatic function because safe use of the drug in the presence of these conditions has not been established. Although abnormally high blood concentrations of vitamin A do not occur during beta carotene therapy, patients receiving beta carotene should be advised against taking supplementary vitamin A because beta carotene will fulfill normal vitamin A requirements. Patients should be cautioned that large quantities of green or yellow vegetables or their juices or extracts are not suitable substitutes for crystalline beta carotene because consumption of excessive quantities of these vegetables may cause adverse effects such as leukopenia or menstrual disorders. Patients should be warned that the protective effect of beta carotene is not total and that they may still develop considerable burning and edema after sufficient exposure to sunlight. Each patient must establish his own time limit of exposure.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

There are no adequate and controlled studies to date in humans. Beta carotene should be used during pregnancy only when the potential benefits justify the possible risks to the fetus. The effect of beta carotene on fertility in humans is not known.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

Since it is not known whether beta carotene is distributed into milk, the drug should be used with caution in nursing women.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

FDA Pregnancy Risk Category: C /RISK CANNOT BE RULED OUT. Adequate, well controlled human studies are lacking, and animal studies have shown risk to the fetus or are lacking as well. There is a chance of fetal harm if the drug is given during pregnancy; but the potential benefits may outweigh the potential risk./
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Yellow discoloration of skin is to be expected; if taking as nutritional supplement, may be a sign that the dose is too high.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Data from two large studies indicate an increased incidence of lung cancers when beta-carotene supplements were given to individuals with a history of smoking and/or asbestos exposure; use of beta-carotene supplements in these subgroups is not recommended.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

The use of beta-carotene for the treatment of vitamin A deficiency requires medical management.

[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Smokers should be made aware that supplemental intake of beta-carotene of 20 mg daily or greater were associated with a higher incidence of lung cancer in smokers. Smokers should avoid beta-carotene supplementation pending the establishment of a safe dose for smokers.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Pregnant women and nursing mothers should avoid intakes of beta-carotene greater than 6 mg/day from nutritional supplements.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

**Populations at Special Risk:**
Two large studies have found an increased incidence in lung cancers when beta-carotene supplements were given to individuals with a history of smoking and/or asbestos exposure. One study of 29,000 males with a history of smoking found an 18% increase in the incidence of lung cancer in the group receiving 20 mg of beta-carotene a day for 5 to 8 years as compared with those receiving placebo . Another study of 18,000 individuals found 28% more lung cancers in individuals with a history of smoking and/or asbestos exposure who took 30 mg of beta-carotene in addition to 25,000 Units of retinol a day for 4 years as compared with those receiving placebo . However, one study of 22,000 male physicians, some of them smokers and former smokers, found no increased risk of lung cancer at doses of 50 mg of beta-carotene every other day for 12 years .
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

## Emergency Medical Treatment:

### Emergency Medical Treatment:

### EMT Copyright Disclaimer:

The following Overview, *** VITAMIN A ***, is relevant for this HSDB record chemical.

**Life Support:**

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o   This overview assumes that basic life support measures
       have been instituted.
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**Clinical Effects:**

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0.2.1 SUMMARY OF EXPOSURE
    A)  USES: Used as a dietary supplement and found in some
        topical preparations to promote wound healing. Naturally
        present in high concentrations in some foods.
    B)  PHARMACOLOGY: Essential nutrient required for bone
        development, vision, reproduction, and differentiation
        and maintenance of epithelial tissue. Required cofactor
        for glycosylation of glycoproteins.
    C)  TOXICOLOGY: High doses stimulate bone resorption and
        inhibit keratinization. Excessive doses are stored in
        hepatic Ito cells, which become hypertrophied and
        obstruct sinusoidal blood flow eventually causing portal
        hypertension.
    D)  EPIDEMIOLOGY: Poisoning is rare, with acute toxicity
        even more unusual than chronic toxicity.
    E)  WITH POISONING/EXPOSURE
     1)  ACUTE: Ingestion or parenteral overdose may produce
         significant increases in intracranial pressure, which
         may result in bulging fontanelles (in infants),
         nausea/vomiting, abdominal pain, headache, blurred
         vision, irritability and other effects associated with
         increased intracranial pressure. Exfoliation of the
         skin has also been reported.
     2)  CHRONIC: Signs and symptoms of toxicity include
         nausea/vomiting, abdominal pain, anorexia, fatigue,
         irritability, diplopia, headache, bone pain, alopecia,
         skin lesions, cheilosis, and signs of increased
         intracranial pressure (e.g. papilledema).
      a)  Laboratory findings include elevated liver enzymes and
          bilirubin, increased INR, hypercalcemia, elevated
          erythrocyte sedimentation rate and periosteal
          calcification on radiographs. Increased opening
          pressure may be noted on lumbar puncture.
      b)  Symptoms usually begin to resolve within days to weeks
          after discontinuation of vitamin A use. The prognosis
          is usually excellent with few, if any, long term
          sequelae.
     3)  BETA CAROTENE: There are no known cases of vitamin A
         toxicity associated with beta-carotene ingestion,
         although excessive beta-carotene ingestion may result
         in carotenemia (yellow skin discoloration).
  0.2.4 HEENT
    A)  WITH POISONING/EXPOSURE
     1)  Diplopia, nystagmus, tinnitus and papilledema may be
         noted (pseudotumor cerebri) as a result of vitamin A
         intoxication.
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0.2.7 NEUROLOGIC
A) WITH POISONING/EXPOSURE
1) Fatigue, irritability, headache, lethargy, papilledema, and increased intracranial pressure may be noted. Unusual effects include seizures and cranial nerve palsy.

0.2.8 GASTROINTESTINAL
A) WITH POISONING/EXPOSURE
1) Nausea, vomiting, abdominal pain and anorexia may be noted.

0.2.9 HEPATIC
A) WITH POISONING/EXPOSURE
1) Chronic hypervitaminosis A may result in elevation of liver enzymes, hepatic fibrosis, hepatosplenomegaly and hepatitis. In severe cases, it may progress to cirrhosis, portal hypertension and ascites. Liver transplant was necessary in one patient following chronic toxicity. One report of fulminant hepatic failure was reported in an adult following acute acitretin (metabolite of vitamin A) ingestion.

0.2.12 FLUID-ELECTROLYTE
A) WITH POISONING/EXPOSURE
1) Hypercalcemia may occur as a result of chronic vitamin A intoxication.

0.2.13 HEMATOLOGIC
A) WITH POISONING/EXPOSURE
1) Elevated erythrocyte sedimentation rate is common. Hypoprothrombinemia may develop in patients with hepatic injury.

0.2.14 DERMATOLOGIC
A) WITH POISONING/EXPOSURE
1) The dermal changes are frequently among the first signs of hypervitaminosis A and are likely to include cheilosis, dryness, pruritus, desquamation, seborrhea-like eruptions, skin pigmentation, brittle nails and alopecia. Facial swelling associated with palmar-plantar desquamation may be noted following overdose with isotretinoin (Accutane(R)).

0.2.15 MUSCULOSKELETAL
A) WITH POISONING/EXPOSURE
1) Subcutaneous swelling, pain in bones and joints, with tenderness over the long bones commonly occurs.

0.2.20 REPRODUCTIVE
A) Vitamin A is classified as FDA pregnancy category X. The safety of vitamin A exceeding 6000 units/day during pregnancy has not been established. Animal reproduction studies have demonstrated fetal abnormalities associated with vitamin A overdose in several species.

## Laboratory:

A) Plasma vitamin A concentrations may be helpful in diagnosis, but are not clinically useful in treatment and are not available at most institutions. Obtain serum aminotransferase level, bilirubin, INR, and calcium concentrations in patients with chronic overdose.
B) Lumbar puncture may be necessary to confirm the diagnosis of benign intracranial hypertension and relieve symptoms.

C) Radiographic changes include: pericapsular, ligamentous, and subperiosteal calcification; cortical thickening in the shafts of long bones; diffuse osteopenia; and widened skull sutures in infants.

**Treatment Overview:**

0.4.2 ORAL/PARENTERAL EXPOSURE

A) MANAGEMENT OF MILD TO MODERATE TOXICITY

1) Care is symptomatic and supportive, most patients recover with cessation of vitamin A exposure.

2) Immediately discontinue exposure to vitamin A. Signs and symptoms of vitamin A toxicity generally resolve within days to weeks following withdrawal of vitamin A.

B) MANAGEMENT OF SEVERE TOXICITY

1) Symptoms may persist for a prolonged period following the chronic use of vitamin A due to its highly fat-soluble nature.

C) DECONTAMINATION

1) PREHOSPITAL: In general, doses of less than 300,000 International Units in children and less than 1,000,000 International Units in adults do NOT require decontamination. Activated charcoal should be administered for ingestions above 300,000 International Units in children and greater than 1,000,000 International Units in adults.

D) BENIGN INTRACRANIAL HYPERTENSION

1) The majority of patients with pseudotumor cerebri improve with discontinuation of vitamin A. In rare cases, lumbar puncture with drainage of CSF may be required to alleviate symptoms.

E) PATIENT DISPOSITION

1) HOME CRITERIA: Acute unintentional ingestions of less than 300,000 International Units in children and 1,000,000 International Units in adults do not require gastric decontamination. Acute pediatric ingestions of more than 300,000 International Units can be treated at home with gastric decontamination.

2) OBSERVATION CRITERIA: Symptomatic patients, and those with deliberate ingestion should be referred to a healthcare facility for evaluation.

3) ADMISSION CRITERIA: ACUTE: Children who are symptomatic (ie, vomiting, irritability, bulging fontanelle, and other signs of increased intracranial pressure) should be admitted for observation. CHRONIC: Pediatric and adult patients with chronic hypervitaminosis A should be removed from the source of exposure and admitted based on evaluation of liver enzymes, INR, electrolytes, neurologic status, and dermatologic problems. The need for admission is based upon the severity of clinical illness and laboratory abnormalities.

4) CONSULT CRITERIA: Consult a medical toxicologist or poison center in patients with severe toxicity or in whom the diagnosis is unclear.

F) PITFALLS

1) Acute overdose rarely causes clinical toxicity; avoid over treatment.

G) PHARMACOKINETICS
1) Vitamin A is converted to retinol prior to absorption, primarily in the small intestine. In the plasma its carried by retinol binding protein. It is distributed primarily (90%) to the liver.
H) DIFFERENTIAL DIAGNOSIS
1) Other conditions causing increased intracranial pressure and papilledema (ie, intracranial tumor, malignant hypertension, optic neuropathy, cerebral venous sinus thrombosis). Other disorders causing elevations of liver enzymes (acetaminophen overdose, viral hepatitis).

**Range of Toxicity:**

A) TOXICITY: Significant individual variation (e.g., age, diet, and preexisting disease) may reduce the amount of dietary and non-dietary vitamin A necessary to produce toxicity.
B) ACUTE: Ingestion of more than 1 million International Units in adults, and more than 300,000 International Units in children has caused acute toxicity.
C) CHRONIC: Signs and symptoms of vitamin A toxicity are most commonly associated with chronic ingestion of greater than 10 times the RDA for weeks to months, or more than 50,000 International Units/day by adults and more than 25,000 International Units/day by children.
D) There are no known cases of vitamin A toxicity associated with beta-carotene ingestion.
E) THERAPEUTIC: RECOMMENDED DIETARY ALLOWANCE: ADULT: Female 2,310 International Units/day; Male: 3,000 International Units/day. CHILD: 1 to 3 years old: 1,000 International Units/day.

[Rumack BH POISINDEX(R) Information System Micromedex, Inc., Englewood, CO, 2011; CCIS Volume 149, edition expires Nov, 2011. Hall AH & Rumack BH (Eds): TOMES(R) Information System Micromedex, Inc., Englewood, CO, 2011; CCIS Volume 149, edition expires Nov, 2011.] **PEER REVIEWED**

**Antidote and Emergency Treatment:**

/SRP:/ Basic treatment: Establish a patent airway (oropharyngeal or nasopharyngeal airway, if needed). Suction if necessary. Watch for signs of respiratory insufficiency and assist ventilations if needed. Administer oxygen by nonrebreather mask at 10 to 15 L/min. Monitor for pulmonary edema and treat if necessary ... . Monitor for shock and treat if necessary ... . Anticipate seizures and treat if necessary ... . For eye contamination, flush eyes immediately with water. Irrigate each eye continuously with 0.9% saline (NS) during transport ... . Do not use emetics. For ingestion, rinse mouth and administer 5 ml/kg up to 200 ml of water for dilution if the patient can swallow, has a strong gag reflex, and does not drool ... . Cover skin burns with dry sterile dressings after decontamination ... . /Poisons A and B/

[Currance, P.L. Clements, B., Bronstein, A.C. (Eds).; Emergency Care For Hazardous Materials Exposure. 3Rd edition, Elsevier Mosby, St. Louis, MO 2005, p. 160] **PEER REVIEWED**

/SRP:/ Advanced treatment: Consider orotracheal or nasotracheal intubation for airway control in the patient who is unconscious, has severe pulmonary edema, or is in severe respiratory distress. Positive-pressure ventilation techniques with a bag valve mask device may be beneficial. Consider drug therapy for pulmonary edema ... . Consider administering a beta agonist such as albuterol for severe bronchospasm ... . Monitor cardiac rhythm and treat arrhythmias as necessary ... . Start IV administration of D5W /SRP: "To keep open", minimal flow rate/. Use 0.9% saline (NS) or lactated Ringer�s if signs of hypovolemia are present. For hypotension with signs of hypovolemia, administer fluid cautiously. Watch for signs of fluid overload ... . Treat seizures with diazepam or lorazepam ... . Use proparacaine hydrochloride to assist eye irrigation ... . /Poisons A and B/
[Currance, P.L. Clements, B., Bronstein, A.C. (Eds).; Emergency Care For Hazardous Materials Exposure. 3Rd edition, Elsevier Mosby, St. Louis, MO 2005, p. 160-1] **PEER REVIEWED**

## Animal Toxicity Studies:

**Non-Human Toxicity Excerpts:**
/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ The inhibitory effects of beta-carotene on preneoplastic lesions induced in male Wistar rats by the resistant hepatocyte model was investigated. Rats were divided into six groups. Initiation was performed in all animals by a single injection of diethylnitrosamine. During the selection/promotion period five doses of 2-acetylaminofluorene were administered to the rats and a partial hepatectomy was performed. To three different groups beta-carotene was given by gavage throughout the experiment, before the initiation or during the selection/promotion period respectively. Three other groups served as controls and received corn oil instead of the carotenoid. At the end of the study (8 weeks), beta- carotene administration throughout the experiment reduced the incidence ($p< 0.005$), multiplicity as well as the total number and size of hepatocyte nodules. Furthermore, it significantly decreased the number of foci per sq cm ($p< 0.05$), the average focal area ($p< 0.01$) and the percentage of liver parenchyma occupied ($p< 0.01$). Similar results were observed when beta-carotene was given only before the initiation. However, the administration of the carotenoid during the selection/promotion period did not result in significant decreases of these parameters. These results suggest that the inhibitory effects of beta-carotene are primarily exerted on the initiation phase of the hepatocarcinogenic process. Nevertheless, continuous long term exposure to the carotenoid would confer a greater degree of protection. In addition, by means of an analysis of correlation a positive relationship was found between the number of hepatocyte nodules and the hepatic concentration of beta-carotene. In contrast, an inverse relationship was observed between the number of nodules and the hepatic concentration of total vitamin A.
[Moreno FS et al; Carcinogenesis 12 (10): 1817-22 (1991)] **PEER REVIEWED**
PubMed Abstract

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ In this study, the inhibitory effect of natural alpha-carotene, obtained from palm oil was compared with that of beta-carotene on spontaneous liver carcinogenesis in C3H/He male mice. The mean number of hepatomas per mouse was significantly decreased by alpha-carotene supplementation (oral administration in

drinking water at a concentration of 0.05%, at pleasure) as compared with that in the control group (p< 0.001, Student's t test). On the other hand, beta-carotene, at the same dose as alpha-carotene, did not show any such significant difference from the control group.
[Murakoshi M et al; Cancer Res 52 (23): 6583-7 (1992)] **PEER REVIEWED**
PubMed Abstract

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ The effects of beta-carotene (BC) on ventricular remodeling after myocardial infarction /were studied/. Myocardial infarction was induced in Wistar rats that were then treated with a BC diet (500 mg/kg of diet per day; MI-BC; n = 27) or a regular diet (MI; n = 27). Hearts were analyzed in vivo and in vitro after 6 mo. BC caused decreased left ventricular wall thickness (MI = 1.49 + or - 0.3 mm, MI-BC = 1.23 + or - 0.2 mm, P = 0.027) and increased diastolic (MI = 0.83 + or - 0.15 cm2, MI-BC = 0.98 + or - 0.14 cm2, P = 0.020) and systolic (MI = 0.56 + or - 0.12 cm2, MI-BC = 0.75 + or - 0.13 cm2, P = 0.002) left ventricular chamber areas. With respect to systolic function, the BC group presented less change in fractional area than did controls (MI = 32.35 + or - 6.67, MI-BC = 23.77 + or - 6.06, P = 0.004). There was no difference in transmitral diastolic flow velocities between groups. In vitro results showed decreased maximal isovolumetric systolic pressure (MI = 125.5 + or - 24.1 mm Hg, MI-BC = 95.2 + or - 28.4 mmHg, P = 0.019) and increased interstitial myocardial collagen concentration (MI = 3.3 + or - 1.2%, MI-BC = 5.8 + or - 1.7%, P = 0.004) in BC-treated animals. Infarct sizes were similar between groups (MI = 45.0 + or - 6.6%, MI-BC = 48.0 + or - 5.8%, P = 0.246). Taken together, these data suggest that BC has adverse effects on ventricular remodeling after myocardial infarction.
[Zornoff LA et al; Nutrition 22 (2): 146-51 (2006)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ A 3 generation reproduction study in rats receiving beta carotene at a dietary concentration of 0.1% has revealed no evidence of harm to the fetus.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Reproduction studies in rats using beta carotene dosages 300-400 times the maximum usual human dosage have shown the drug to be fetotoxic (an increase in resorption rate) but not teratogenic; at 75 times the maximum usual human dosage or less, no such fetotoxicity was observed.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ No evidence of impaired fertility has been observed in a 3 generation reproduction study in rats receiving the drug at a dietary concentration of 0.1%, nor in male rats receiving 100 times the recommended human dosage.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

/GENOTOXICITY/ Chromosomal aberrations induced by beta-carotene (a natural food color) were studied on bone marrow cells of mice in vivo. Chromosome aberrations induced by beta-carotene were not significantly higher than those of the control (olive oil) in the dose range 0.27 to 27 mg/kg body weight. The genotoxicity can be attributed to the chemical composition of the dye. In so far as genotoxicity is concerned the carotenoid beta-carotene can be safely used as a food colorant.
[Agarwal K et al; Cytobios 74 (296): 23-8 (1993)] **PEER REVIEWED** PubMed Abstract

/GENOTOXICITY/ The anticlastogenic activity of beta-carotene against cyclophosphamide was studied in bone marrow cells of mice in vivo. Seven days' oral priming with beta-carotene (2.7 and 27 mg/kg body weight) followed by an acute treatment with cyclophosphamide (25 mg/kg body weight; ip) inhibited clastogenicity. The values of chromosomal aberrations and micronucleated polychromatic erythrocytes were consistently lower than the sum of the expected values of beta-carotene and cyclophosphamide given individually. This antagonistic response indicates anticlastogenic activity of beta-carotene against cyclophosphamide.
[Mukherjee A et al; Mutat Res 263 (1): 41-6 (1991)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ This study investigated the individual and combined effects of beta-carotene with a common flavonoid (naringin, quercetin or rutin) on DNA damage induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a potent tobacco-related carcinogen in human. A human lung cancer cell line, A549, was pre-incubated with beta-carotene, a flavonoid, or both for 1h followed by incubation with NNK for 4 h. Then, DNA strand breaks and the level of 7-methylguanine (7-mGua), a product of NNK metabolism by cytochrome P450 (CYP) /were determined/ that beta-carotene at 20 microM significantly enhanced NNK-induced DNA strand breaks and 7-mGua levels by 90% ($p < 0.05$) and 70% ($p < 0.05$), respectively, and that the effect of beta-carotene was associated with an increased metabolism of NNK by CYP because the concomitant addition of 1-aminobenzotriazole, a CYP inhibitor, with beta-carotene to cells strongly inhibited NNK-induced DNA strand breaks. In contrast to beta-carotene, incubation of cells with naringin, quercetin or rutin added at 23 uM led to significant inhibition of NNK-induced DNA strand breaks, and the effect was in the order of quercetin > naringin > rutin. However, these flavonoids did not significantly affect the level of 7-mGua induced by NNK. Co-incubation of beta-carotene with any of these flavonoids significantly inhibited the enhancing effect of beta-carotene on NNK-induced DNA strand breaks; the effects of flavonoids were dose-dependent and were also in the order of quercetin > naringin > rutin. Co-incubation of beta-carotene with any of these flavonoids also significantly inhibited the loss of beta-carotene incorporated into the cells, and the effects of the flavonoids were also in the order of quercetin > naringin > rutin. The protective effects of these flavonoids may be attributed to their antioxidant activities because they significantly decreased intracellular ROS, and the effects were also in the order of quercetin > naringin > rutin. These in vitro results suggest that a combination of beta-carotene with naringin, rutin, or quercetin may increase the safety of beta-carotene.
[Yeh SL et al; Chem Biol Interact 160 (2): 175-82 (2006)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ Since it has to be expected that individuals exposed to oxidative stress who take supplements of beta-carotene are simultaneously exposed to both beta-carotene cleavage products (CPs) and oxidative stress, and both exposures have been demonstrated to cause genotoxic effects in primary rat hepatocytes, cyto- and genotoxic effects on primary rat hepatocytes after supplementation of the medium with increasing concentrations of a CP mixture during exposure to oxidative stress by treatment with either DMNQ (2,3-dimethoxy-1,4-naphthoquinone) or hypoxia/reoxygenation (Hy/Reox) was investigated. The cytological endpoints analysed were the mitotic indices, the percentages of apoptotic and necrotic cells, the percentages of micronucleated (MN) cells and the number of chromosomal aberrations (CAs) and sister chromatid exchanges (SCE). The results obtained clearly demonstrate that the CP mixture enhances the genotoxic effects of oxidative stress exposure, whereas it had no effect at all on the endpoints of cytotoxicity studied. These results further support the hypothesis that CP might be responsible for the reported carcinogenic response in the beta-CArotene and Retinol Efficacy Trial (CARET) and Alpha-Tocopherol Beta-carotene Cancer prevention (ATBC) chemoprevention trials.
[Alija AJ et al; Carcinogenesis 27 (6): 1128-33 (2006)] **PEER REVIEWED**
PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ This article reports the first evidence that beta-carotene, combined with cigarette smoke condensate (TAR), regulates heme oxygenase-1 (HO-1) via its transcriptional factor Bach1 and modulates cell growth. Both immortalized rat fibroblasts (RAT-1) and human lung cancer cells (Mv1Lu) exposed to TAR (25 microg/ml), exhibited an initial (6 h) induction of HO-1, followed by a late (24 h) repression due to the activation of Bach1. Heme oxygenase-1 repression was much more consistent when TAR was administered in combination with beta-carotene (1 microM) for 24 h; at this concentration the carotenoid per se did not have any effect on HO-1. Interestingly, the HO-1 repression following TAR plus beta-carotene treatment caused a resynchronization of RAT-1 cell-cycle with a significant increase in the S-phase, and this was probably due to the decreased intracellular levels of carbon monoxide and bilirubin, both of which have antiproliferative effects. The role of HO-1 repression in increasing cell growth was also confirmed in Mv1Lu cells by the "knock down" of the Bach1 gene, thus demonstrating as HO-1 repression is a conserved mechanism by which cells can react to oxidative stress.
[Palozza P et al; Antioxid Redox Signal 8 (5-6): 1069-80 (2006)] **PEER REVIEWED** PubMed Abstract

/OTHER TOXICITY INFORMATION/ The anticarcinogenic properties of beta-carotene have so far been attributed to its scavenger properties in deactivating or trapping reactive chemical species such as singlet oxygen and certain organic free radicals. Smoking results in increased excretion of detoxification products of electrophilic agents (mercapturic acids) in urine. Since reactive electrophilic intermediates are involved in carcinogenesis, a double blind, placebo controlled intervention trial was performed to investigate whether the intake of beta-carotene by smokers would affect urinary thioether excretion. Before the intervention the beta-carotene group (n = 62) and the placebo group (n = 61) had similar thioether excretion levels in urine (4.2 vs 4.3 mmol/mol creatinine). During the intervention (20 mg beta-carotene daily for 14 wk) the placebo group showed a 12% increase, whereas the beta-carotene group showed a 5% decrease (p= 0.004). After the intervention the beta-carotene group had a 15% lower thioether excretion level

than the placebo group (4.1 vs 4.7 mmol/mol creatinine; p= 0.0017). Our study shows that urinary thioether excretion varies considerably over time, and that smokers have a decreased excretion of thioethers in urine after the use of beta-carotene.
[Bos RP et al; Int Arch Occup Environ Health 64 (3): 189-93 (1992)] **PEER REVIEWED** PubMed Abstract

## Metabolism/Pharmacokinetics:

### Metabolism/Metabolites:

A portion of the beta-carotene is converted to retinol in the wall of the small intestine, principally by its initial cleavage at the 15,15' double bond to form two molecules of retinal. Some of the retinal is further oxidized to retinoic acid; only one-half is reduced to retinol, which is then esterified and transported in the lymph. ...
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1781] **PEER REVIEWED**

Approximately 20 to 60% of beta-carotene is metabolized to retinaldehyde and then converted to retinol, primarily in the intestinal wall. A small amount of beta-carotene is converted to vitamin A in the liver. The proportion of beta-carotene converted to vitamin A diminishes inversely to the intake of beta-carotene, as long as the dosages are higher than one to two times the daily requirements. High doses of beta-carotene do not lead to abnormally high serum concentrations of vitamin A.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Beta carotene may be converted to 2 molecules of retinal by cleavage at the 15-15' double bond in the center of the molecule. Most of the retinal is reduced to retinol which is then conjugated with glucuronic acid and excreted in urine and feces. Some retinal may be further oxidized to retinoic acid which can be decarboxylated and further metabolized, secreted into bile, and excreted in feces as the glucuronide.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

Two pathways have been suggested for the conversion of carotenoids to vitamin A in mammals, central cleavage and excentric cleavage. An enzyme, beta-carotenoid-15,15'-dioxygenase, has been partly purified from the intestines of several species and has been identified in several other organs and species. The enzyme, which converts beta-carotene into two molecules of retinal in good yield, requires molecular oxygen and is inhibited by sulfhydryl binding reagents and iron binding reagents. Most provitamin A carotenoids, including the beta-apo-carotenals, are cleaved to retinal by this enzyme. Its maximal activity in the rabbit is approximately 200 times that required to meet nutritional needs but is less than 50% of that expected to produce signs of vitamin A toxicity. Excentric cleavage unquestionably occurs in plants and some microorganisms and might occur in mammals. Thus far, however, carotenoid dioxygenase with excentric bond specificity has been identified in mammals, the yield of beta-apo-carotenals from

beta-carotene in vivo and in vitro is very low, and beta-apo-carotenals are formed nonbiologically from beta-carotene.
[Olson JA; J Nutr 119 (1): 105-8 (1989)] **PEER REVIEWED** PubMed Abstract

The carotenes are not converted to retinol very rapidly, so that overdoses of the carotenes do not cause vitamin A toxicity. /Carotenes/
[Shoden & Griffin; Fundamentals of Clinical Nutrition: 77 (1980)] **PEER REVIEWED**

**Absorption, Distribution & Excretion:**
Carotenoids are absorbed and transported via lymphatics to the liver. They circulate in association with lipoproteins, and are found in liver, adrenal, testes, and adipose tissue, and can be converted to vitamin A in numerous tissues, including the liver. Some beta carotene is absorbed as such and circulates in association with lipoproteins; it apparently partitions into body lipids and can be converted to vitamin A in numerous tissues, including the liver.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1781] **PEER REVIEWED**

Absorption of beta-carotene depends on the presence of dietary fat and bile in the intestinal tract.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Unchanged beta-carotene is found in various tissues, primarily fat tissues, adrenal glands, and ovaries. Small concentrations are found in the liver.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Only about one-third of beta-carotene or other carotenoids is absorbed by human beings. The absorption of carotenoids takes place in a relatively nonspecific fashion and depends upon the presence of bile and absorbable fat in the intestinal tract; it is greatly decreased by steatorrhea, chronic diarrhea, and very-low-fat diets.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1781] **PEER REVIEWED**

Absorption of dietary beta carotene depends on the presence of bile and absorbable fat in the intestinal tract and is greatly decreased by steatorrhea and chronic diarrhea. These factors may have a similar effect on absorption of therapeutic doses of beta carotene. During absorption, dietary beta carotene is metabolized to vitamin A in the wall of the small intestine. Studies utilizing 50 ug doses of radiolabeled beta carotene indicated that only 20-30% of the drug was absorbed unchanged. Blood carotene concentrations reach a maximum and carotenodermia usually develops about 4-6 wk after beginning beta carotene therapy.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

Beta carotene is widely distributed in the body and accumulates in the skin. An appreciable amount is stored in various tissues, particularly depot fat. It is not known whether beta carotene is distributed into milk.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

Elimination: Primarily fecal.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

A physiologic compartmental model of beta-carotene metabolism was constructed and tested. This model suggests that 22% of the beta-carotene dose is absorbed: 17.8% as intact beta-carotene and 4.2% as retinoid. Also, it suggests that both liver and enterocyte are important in converting beta-carotene to retinoid; 43% is converted in liver and 57% in enterocyte. Finally, it suggests that the mean residence time for beta-carotene is 51 days and that the 73 mumole dose does not alter the fractional transfer coefficients of the system after absorption takes place. The issue of central versus eccentric cleavage of beta-carotene in humans can be studied with further modeling combined with use of appropriately labeled beta-carotene.
[Novotny JA et al; J Lipid Res 36 (8): 1825-38 (1995)] **PEER REVIEWED**
PubMed Abstract

Surgically excised human abdominal skin was mounted on Franz perfusion chambers to assess the cutaneous penetration of topical beta-carotene as well as its metabolism, after a 24-hr incubation period, whereas hairless mice received topical beta-carotene 24 hr before assaying epidermal beta-carotene and retinoid concentrations. Epidermal retinoid and beta-carotene concentrations were determined by high-pressure liquid chromatography. Topical beta-carotene penetrated well into human and mouse epidermis and induced a 10-fold (human) and a threefold (mouse) increase of epidermal retinyl esters, which demonstrates that topical beta-carotene is converted into retinyl esters by human and mouse epidermis and thus appears as a precursor of epidermal vitamin A.
[Antille C et al; Exp Dermatol 13 (9): 558-61 (2004)] **PEER REVIEWED**
PubMed Abstract

**Mechanism of Action:**
IN HEMATOPORPHYRIN PHOTOSENSITIZED MICE BETA-CAROTENE SHOWED PHOTOPROTECTION WAS DUE TO FREE RADICAL SCAVENGING OR SINGLET O QUENCHING BUT ALSO A POSSIBLE ROLE OF 400 NM LIGHT ABSORPTION, A PROPERTY OF BETA-CAROTENE.
[MOSHELL AN, BJORNSON L; J INVEST DERMATOL 68 (3): 157 (1977)] **PEER REVIEWED** PubMed Abstract

Beta carotene protects patients with erythropoietic protoporphyria against severe photosensitivity reactions (burning sensation, edema, erythema, pruritus, and/or cutaneous lesions). The drug has no effect on the basic biochemical abnormality of erythropoietic protoporphyria (eg, erythrocyte, plasma, and stool concentrations of protoporphyrins are not altered by the drug). The precise

mechanism by which the drug exerts photoprotection has not been established. There is some evidence that photosensitizers may act through the formation of singlet excited oxygen and/or free radicals. Since in vitro studies indicate that beta carotene can quench free radicals and singlet excited oxygen, this may be the mechanism by which the drug acts. It is unlikely that beta carotene acts simply as a filter for the wavelengths of light that induce phototoxic effects.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

beta-Carotene inhibits UV-B carcinogenesis. beta-Carotene is an excellent quencher of singlet oxygen, and can quench free radicals. beta-Carotene has been shown to quench singlet oxygen/free radical reactions in the skin of porphyric mice, and has been found to quench excited species formed on irradiation of mouse skin by UV-B.
[Black HS, Mathews-Roth MM; Photochem Photobiol 53 (5): 707-16 (1991)] **PEER REVIEWED** PubMed Abstract

**Interactions:**
Cigarette smoking is associated with decreased plasma levels of ascorbate and beta-carotene, which indicates that the smoking related chronic inflammatory response leads to an imbalance of oxidant/antioxidant homeostasis and possible predisposition to oxidant inflicted tissue damage and disease.
[Anderson R; Am J Clin Nutr 53 (1): 358S-61S (1991)] **PEER REVIEWED**

Weanling male Sprague-Dawley rats were pair-fed beta-carotene (56.5 mg/L of diet) for 8 weeks, with and without ethanol. As expected, ethanol increased CYP2E1 (measured by Western blots) from 67 + or - 8 to 317 + or - 27 densitometric units ($p < 0.001$). Furthermore, beta-carotene potentiated the ethanol induction to 442 + or - 38 densitometric units ($p < 0.01$) with a significant interaction ($p = 0.012$). The rise was confirmed by a corresponding increase in the hydroxylation of p-nitrophenol, a specific substrate for CYP2E1, and by the inhibition with diethyl dithiocarbamate (50 microM). Beta-carotene alone also significantly induced CYP4A1 protein (328 + or - 49 vs. 158 + or - 17 densitometric units, $p < 0.05$). The corresponding CYP4A1 mRNA (measured by Northern blots) was also increased ($p < 0.05$) and there was a significant interaction of the two treatments ($p = 0.015$). The combination of ethanol and beta-carotene had no significant effect on either total cytochrome P-450 or CYP1A1/2, CYP2B, CYP3A, and CYP4A2/3 contents. Beta-carotene potentiates the CYP2E1 induction by ethanol in rat liver and also increases CYP4A1, which may, at least in part, explain the associated hepatotoxicity.
[Kessova IG et al; Alcohol Clin Exp Res 25 (9): 1368-72 (2001)] **PEER REVIEWED** PubMed Abstract

AFLATOXIN B1 (4 MG/KG/DAY, ORALLY) ADMIN TO RATS FOR 26 DAYS INHIBITED THE FORMATION OF VITAMIN A FROM BETA-CAROTENE IN THE INTESTINAL MUCOSA.
[HIKARAISHI S; KANAGAWA KENRITSU EIYO TANKI DAIGAKU KIYO 9: 20 (1977)] **PEER REVIEWED**

SULFITE-MEDIATED BETA-CAROTENE DESTRUCTION WAS INVESTIGATED; IT WAS INHIBITED BY ALPHA-TOCOPHEROL, 1,2-DIHYDROXYBENZENE-3,5-DISULFONIC ACID & BUTYLATED HYDROXYTOLUENE
[PEISER GD, YANG SF; J AGRIC FOOD CHEM 27 (2): 446 (1979)] **PEER REVIEWED**

SENCAR mice were used to determine the effects of the provitamin A compound beta-carotene on papilloma formation and the conversion of papillomas to carcinomas in a two stage protocol with one application of the initiator 7,12-dimethylbenz(a)anthracene (20 ug) and 20 weekly applications of the promotor 12-O-tetradecanoylphorbol-13-acetate (2 ug). A purified vitamin A-free diet was supplemented with beta-carotene at four levels (0.6, 6, 60 and 600 ug/g of diet) for female mice and two levels (60 and 600 ug/g) for male mice. Dietary supplementations of beta-carotene did not result in significant changes in body weight and survival of female and male mice. However, papillomas developed more rapidly and papilloma incidence (% mice with papillomas) reached its maximum (100%) sooner in male mice fed 600 ug of beta-carotene/g of diet than those fed 60 ug/g. There were smaller differences in papilloma incidence among the dietary groups in female mice, but the papilloma incidence again reached 100% sooner in mice fed 600 ug of beta-carotene/g of diet. Female and male mice fed 600 ug of beta-carotene/g of diet had significantly higher papilloma yields (average number of papillomas/mouse) than other dietary groups and a very low percentage of these papillomas converted to carcinomas in these mice. Thus, beta-carotene at 600 ug/g inhibited the conversion of papillomas to carcinomas in both sexes. In addition, papilloma yields were higher in female mice and these papillomas regressed more quickly than those in the corresponding groups of male mice. In conclusion, dietary beta-carotene caused differential effects on papilloma and carcinoma yields and sex-dependent differences in papilloma formation in female and male SENCAR mice treated with 7,12-dimethylbenz(a)anthracene and 12-O- tetradecanoylphorbol-13-acetate in a two-stage carcinogenesis protocol.
[Chen LC et al; Carcinogenesis 14 (4): 713-7 (1993)] **PEER REVIEWED** PubMed Abstract

Preventive effects of artificial beta-carotene on the development of rat mammary gland adenocarcinomas induced by 7,12-dimethylbenz(a)anthracene were studied in rats maintained on a diet containing beta-carotene at a dose of 2.5 mg/animal within 10 wk, which was initiated after the carcinogen administration. The carotenoid treatment course caused the following effects: manifestation of adenocarcinomas induced by 7,12-dimethylbenz(a)anthracene was decreased, latent period of neoplasm development as well as the rate of tumor differentiation were increased and metastatic spreading into the regional lymph nodes was inhibited.
[Sergeeva TI et al; Vopr Med Khim 38 (6): 14-6 (1992)] **PEER REVIEWED**

The antitumor-promoting activity of alpha-carotene was also compared with that of beta-carotene against two stage mouse lung carcinogenesis (initiator, 4-nitroquinoline 1-oxide; promoter, glycerol). alpha-Carotene, but not beta-carotene, reduced the number of lung tumors per mouse to about 30% of that in the control group ($p < 0.001$, Student's t test). The higher potency of the antitumor promoting action of alpha-carotene compared to beta-carotene was confirmed in other experimental systems; eg, alpha-carotene was also found to have a stronger effect than beta-carotene in suppressing the promoting activity of 12-O-tetradecanoylphorbol-13-acetate on skin carcinogenesis in 7,12-dimethylbenz(a)anthracene initiated mice.
[Murakoshi M et al; Cancer Res 52 (23): 6583-7 (1992)] **PEER REVIEWED** PubMed Abstract

The inhibitory effects of beta-carotene on cyclophosphamide induced chromosomal aberrations in mouse bone marrow cells were investigated. Male Balb C mice, 8-10 wk old, were treated with beta-carotene (0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100, and 200 mg/kg) or with corn oil (0.05 ml/10 g body weight) by gavage for 5 consecutive days. Four hr after the last treatment with or without beta-carotene, the animals were ip injected with cyclophosphamide and killed 24 hr later for cytological preparations and analysis. The results obtained show that beta-carotene provides significant protection against the clastogenicity of cyclophosphamide. The maximum reduction in the frequency of aberrant metaphases (26.9%) and in total number of chromosomal aberrations were observed when beta-carotene was used at 50 mg/kg. Nevertheless, no direct dose response relationship was detected, suggesting that beta-carotene might act through different mechanisms at different doses.
[Salvadori DM et al; Environ Mol Mutagen 20 (3): 206-10 (1992)] **PEER REVIEWED** PubMed Abstract

Of the several models for lung carcinogenesis, two appear appropriate for chemoprevention studies based upon dose response, tumor type, and tumor localization. One model utilizes the direct-acting carcinogen methylnitrosourea, and the other utilizes a carcinogen (diethylnitrosamine) requiring metabolic activation. Tumors appear rapidly in both models (within 6 months), and the model systems are responsive to modulation by several classes of potential chemopreventive agents. For example, the retinoid N-(4-hydroxyphenyl) retinamide reduces the incidence of lung adenosquamous carcinoma, but retinol or beta-carotene are ineffective when administered alone. However, concomitant administration of these compounds reduces the incidence of non-neoplastic dysplasias as well as adenosquamous carcinomas of the lung. In the methylnitrosourea system, retinoids in general have been ineffective in reducing the incidence of tracheobronchial squamous cell carcinomas.
[Moon RC et al; Monogr Natl Cancer Inst 13: 45-9 (1992)] **PEER REVIEWED** PubMed Abstract

The putative cancer preventive potential of beta-carotene may be explained by its antioxidant capacity to prevent free radical induced DNA damage. To evaluate this hypothesis, the effect of 14 wk of beta-carotene supplementation on the frequency of sister chromatid exchanges in lymphocytes was studied in 143 heavy smokers in a randomized, double blind, placebo controlled intervention trial. Age, smoking habits and pretreatment blood levels of cotinine, beta-carotene, retinol and vitamins C and E were similar in the placebo group (n = 73) and the treatment group (n = 70). Plasma beta-carotene levels increased 13-fold in the treatment group during intervention, whereas the other parameters remained stable in both groups. Initial sister chromatid exchange levels were similar in the treatment and placebo groups (5.10 + or - 0.98 vs 5.00 + or - 0.99 sister chromatid exchange/lymphocyte). During the intervention, both groups showed an almost identical decrease, and at the end of the intervention period there was no difference in sister chromatid exchange levels between the treatment and the placebo groups (4.37 + or - 0.38 vs 4.24 + or - 0.37 sister chromatid exchange/lymphocyte). This study shows no protective effect of beta-carotene on DNA damage as reflected by sister chromatid exchanges in lymphocytes. These results thus do not yield support for a cancer preventive mechanism of beta-carotene involving this form of DNA damage. It cannot be excluded, however, that beta-carotene prevents other forms of smoking induced DNA damage, affects other tissues, or is preventive in later stages of carcinogenesis.

[Van Poppel G et al; Int J Cancer 51 (3): 355-8 (1992)] **PEER REVIEWED**
PubMed Abstract

Bladder cancer was induced in male B6D2F1 strain mice by the administration of N-butyl-N-(4-hydroxybutyl)nitrosamine. Mice supplemented with beta-carotene for 5 wk before receiving the carcinogen and maintained on beta-carotene for an additional 26 wk developed significantly fewer tumors than did unsupplemented mice. Mice receiving canthaxanthin for the same time period showed no protection against the development of bladder cancer.
[Mathews-Roth MM et al; Oncology 48 (3): 177-9 (1991)] **PEER REVIEWED**
PubMed Abstract

A study was made on the effects of long term dietary administration of beta-carotene, vitamin C, vitamin E and selenium, either alone or in combination, on azaserine-induced pancreatic carcinogenesis in rats. Male Wistar rats were given two ip injections of 30 mg azaserine/kg body weight at 19 and 26 days of age. The rats were allocated to eight groups of 40 animals each and were fed an AIN-76 diet rich in saturated fat (20% lard), either as such or after supplementation with beta-carotene, vitamin C, beta-carotene + vitamin C, vitamin E, selenium, vitamin E + selenium, or the combination of all micronutrients investigated. Fifteen months after the last treatment with azaserine the survivors were killed. The pancreata were examined for the number and size of advanced putative preneoplastic lesions and the number of neoplasms as well. Rats maintained on a diet high in either beta-carotene, vitamin C or selenium developed significantly less atypical acinar cells nodules, adenomas and carcinomas as compared to controls. The number of tumor bearing animals was significantly lower in the groups fed the diet high in beta-carotene or selenium. In animals of the group given a diet high in all micronutrients investigated, both the number and incidence of pancreatic tumours was lower than in all other groups. It was concluded that selenium, beta-carotene and vitamin C, alone as well as in combination, have an inhibitory effect on pancreatic carcinogenesis induced in rats by azaserine.
[Appel MJ et al; Carcinogenesis 12 (11): 2157-61 (1991)] **PEER REVIEWED**
PubMed Abstract

Effects of topically applied betel leaf extract and its constituents, beta-carotene, alpha-tocopherol, eugenol and hydroxychavicol on 7,12-dimethylbenz(a)anthracene induced skin tumors were evaluated in two strains of mice. Betel leaf extract, beta-carotene and alpha-tocopherol, significantly inhibited the tumor formation by 83, 86, 86% in Swiss mice and 92, 94 and 89% in male Swiss bare mice respectively. Hydroxychavicol showed 90% inhibition in Swiss bare mice at 24 wk of treatment. Eugenol showed minimal protection in both strains of mice. The mean latency period and survivors in betel leaf extract, beta-carotene, alpha-tocopherol and hydroxychavicol treated groups were remarkably high as compared to 7,12-dimethylbenz(a)anthracene alone treated group. Ip injection of betal leaf constituents showed a significant effect on both glutathione and glutathione S-transferase levels in the Swiss mouse skin.
[Azuine MA et al; Indian J Exp Biol 29 (4): 346-51 (1991)] **PEER REVIEWED**
PubMed Abstract

In 14 baboons fed ethanol (50% of total energy) for 2 to 5 yr with a standard amount of beta-carotene (one 200 g carrot/day), levels of beta-carotene were much higher than in controls fed isocaloric carbohydrate, both in plasma (122.5 : 30.9 nmol/dL vs 6.3:1.4 nmol/dL; p< 0.005) and in liver (7.9:1.1 nmol/g vs 1.8:0.5 nmol/g; p< 0.001). Even 20 days after withdrawal of the

carrots, plasma beta-carotene levels remained higher in alcohol fed baboons than in controls (10.1:3.8 nmol/dl vs < 0.1 nmol/dL). Next, the diet was supplemented with beta-carotene beadlets: in four pairs of baboons given a low dose of beta-carotene (3 mg/1,000 kcal), plasma levels were significantly higher in alcohol fed animals than in controls, even when expressed per cholesterol (although the latter increased with alcohol intake). Seven pairs of animals were given a high dose (30 mg/1,000 kcal) of beta-carotene for 1 mo, followed, in four pairs, by 45 mg for another mo. On cessation of beta-carotene treatment, plasma levels decreased more slowly in the alcohol fed baboons than in the controls. Percutaneous liver biopsy specimens revealed that liver concentrations of beta-carotene correlated with plasma levels but were higher in the alcohol fed baboons than in the control baboons, whereas the beta-carotene induced increase in liver retinoids was lower (p< 0.0). Furthermore, the ethanol induced liver depletion of total retinoids (432:103 nmol/g vs 1,711:103 in controls; p< 0.001) was not corrected (637:147 vs 2,404:74; p< 0.001), despite the massive supplementation with beta-carotene. Moreover, in the animals fed alcohol with beta-carotene, multiple ultrastructural lesions appeared, with autophagic vacuoles, abundant myelin figures, degenerated mitochondria and increased blood levels of the mitochondrial enzyme glutamic dehydrogenase. The histological changes were either absent or much less prominent in the baboons given beta-carotene with the control diet or in animals fed the ethanol or control diets without beta-carotene. Thus the combination of an increase in plasma and liver beta-carotene after ethanol and a relative lack of a corresponding rise in retinol suggests interference with the conversion of beta-carotene to vitamin A.
[Leo MA et al; Hepatology 15 (5): 883-91 (1992)] **PEER REVIEWED** PubMed Abstract

Concurrent use of vitamin E may facilitate absorption and utilization of beta-carotene and may reduce toxicity of vitamin A.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Concurrent use /with cholestyramine, colestipol, mineral oil, or neomycin/ may interfere with the absorption of beta-carotene or vitamin A; requirements for vitamin A may be increased in patients receiving these medications.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Concomitant intake of olestra and beta-carotene may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of pectin and beta-carotene may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of the carotenoid lutein and beta-carotene may decrease the absorption of lutein.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Orlistat may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical

Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of mineral oil and beta-carotene may reduce the absorption of beta-carotene. [Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of colestipol and beta-carotene may decrease the absorption of beta-carotene. [Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

/It was/ demonstrated previously that smoke exposure and/or high-dose beta-carotene supplementation decreases levels of retinoic acid and retinoic acid receptor beta (RARbeta) protein, but increase levels of c-Jun and proliferating cellular nuclear antigen protein in the lungs of ferrets. In contrast, low-dose beta-carotene can prevent the decreased lung retinoic acid and the smoke-induced lung lesions.The present study investigated whether smoke exposure and/or beta-carotene supplementation could affect Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and p53 in the lungs of ferrets. Ferrets were subjected to cigarette smoke exposure and either a high or low dose of beta-carotene (2 x 3 factorial design) for 6 mo. There were greater protein levels of phosphorylated JNK, p38, and c-Jun, but lower levels of MAPK phophatase-1 (MKP-1) in groups exposed to smoke and/or high dose beta-carotene. Both phosphorylated-p53 and total p53 were substantially increased in the lungs of these groups. In contrast, low-dose beta-carotene greatly attenuated the smoke-induced phosphorylation of JNK, p38, c-Jun, p53, and total p53, accompanied by upregulated MKP-1. Smoke exposure increased MAPK kinase-4 (MKK4) phosphorylation regardless of beta-carotene supplementation. These data indicate that restoration of retinoic acid and MKP-1 by low-dose beta-carotene in the lungs of ferrets may prevent the smoke-induced activation of the JNK-dependent signaling pathway, p38 MAPK, and the associated phosphorylation of p53, thereby lowering the risk of the smoke-related lung lesions. These data provide supportive evidence that the beneficial vs. detrimental effects of beta-carotene supplementation are related to the dosage of beta-carotene administered. [Liu C et al; J Nutr 134 (10): 2705-10 (2004)] **PEER REVIEWED** PubMed Abstract

Deficiencies of vitamin A, iron, and zinc are prevalent in women and infants in developing countries. Supplementation during pregnancy can benefit mother and infant. We examined whether supplementation during pregnancy with iron and folic acid plus beta-carotene or zinc or both improves the micronutrient status of mothers and infants postpartum. Pregnant women (n = 170) were supplemented daily only during pregnancy with beta-carotene (4.5 mg), zinc (30 mg), or both or placebo plus iron (30 mg) and folic acid (0.4 mg) in a randomized, double-blind, placebo-controlled trial. Micronutrient status was assessed 1 and 6 mo postpartum. Six months postpartum, plasma retinol concentrations were higher in the women who received zinc during pregnancy than in women who did not. Infants born to mothers supplemented with beta-carotene + zinc had higher plasma retinol concentrations, with the frequency of vitamin A deficiency reduced by >30% compared with the other 3 groups. Breast-milk beta-carotene concentrations were higher in all women supplemented with beta-carotene, but breast-milk retinol concentrations were higher only in women who received beta-carotene + zinc. Zinc concentrations did not differ among groups in mothers and infants. /It was concluded that/ Zinc supplementation during pregnancy improved the vitamin A status of mothers and infants postpartum, which indicates a specific role of zinc in vitamin A metabolism. Addition of both

beta-carotene and zinc to iron supplements during pregnancy could be effective in improving the vitamin A status of mothers and infants.
[Dijkhuizen MA et al; Am J Clin Nutr 80 (5): 1299-307 (2004)] **PEER REVIEWED** PubMed Abstract

The objectives were to analyze the cardiac effects of exposure to tobacco smoke (ETS), for a period of 30 days, alone and in combination with beta-carotene supplementation (BC). Research methods and procedures: Rats were allocated into: Air (control, n = 13); Air + BC (n = 11); ETS (n = 11); and BC + ETS (n = 9). In Air + BC and BC + ETS, 500 mg of BC were added to the diet. After three months of randomization, cardiac structure and function were assessed by echocardiogram. After that, animals were euthanized and morphological data were analyzed post-mortem. One-way and two-way ANOVA were used to assess the effects of ETS, BC and the interaction between ETS and BC on the variables. ETS presented smaller cardiac output (0.087 + or - 0.001 vs. 0.105 + or - 0.004 l/min; p = 0.007), higher left ventricular diastolic diameter (19.6 + or - 0.5 vs. 18.0 + or - 0.5 mm/kg; p = 0.024), higher left ventricular (2.02 + or - 0.05 vs. 1.70 + or - 0.03 g/kg; p < 0.001) and atrium (0.24 + or - 0.01 vs. 0.19 + or - 0.01 g/kg; p = 0.003) weight, adjusted to body weight of animals, and higher values of hepatic lipid hydroperoxide (5.32 + or - 0.1 vs. 4.84 + or - 0.1 nmol/g tissue; p = 0.031) than Air. However, considering those variables, there were no differences between Air and BC + ETS (0.099 + or - 0.004 l/min; 19.0 + or - 0.5 mm/kg; 1.83 + or - 0.04 g/kg; 0.19 + or - 0.01 g/kg; 4.88 + or - 0.1 nmol/g tissue, respectively; p > 0.05). Ultrastructural alterations were found in ETS: disorganization or loss of myofilaments, plasmatic membrane infolding, sarcoplasm reticulum dilatation, polymorphic mitochondria with swelling and decreased cristae. In BC + ETS, most fibers showed normal morphological aspects. One-month tobacco-smoke exposure induces functional and morphological cardiac alterations and BC supplementation attenuates this ventricular remodeling process.
[Zornoff LA et al; Toxicol Sci 90 (1): 259-66 (2006)] **PEER REVIEWED** PubMed Abstract

## Pharmacology:

### Therapeutic Uses:

Antioxidants
[National Library of Medicine's Medical Subject Headings online file (MeSH, 1999)] **PEER REVIEWED**

THERAPY WITH ORAL BETA-CAROTENE IN PATIENT WITH POLYMORPHOUS LIGHT ERUPTION; COMPLETE REMISSION OCCURRED IN 32% (6/19) TREATED WITH BETA-CAROTENE.
[PARRISH JA ET AL; BR J DERMATOL 100 (2): 187 (1979)] **PEER REVIEWED** PubMed Abstract

MEDICATION (VET): VITAMIN A PRECURSOR FOR ALL SPECIES EXCEPT CATS.
[Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989., p. 282] **PEER REVIEWED**

The effects of chronic oral administration of beta-carotene, a carotenoid partially metabolized to retinol, on plasma lipid concentrations have not been well studied; therefore, 61 subjects were studied over 12 mo while they were enrolled in a skin cancer prevention study in which patients were randomly assigned to receive either placebo (n = 30) or 50 mg beta-carotene/day orally (n = 31). At study entry and 1 yr later, fasting blood samples were obtained for measurement of triglycerides, total cholesterol, high density lipoprotein cholesterol, retinol, and beta-carotene. Retinol concentrations changed minimally in both groups; beta-carotene concentration increased an average of 12.1 + or - 47 nmol/L in the placebo group and 4279 + or - 657 nmol/l in the active treatment group. Both groups experienced similar small increases in triglyceride and total cholesterol concentrations and small decreases in high density lipoprotein cholesterol. Daily oral administration of 50 mg beta-carotene/day did not affect plasma lipid concentrations.
[Nierenberg DW et al; Am J Clin Nutr 53 (3): 652-4 (1991)] **PEER REVIEWED**
PubMed Abstract

Beta carotene is used to reduce the severity of photosensitivity reactions in patients with erythropoietic protoporphyria. When patients with erythropoietic protoporphyria were phototested with artificial light, beta carotene therapy increased the development time for minimal erythema in nearly all patients. Similarly, most patients with erythropoietic protoporphyria have been able to tolerate exposure to sunlight without discomfort for much longer periods of time during beta carotene therapy. Some patients have been able to remain in the sun all day without experiencing photosensitivity reactions. The protective effect of beta carotene is not total, and each patient must determine his own time limit of exposure to the sun. Patients who respond to beta carotene usually develop enough protection so that they can remain in the sun without discomfort for about the same length of time as normal individuals. After beta carotene therapy is discontinued, patients exhibit decreased tolerance to artificial light and or sunlight, usually returning to pretreatment hypersensitivity.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3555] **PEER REVIEWED**

Beta carotene also has been used with variable success in the management of polymorphous light eruptions and photosensitivity caused by diseases other than erythropoietic protoporphyria. Further studies are required to determine the value of the drug in these conditions. In some patients with polymorphous light eruption, concomitant use of beta carotene and a sunscreen (e.g., aminobenzoic acid, sulisobenzone) may protect against photosensitivity. Beta carotene is not effective as a sunscreen in normal individuals and should not be used for that purpose. A combination preparation containing beta carotene and canthaxanthin has been used orally for cosmetic "tanning" by coloring the skin (carotenodermia).
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3555] **PEER REVIEWED**

Beta-carotene may be used for prevention of vitamin A deficiency states in most individuals. Vitamin A deficiency may occur as a result of inadequate nutrition or intestinal malabsorption but does not occur in healthy individuals receiving an adequate balanced diet. For prophylaxis of vitamin A deficiency, dietary improvement, rather than supplementation, is advisable. For treatment of vitamin A deficiency, supplementation with vitamin A is preferred. /Included in

product labeling/
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Beta-carotene is used in the prophylaxis and treatment of severe cases of polymorphous light eruption. /NOT included in US product labeling/
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Beta-carotene is indicated to reduce the severity of photosensitivity reactions in patients with erythropoietic protoporphyria (EPP). /NOT included in US product labeling/
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Vitamin E, beta-carotene, and vitamin C are micronutrient antioxidants that protect cells from oxidative damage involved in prostate carcinogenesis. In separate trials, supplemental vitamin E was associated with a decreased risk of prostate cancer among smokers and supplemental beta-carotene was associated with a decreased risk of prostate cancer among men with low baseline plasma beta-carotene levels. The association between intake of these micronutrient antioxidants from foods and supplements and the risk of prostate cancer among men in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial /were evaluated/.. At baseline, trial participants completed a 137-item food frequency questionnaire that included detailed questions on 12 individual supplements. Cox proportional hazards models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs). All statistical tests were two-sided. 1338 cases of prostate cancer among 29 361 men during up to 8 years of follow-up /were identified/. Overall, there was no association between prostate cancer risk and dietary or supplemental intake of vitamin E, beta-carotene, or vitamin C. However, among current and recent (i.e., within the previous 10 years) smokers, decreasing risks of advanced prostate cancer (i.e., Gleason score > or = 7 or stage III or IV) were associated with increasing dose (RR for > 400 IU/day versus none = 0.29, 95% CI = 0.12 to 0.68; Ptrend = .01) and duration (RR for > or = 10 years of use versus none = 0.30, 95% CI = 0.09 to 0.96; Ptrend = .01) of supplemental vitamin E use. Supplemental beta-carotene intake at a dose level of at least 2000 microg/day was associated with decreased prostate cancer risk in men with low (below the median of 4129 microg/day) dietary beta-carotene intake (RR = 0.52, 95% CI = 0.33 to 0.81). Among smokers, the age-adjusted rate of advanced prostate cancer was 492 per 100,000 person-years in those who did not take supplemental vitamin E, 153 per 100,000 person-years in those who took more than 400 IU/day of supplemental vitamin E, and 157 per 100,000 person-years in those who took supplemental vitamin E for 10 or more years. Among men with low dietary beta-carotene intake, the age-adjusted rate of prostate cancer was 1122 per 100,000 person-years in those who did not take supplemental beta-carotene, and 623 per 100,000 person-years in those who took at least 2000 microg/day of supplemental beta-carotene. Our results do not provide strong support for population-wide implementation of high-dose antioxidant supplementation for the prevention of prostate cancer. However, vitamin E supplementation in male smokers and beta-carotene supplementation in men with low dietary beta-carotene intakes were associated with reduced risk of this disease.
[Kirsh VA et al; J Natl Cancer Inst 98 (4): 245-54 (2006)] **PEER REVIEWED**
PubMed Abstract

**Drug Warnings:**
NOT EFFECTIVE AS SUNSCREEN IN NORMAL INDIVIDUALS & SHOULD NOT BE USED FOR THAT PURPOSE ... USED WITH CAUTION IN PT WITH IMPAIRED RENAL OR HEPATIC FUNCTION BECAUSE SAFE USE ... HAS NOT BEEN ESTABLISHED.
[American Society of Hospital Pharmacists. Data supplied on contract from American Hospital Formulary Service and other current ASHP sources., p. 1976] **PEER REVIEWED**

Beta carotene is well tolerated. Carotenodermia is usually the only adverse effect. Patients should be forewarned that carotenodermia will develop after 2-6 weeks of therapy, usually first noticed as yellowness of the palms of the hands or soles of the feet and to a lesser extent of the face. Some patients may experience loose stools during beta carotene therapy, but this is sporadic and may not require discontinuance of therapy. Ecchymoses and arthralgia have been reported rarely
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3555] **PEER REVIEWED**

Beta carotene should be used with caution in patients with impaired renal or hepatic function because safe use of the drug in the presence of these conditions has not been established. Although abnormally high blood concentrations of vitamin A do not occur during beta carotene therapy, patients receiving beta carotene should be advised against taking supplementary vitamin A because beta carotene will fulfill normal vitamin A requirements. Patients should be cautioned that large quantities of green or yellow vegetables or their juices or extracts are not suitable substitutes for crystalline beta carotene because consumption of excessive quantities of these vegetables may cause adverse effects such as leukopenia or menstrual disorders. Patients should be warned that the protective effect of beta carotene is not total and that they may still develop considerable burning and edema after sufficient exposure to sunlight. Each patient must establish his own time limit of exposure.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

There are no adequate and controlled studies to date in humans. Beta carotene should be used during pregnancy only when the potential benefits justify the possible risks to the fetus. The effect of beta carotene on fertility in humans is not known.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

Since it is not known whether beta carotene is distributed into milk, the drug should be used with caution in nursing women.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

FDA Pregnancy Risk Category: C /RISK CANNOT BE RULED OUT. Adequate, well controlled human studies are lacking, and animal studies have shown risk to the fetus or are

lacking as well. There is a chance of fetal harm if the drug is given during pregnancy; but the potential benefits may outweigh the potential risk./
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Yellow discoloration of skin is to be expected; if taking as nutritional supplement, may be a sign that the dose is too high.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Data from two large studies indicate an increased incidence of lung cancers when beta-carotene supplements were given to individuals with a history of smoking and/or asbestos exposure; use of beta-carotene supplements in these subgroups is not recommended.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

The use of beta-carotene for the treatment of vitamin A deficiency requires medical management.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Smokers should be made aware that supplemental intake of beta-carotene of 20 mg daily or greater were associated with a higher incidence of lung cancer in smokers. Smokers should avoid beta-carotene supplementation pending the establishment of a safe dose for smokers.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Pregnant women and nursing mothers should avoid intakes of beta-carotene greater than 6 mg/day from nutritional supplements.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

**Interactions:**
Cigarette smoking is associated with decreased plasma levels of ascorbate and beta-carotene, which indicates that the smoking related chronic inflammatory response leads to an imbalance of oxidant/antioxidant homeostasis and possible predisposition to oxidant inflicted tissue damage and disease.
[Anderson R; Am J Clin Nutr 53 (1): 358S-61S (1991)] **PEER REVIEWED**

Weanling male Sprague-Dawley rats were pair-fed beta-carotene (56.5 mg/L of diet) for 8 weeks, with and without ethanol. As expected, ethanol increased CYP2E1 (measured by Western blots) from 67 + or - 8 to 317 + or - 27 densitometric units ($p < 0.001$). Furthermore, beta-carotene potentiated the ethanol induction to 442 + or - 38 densitometric units ($p < 0.01$) with a significant interaction ($p = 0.012$). The rise was confirmed by a corresponding increase in the hydroxylation of p-nitrophenol, a specific substrate for CYP2E1, and by the inhibition with diethyl dithiocarbamate (50 microM). Beta-carotene alone also significantly induced CYP4A1 protein (328 + or - 49 vs. 158 + or - 17 densitometric units, $p < 0.05$). The corresponding CYP4A1 mRNA (measured by Northern blots) was also increased ($p < 0.05$) and there was a

significant interaction of the two treatments (p = 0.015). The combination of ethanol and beta-carotene had no significant effect on either total cytochrome P-450 or CYP1A1/2, CYP2B, CYP3A, and CYP4A2/3 contents. Beta-carotene potentiates the CYP2E1 induction by ethanol in rat liver and also increases CYP4A1, which may, at least in part, explain the associated hepatotoxicity.
[Kessova IG et al; Alcohol Clin Exp Res 25 (9): 1368-72 (2001)] **PEER REVIEWED** PubMed Abstract

AFLATOXIN B1 (4 MG/KG/DAY, ORALLY) ADMIN TO RATS FOR 26 DAYS INHIBITED THE FORMATION OF VITAMIN A FROM BETA-CAROTENE IN THE INTESTINAL MUCOSA.
[HIKARAISHI S; KANAGAWA KENRITSU EIYO TANKI DAIGAKU KIYO 9: 20 (1977)] **PEER REVIEWED**

SULFITE-MEDIATED BETA-CAROTENE DESTRUCTION WAS INVESTIGATED; IT WAS INHIBITED BY ALPHA-TOCOPHEROL, 1,2-DIHYDROXYBENZENE-3,5-DISULFONIC ACID & BUTYLATED HYDROXYTOLUENE
[PEISER GD, YANG SF; J AGRIC FOOD CHEM 27 (2): 446 (1979)] **PEER REVIEWED**

SENCAR mice were used to determine the effects of the provitamin A compound beta-carotene on papilloma formation and the conversion of papillomas to carcinomas in a two stage protocol with one application of the initiator 7,12-dimethylbenz(a)anthracene (20 ug) and 20 weekly applications of the promotor 12-O-tetradecanoylphorbol-13-acetate (2 ug). A purified vitamin A-free diet was supplemented with beta-carotene at four levels (0.6, 6, 60 and 600 ug/g of diet) for female mice and two levels (60 and 600 ug/g) for male mice. Dietary supplementations of beta-carotene did not result in significant changes in body weight and survival of female and male mice. However, papillomas developed more rapidly and papilloma incidence (% mice with papillomas) reached its maximum (100%) sooner in male mice fed 600 ug of beta-carotene/g of diet than those fed 60 ug/g. There were smaller differences in papilloma incidence among the dietary groups in female mice, but the papilloma incidence again reached 100% sooner in mice fed 600 ug of beta-carotene/g of diet. Female and male mice fed 600 ug of beta-carotene/g of diet had significantly higher papilloma yields (average number of papillomas/mouse) than other dietary groups and a very low percentage of these papillomas converted to carcinomas in these mice. Thus, beta-carotene at 600 ug/g inhibited the conversion of papillomas to carcinomas in both sexes. In addition, papilloma yields were higher in female mice and these papillomas regressed more quickly than those in the corresponding groups of male mice. In conclusion, dietary beta-carotene caused differential effects on papilloma and carcinoma yields and sex-dependent differences in papilloma formation in female and male SENCAR mice treated with 7,12-dimethylbenz(a)anthracene and 12-O- tetradecanoylphorbol-13-acetate in a two-stage carcinogenesis protocol.
[Chen LC et al; Carcinogenesis 14 (4): 713-7 (1993)] **PEER REVIEWED** PubMed Abstract

Preventive effects of artificial beta-carotene on the development of rat mammary gland adenocarcinomas induced by 7,12-dimethylbenz(a)anthracene were studied in rats maintained on a diet containing beta-carotene at a dose of 2.5 mg/animal within 10 wk, which was initiated after the carcinogen administration. The carotenoid treatment course caused the following effects: manifestation of adenocarcinomas induced by 7,12-dimethylbenz(a)anthracene was

decreased, latent period of neoplasm development as well as the rate of tumor differentiation were increased and metastatic spreading into the regional lymph nodes was inhibited.
[Sergeeva TI et al; Vopr Med Khim 38 (6): 14-6 (1992)] **PEER REVIEWED**

The antitumor-promoting activity of alpha-carotene was also compared with that of beta-carotene against two stage mouse lung carcinogenesis (initiator, 4-nitroquinoline 1-oxide; promoter, glycerol). alpha-Carotene, but not beta-carotene, reduced the number of lung tumors per mouse to about 30% of that in the control group ($p< 0.001$, Student's t test). The higher potency of the antitumor promoting action of alpha-carotene compared to beta-carotene was confirmed in other experimental systems; eg, alpha-carotene was also found to have a stronger effect than beta-carotene in suppressing the promoting activity of 12-O-tetradecanoylphorbol-13-acetate on skin carcinogenesis in 7,12-dimethylbenz(a)anthracene initiated mice.
[Murakoshi M et al; Cancer Res 52 (23): 6583-7 (1992)] **PEER REVIEWED**
PubMed Abstract

The inhibitory effects of beta-carotene on cyclophosphamide induced chromosomal aberrations in mouse bone marrow cells were investigated. Male Balb C mice, 8-10 wk old, were treated with beta-carotene (0.5, 1.0, 2.0, 5.0, 10, 25, 50, 100, and 200 mg/kg) or with corn oil (0.05 ml/10 g body weight) by gavage for 5 consecutive days. Four hr after the last treatment with or without beta-carotene, the animals were ip injected with cyclophosphamide and killed 24 hr later for cytological preparations and analysis. The results obtained show that beta-carotene provides significant protection against the clastogenicity of cyclophosphamide. The maximum reduction in the frequency of aberrant metaphases (26.9%) and in total number of chromosomal aberrations were observed when beta-carotene was used at 50 mg/kg. Nevertheless, no direct dose response relationship was detected, suggesting that beta-carotene might act through different mechanisms at different doses.
[Salvadori DM et al; Environ Mol Mutagen 20 (3): 206-10 (1992)] **PEER REVIEWED** PubMed Abstract

Of the several models for lung carcinogenesis, two appear appropriate for chemoprevention studies based upon dose response, tumor type, and tumor localization. One model utilizes the direct-acting carcinogen methylnitrosourea, and the other utilizes a carcinogen (diethylnitrosamine) requiring metabolic activation. Tumors appear rapidly in both models (within 6 months), and the model systems are responsive to modulation by several classes of potential chemopreventive agents. For example, the retinoid N-(4-hydroxyphenyl) retinamide reduces the incidence of lung adenosquamous carcinoma, but retinol or beta-carotene are ineffective when administered alone. However, concomitant administration of these compounds reduces the incidence of non-neoplastic dysplasias as well as adenosquamous carcinomas of the lung. In the methylnitrosourea system, retinoids in general have been ineffective in reducing the incidence of tracheobronchial squamous cell carcinomas.
[Moon RC et al; Monogr Natl Cancer Inst 13: 45-9 (1992)] **PEER REVIEWED**
PubMed Abstract

The putative cancer preventive potential of beta-carotene may be explained by its antioxidant capacity to prevent free radical induced DNA damage. To evaluate this hypothesis, the effect of 14 wk of beta-carotene supplementation on the frequency of sister chromatid exchanges in lymphocytes was studied in 143 heavy smokers in a randomized, double blind, placebo controlled intervention trial. Age, smoking habits and pretreatment blood levels of cotinine, beta-

carotene, retinol and vitamins C and E were similar in the placebo group (n = 73) and the treatment group (n = 70). Plasma beta-carotene levels increased 13-fold in the treatment group during intervention, whereas the other parameters remained stable in both groups. Initial sister chromatid exchange levels were similar in the treatment and placebo groups (5.10 + or - 0.98 vs 5.00 + or - 0.99 sister chromatid exchange/lymphocyte). During the intervention, both groups showed an almost identical decrease, and at the end of the intervention period there was no difference in sister chromatid exchange levels between the treatment and the placebo groups (4.37 + or - 0.38 vs 4.24 + or - 0.37 sister chromatid exchange/lymphocyte). This study shows no protective effect of beta-carotene on DNA damage as reflected by sister chromatid exchanges in lymphocytes. These results thus do not yield support for a cancer preventive mechanism of beta-carotene involving this form of DNA damage. It cannot be excluded, however, that beta-carotene prevents other forms of smoking induced DNA damage, affects other tissues, or is preventive in later stages of carcinogenesis.
[Van Poppel G et al; Int J Cancer 51 (3): 355-8 (1992)] **PEER REVIEWED**
PubMed Abstract

Bladder cancer was induced in male B6D2F1 strain mice by the administration of N-butyl-N-(4-hydroxybutyl)nitrosamine. Mice supplemented with beta-carotene for 5 wk before receiving the carcinogen and maintained on beta-carotene for an additional 26 wk developed significantly fewer tumors than did unsupplemented mice. Mice receiving canthaxanthin for the same time period showed no protection against the development of bladder cancer.
[Mathews-Roth MM et al; Oncology 48 (3): 177-9 (1991)] **PEER REVIEWED**
PubMed Abstract

A study was made on the effects of long term dietary administration of beta-carotene, vitamin C, vitamin E and selenium, either alone or in combination, on azaserine-induced pancreatic carcinogenesis in rats. Male Wistar rats were given two ip injections of 30 mg azaserine/kg body weight at 19 and 26 days of age. The rats were allocated to eight groups of 40 animals each and were fed an AIN-76 diet rich in saturated fat (20% lard), either as such or after supplementation with beta-carotene, vitamin C, beta-carotene + vitamin C, vitamin E, selenium, vitamin E + selenium, or the combination of all micronutrients investigated. Fifteen months after the last treatment with azaserine the survivors were killed. The pancreata were examined for the number and size of advanced putative preneoplastic lesions and the number of neoplasms as well. Rats maintained on a diet high in either beta-carotene, vitamin C or selenium developed significantly less atypical acinar cells nodules, adenomas and carcinomas as compared to controls. The number of tumor bearing animals was significantly lower in the groups fed the diet high in beta-carotene or selenium. In animals of the group given a diet high in all micronutrients investigated, both the number and incidence of pancreatic tumours was lower than in all other groups. It was concluded that selenium, beta-carotene and vitamin C, alone as well as in combination, have an inhibitory effect on pancreatic carcinogenesis induced in rats by azaserine.
[Appel MJ et al; Carcinogenesis 12 (11): 2157-61 (1991)] **PEER REVIEWED**
PubMed Abstract

Effects of topically applied betel leaf extract and its constituents, beta-carotene, alpha-tocopherol, eugenol and hydroxychavicol on 7,12-dimethylbenz(a)anthracene induced skin tumors were evaluated in two strains of mice. Betel leaf extract, beta-carotene and alpha-tocopherol, significantly inhibited the tumor formation by 83, 86, 86% in Swiss mice and 92, 94 and 89% in male Swiss bare mice respectively. Hydroxychavicol showed 90% inhibition in

Swiss bare mice at 24 wk of treatment. Eugenol showed minimal protection in both strains of mice. The mean latency period and survivors in betel leaf extract, beta-carotene, alpha-tocopherol and hydroxychavicol treated groups were remarkably high as compared to 7,12-dimethylbenz(a)anthracene alone treated group. Ip injection of betal leaf constituents showed a significant effect on both glutathione and glutathione S-transferase levels in the Swiss mouse skin.
[Azuine MA et al; Indian J Exp Biol 29 (4): 346-51 (1991)] **PEER REVIEWED**
PubMed Abstract

In 14 baboons fed ethanol (50% of total energy) for 2 to 5 yr with a standard amount of beta-carotene (one 200 g carrot/day), levels of beta-carotene were much higher than in controls fed isocaloric carbohydrate, both in plasma (122.5 : 30.9 nmol/dL vs 6.3:1.4 nmol/dL; p< 0.005) and in liver (7.9:1.1 nmol/g vs 1.8:0.5 nmol/g; p< 0.001). Even 20 days after withdrawal of the carrots, plasma beta-carotene levels remained higher in alcohol fed baboons than in controls (10.1:3.8 nmol/dl vs < 0.1 nmol/dL). Next, the diet was supplemented with beta-carotene beadlets: in four pairs of baboons given a low dose of beta-carotene (3 mg/1,000 kcal), plasma levels were significantly higher in alcohol fed animals than in controls, even when expressed per cholesterol (although the latter increased with alcohol intake). Seven pairs of animals were given a high dose (30 mg/1,000 kcal) of beta-carotene for 1 mo, followed, in four pairs, by 45 mg for another mo. On cessation of beta-carotene treatment, plasma levels decreased more slowly in the alcohol fed baboons than in the controls. Percutaneous liver biopsy specimens revealed that liver concentrations of beta-carotene correlated with plasma levels but were higher in the alcohol fed baboons than in the control baboons, whereas the beta-carotene induced increase in liver retinoids was lower (p< 0.0). Furthermore, the ethanol induced liver depletion of total retinoids (432:103 nmol/g vs 1,711:103 in controls; p< 0.001) was not corrected (637:147 vs 2,404:74; p< 0.001), despite the massive supplementation with beta-carotene. Moreover, in the animals fed alcohol with beta-carotene, multiple ultrastructural lesions appeared, with autophagic vacuoles, abundant myelin figures, degenerated mitochondria and increased blood levels of the mitochondrial enzyme glutamic dehydrogenase. The histological changes were either absent or much less prominent in the baboons given beta-carotene with the control diet or in animals fed the ethanol or control diets without beta-carotene. Thus the combination of an increase in plasma and liver beta-carotene after ethanol and a relative lack of a corresponding rise in retinol suggests interference with the conversion of beta-carotene to vitamin A.
[Leo MA et al; Hepatology 15 (5): 883-91 (1992)] **PEER REVIEWED** PubMed Abstract

Concurrent use of vitamin E may facilitate absorption and utilization of beta-carotene and may reduce toxicity of vitamin A.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Concurrent use /with cholestyramine, colestipol, mineral oil, or neomycin/ may interfere with the absorption of beta-carotene or vitamin A; requirements for vitamin A may be increased in patients receiving these medications.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Concomitant intake of olestra and beta-carotene may decrease the absorption of beta-carotene.

[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of pectin and beta-carotene may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of the carotenoid lutein and beta-carotene may decrease the absorption of lutein.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Orlistat may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of mineral oil and beta-carotene may reduce the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

Concomitant intake of colestipol and beta-carotene may decrease the absorption of beta-carotene.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

/It was/ demonstrated previously that smoke exposure and/or high-dose beta-carotene supplementation decreases levels of retinoic acid and retinoic acid receptor beta (RARbeta) protein, but increase levels of c-Jun and proliferating cellular nuclear antigen protein in the lungs of ferrets. In contrast, low-dose beta-carotene can prevent the decreased lung retinoic acid and the smoke-induced lung lesions.The present study investigated whether smoke exposure and/or beta-carotene supplementation could affect Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and p53 in the lungs of ferrets. Ferrets were subjected to cigarette smoke exposure and either a high or low dose of beta-carotene (2 x 3 factorial design) for 6 mo. There were greater protein levels of phosphorylated JNK, p38, and c-Jun, but lower levels of MAPK phophatase-1 (MKP-1) in groups exposed to smoke and/or high dose beta-carotene. Both phosphorylated-p53 and total p53 were substantially increased in the lungs of these groups. In contrast, low-dose beta-carotene greatly attenuated the smoke-induced phosphorylation of JNK, p38, c-Jun, p53, and total p53, accompanied by upregulated MKP-1. Smoke exposure increased MAPK kinase-4 (MKK4) phosphorylation regardless of beta-carotene supplementation. These data indicate that restoration of retinoic acid and MKP-1 by low-dose beta-carotene in the lungs of ferrets may prevent the smoke-induced activation of the JNK-dependent signaling pathway, p38 MAPK, and the associated phosphorylation of p53, thereby lowering the risk of the smoke-related lung lesions. These data provide supportive evidence that the beneficial vs. detrimental effects of beta-carotene supplementation are related to the dosage of beta-carotene administered.
[Liu C et al; J Nutr 134 (10): 2705-10 (2004)] **PEER REVIEWED** PubMed Abstract

Deficiencies of vitamin A, iron, and zinc are prevalent in women and infants in developing countries. Supplementation during pregnancy can benefit mother and infant. We examined whether supplementation during pregnancy with iron and folic acid plus beta-carotene or zinc or

both improves the micronutrient status of mothers and infants postpartum. Pregnant women (n = 170) were supplemented daily only during pregnancy with beta-carotene (4.5 mg), zinc (30 mg), or both or placebo plus iron (30 mg) and folic acid (0.4 mg) in a randomized, double-blind, placebo-controlled trial. Micronutrient status was assessed 1 and 6 mo postpartum. Six months postpartum, plasma retinol concentrations were higher in the women who received zinc during pregnancy than in women who did not. Infants born to mothers supplemented with beta-carotene + zinc had higher plasma retinol concentrations, with the frequency of vitamin A deficiency reduced by >30% compared with the other 3 groups. Breast-milk beta-carotene concentrations were higher in all women supplemented with beta-carotene, but breast-milk retinol concentrations were higher only in women who received beta-carotene + zinc. Zinc concentrations did not differ among groups in mothers and infants. /It was concluded that/ Zinc supplementation during pregnancy improved the vitamin A status of mothers and infants postpartum, which indicates a specific role of zinc in vitamin A metabolism. Addition of both beta-carotene and zinc to iron supplements during pregnancy could be effective in improving the vitamin A status of mothers and infants.
[Dijkhuizen MA et al; Am J Clin Nutr 80 (5): 1299-307 (2004)] **PEER REVIEWED** PubMed Abstract

The objectives were to analyze the cardiac effects of exposure to tobacco smoke (ETS), for a period of 30 days, alone and in combination with beta-carotene supplementation (BC). Research methods and procedures: Rats were allocated into: Air (control, n = 13); Air + BC (n = 11); ETS (n = 11); and BC + ETS (n = 9). In Air + BC and BC + ETS, 500 mg of BC were added to the diet. After three months of randomization, cardiac structure and function were assessed by echocardiogram. After that, animals were euthanized and morphological data were analyzed post-mortem. One-way and two-way ANOVA were used to assess the effects of ETS, BC and the interaction between ETS and BC on the variables. ETS presented smaller cardiac output (0.087 + or - 0.001 vs. 0.105 + or - 0.004 l/min; p = 0.007), higher left ventricular diastolic diameter (19.6 + or - 0.5 vs. 18.0 + or - 0.5 mm/kg; p = 0.024), higher left ventricular (2.02 + or - 0.05 vs. 1.70 + or - 0.03 g/kg; p < 0.001) and atrium (0.24 + or - 0.01 vs. 0.19 + or - 0.01 g/kg; p = 0.003) weight, adjusted to body weight of animals, and higher values of hepatic lipid hydroperoxide (5.32 + or - 0.1 vs. 4.84 + or - 0.1 nmol/g tissue; p = 0.031) than Air. However, considering those variables, there were no differences between Air and BC + ETS (0.099 + or - 0.004 l/min; 19.0 + or - 0.5 mm/kg; 1.83 + or - 0.04 g/kg; 0.19 + or - 0.01 g/kg; 4.88 + or - 0.1 nmol/g tissue, respectively; p > 0.05). Ultrastructural alterations were found in ETS: disorganization or loss of myofilaments, plasmatic membrane infolding, sarcoplasm reticulum dilatation, polymorphic mitochondria with swelling and decreased cristae. In BC + ETS, most fibers showed normal morphological aspects. One-month tobacco-smoke exposure induces functional and morphological cardiac alterations and BC supplementation attenuates this ventricular remodeling process.
[Zornoff LA et al; Toxicol Sci 90 (1): 259-66 (2006)] **PEER REVIEWED** PubMed Abstract

**Bionecessity:**
Beta-carotene is a precursor to vitamin A, which is essential for normal function of the retina; in the form of retinal, it combines with opsin (red pigment in the retina) to form rhodopsin (visual purple), which is necessary for visual adaptation to darkness. It is also necessary for growth of

bone, testicular and ovarian function, and embryonic development, and for regulation of growth and differentiation of epithelial tissues.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

Of 600 carotenoids from natural sources that have been characterized, fewer than 10% serve as precursors of vitamin A. Many dietary carotenoids, both with and without provitamin A activity, are found in the blood and tissues of humans. beta-Carotene, the most nutritionally active carotenoid, comprises 15-30% of total serum carotenoids. Vitamin A is formed primarily by the oxygen-dependent central cleavage of beta-carotene and other provitamin A carotenoids.
[Bendich A, Olson JA; FASEB J 3 (8): 1927-32 (1989)] **PEER REVIEWED** PubMed Abstract

## Environmental Fate & Exposure:

### Natural Pollution Sources:

ORANGE-YELLOW PIGMENT IN PLANTS, ALGAE, & SOME MARINE ANIMALS, ESP IN LEAVES, VEGETATION, & ROOT CROPS, IN TRACE CONCN. NOTABLY PRESENT IN BUTTER & CARROTS. /CAROTENE/
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 226] **PEER REVIEWED**

RICHEST SOURCES OF CAROTENE ARE YELLOW & GREEN (LEAFY) VEGETABLES & YELLOW FRUITS.
[Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 940] **PEER REVIEWED**

### Milk Concentrations:

EXPERIMENTAL: This study investigated milk carotenoid concentrations during days 4-32 postpartum and assessed the effects of maternal beta-carotene supplementation. Subjects (n = 21; aged 19-39 y) were randomly assigned to receive beta-carotene (30 mg/d) or placebo from days 4 to 32 postpartum. Each subject provided 8 diet records and 8 milk samples during the study. Diet records were analyzed for energy, macronutrients, vitamins A and E, and carotenoids. Milk samples were analyzed with HPLC for concentrations of carotenoids, retinol, and alpha-tocopherol. Data were analyzed by using repeated-measures analysis and orthogonal contrasts. No significant differences in average dietary intakes, body mass index, age, or parity were found between groups at baseline or after supplementation. Milk carotenoid concentrations decreased over time ($P < 0.01$), as did retinol and alpha-tocopherol concentrations ($P < 0.003$). Concentrations of most carotenoids decreased to those reported for mature milk by day 32 postpartum. Milk lutein concentrations remained elevated throughout the study compared with values reported for mature milk, whereas plasma lutein concentrations decreased significantly over time. beta-carotene supplementation did not significantly change the milk concentrations of beta-carotene, the other carotenoids, retinol, or alpha-tocopherol. CONCLUSIONS: The lack of increase in milk beta-carotene despite supplementation suggests that transitional milk may be

already nearly saturated with beta-carotene. The elevated milk lutein concentration and simultaneous decrease in plasma lutein suggest that lutein metabolism may be altered during early lactation.
[Gossage CP et al; Am J Clin Nutr 76 (1): 193-7 (2002)] **PEER REVIEWED**
PubMed Abstract

## Environmental Standards & Regulations:

**FDA Requirements:**
Certification of this color additive when used as a food is not necessary for the protection of the public health and therefore batches thereof are exempt from the requirements of section 706(c) of the Federal Food, Drug, and Cosmetic Act.
[21 CFR 73.95; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 30, 2006: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

Certification of this color additive when used as a drug is not necessary for the protection of the public health and therefore batches thereof are exempt from the requirements of section 706(c) of the Federal Food, Drug, and Cosmetic Act.
[21 CFR 73.1095; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 30, 2006: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

Certification of this color additive when used as a cosmetic is not necessary for the protection of the public health and therefore batches thereof are exempt from the requirements of section 706(c) of the Federal Food, Drug, and Cosmetic Act.
[21 CFR 73.2095; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 30, 2006: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

Substance added directly to human food affirmed as generally recognized as safe (GRAS).
[21 CFR 184.1245; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 30, 2006: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

Beta-Carotene used as a nutrienet and/or dietary supplement in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice.
[21 CFR 582.5245; U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of August 30, 2006: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

The Food and Drug Administration (FDA) is issuing an interim final rule to prohibit the use on foods of a claim relating to the relationship between antioxidant vitamin A and beta-carotene and the risk in adults of atherosclerosis, coronary heart disease, and certain cancers. This interim final rule is in response to a notification of a health claim submitted under section 303 of the FDA Modernization Act of 1997 (FDAMA). FDA has reviewed statements that the petitioner

submitted in that notification, and in conformity with the requirements of FDAMA, the agency is prohibiting the claim because the statements submitted as the basis of the claim are not "authoritative statements" of a scientific body, as required by FDAMA; therefore, section 303 of FDAMA does not authorize use of this claim. As provided for in section 301 of FDAMA, this interim final rule is effective immediately upon publication.
[63 FR 34092 (6/22/1998)] **PEER REVIEWED**

The Approved Drug Products with Therapeutic Equivalence Evaluations List identifies currently marketed discontinued drug products, incl beta-carotene, approved on the basis of safety and effectiveness by FDA under sections 505 of the Federal Food, Drug, and Cosmetic Act.
[DHHS/FDA; Electronic Orange Book-Approved Drug Products with Therapeutic Equivalence Evaluations. Available from, as of November 15, 2006: http://www.fda.gov/cder/ob/ **PEER REVIEWED**

## Chemical/Physical Properties:

### Molecular Formula:

C40-H56
[Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989., p. 282] **PEER REVIEWED**

### Molecular Weight:

536.87
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

### Color/Form:

Deep purple, hexagonal prisms from benzene and methanol
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

Red, rhombic, almost square leaflets from petroleum ether; dil soln are yellow
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

CRYSTALLINE FORM APPEARS DEEP ORANGE OR COPPER-COLORED
[Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 939] **PEER REVIEWED**

Red-brown hexagonal prisms from benzene and methanol
[Lide, D.R. CRC Handbook of Chemistry and Physics 86TH Edition 2005-2006. CRC

Press, Taylor & Francis, Boca Raton, FL 2005, p. 3-88] **PEER REVIEWED**

**Melting Point:**
183 deg C (evacuated tube)
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

**Density/Specific Gravity:**
1.00 at 20 deg C/20 deg C
[Lide, D.R. CRC Handbook of Chemistry and Physics 86TH Edition 2005-2006. CRC Press, Taylor & Francis, Boca Raton, FL 2005, p. 3-88] **PEER REVIEWED**

**Octanol/Water Partition Coefficient:**
log Kow = 17.62 (est)
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Oct 3, 2006: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm **PEER REVIEWED**

**Solubilities:**
Sol in benzene, chloroform, carbon disulfide; moderately sol in ether, petroleum ether, oils; 100 ml hexane dissolve 109 mg at 0 deg C; very sparingly sol in methanol and ethanol; practically insol in water, acids, alkalies
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

SOL IN FAT SOLVENTS
[Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 939] **PEER REVIEWED**

Soluble in acetone
[Lide, D.R. CRC Handbook of Chemistry and Physics 86TH Edition 2005-2006. CRC Press, Taylor & Francis, Boca Raton, FL 2005, p. 3-88] **PEER REVIEWED**

Soluble in vegetable oils
[Ashford, R.D. Ashford's Dictionary of Industrial Chemicals. London, England: Wavelength Publications Ltd., 1994., p. 179] **PEER REVIEWED**

Insoluble in ethanol, glycerol, propylene glycol; slightly soluble in boiling organic solvents such as ether (0.05%), benzene (0.2%), carbon disulfide (1%), methylene chloride (0.5%); solubility in edible oils 0.08% at room temperature, 0.2% at 60 deg C and 0.8% at 100 deg C.
[Kirk-Othmer Encyclopedia of Chemical Technology. 4th ed. Volumes 1: New York, NY. John Wiley and Sons, 1991-Present., p. V6: 927 (1993)] **PEER REVIEWED**

Solubility: 3 mg/L in methyl Cellosolve; 2 mg/L in ethanol
[Green FJ; The Sigma-Aldrich Handbook of Stains, Dyes and Indicators, Aldrich Chemical Company, Inc, Milwaukee, WI, p. 194 (1991)] **PEER REVIEWED**

In water, 0.6 mg/L, temp not specified
[Green FJ; The Sigma-Aldrich Handbook of Stains, Dyes and Indicators, Aldrich Chemical Company, Inc, Milwaukee, WI, p. 194 (1991)] **PEER REVIEWED**

**Spectral Properties:**
Max absorption (chloroform): 497, 466 nm
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

MAX ABSORPTION (BENZENE): 278 NM (LOG E= 4.30), 364 NM (LOG E= 4.62), 463 NM (LOG E= 5.10), 494 NM (LOG E= 4.77)
[Weast, R.C. (ed.). Handbook of Chemistry and Physics. 60th ed. Boca Raton, Florida: CRC Press Inc., 1979., p. C-241] **PEER REVIEWED**

Max Absorption: 456, 484 nm (E=2500, 2200)(in ethanol)
[Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA27: 455 (1996)] **PEER REVIEWED**

IR: 11591 (Sadtler Research Laboratories Prism Collection)
[Lide, D.R., G.W.A. Milne (eds.). Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. 1994., p. 2072] **PEER REVIEWED**

UV: 7-1258 (Organic Electronic Spectral Data, Phillips et al, John Wiley & Sons, New York)
[Lide, D.R., G.W.A. Milne (eds.). Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. 1994., p. 2072] **PEER REVIEWED**

MASS: 280 (Aldermaston, Eight Peak Index of Mass Spectra, UK); 68285 (NIST/EPA/MSDC Mass Spectral database, 1990 version
[Lide, D.R., G.W.A. Milne (eds.). Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. 1994., p. 2072] **PEER REVIEWED**

**Vapor Pressure:**
1.8X10-11 mm Hg at 25 deg C (est)
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Oct 3, 2006: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm **PEER REVIEWED**

**Other Chemical/Physical Properties:**

Sensitive to alkali and very sensitive to air and light, particularly at high temperatures.
[Kirk-Othmer Encyclopedia of Chemical Technology. 4th ed. Volumes 1: New York, NY. John Wiley and Sons, 1991-Present., p. V6: 927 (1993)] **PEER REVIEWED**

IR: 21933 (Sadtler Research Laboratories Prism Collection) /Alpha-carotene/
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 403] **PEER REVIEWED**

UV: 7-1258 (Organic Electronic Spectral Data, Phillips et al, John Wiley & Sons, New York) /Alpha-carotene/
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 403] **PEER REVIEWED**

MASS: 280 (Aldermaston, Eight Peak Index of Mass Spectra, UK) /Alpha-carotene/
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 403] **PEER REVIEWED**

UV: 7-1258 (Organic Electronic Spectral Data, Phillips et al, John Wiley & Sons, New York) /Gamma-carotene/
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 403] **PEER REVIEWED**

MASS: 280 (Aldermaston, Eight Peak Index of Mass Spectra, UK) /Gamma-carotene/
[Weast, R.C. and M.J. Astle. CRC Handbook of Data on Organic Compounds. Volumes I and II. Boca Raton, FL: CRC Press Inc. 1985., p. V1 403] **PEER REVIEWED**

Henry's Law constant = 1.1X10+2 atm-cu m/mol at 25 deg C (est)
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Oct 3, 2006: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm **PEER REVIEWED**

Hydroxyl radical reaction rate constant = 7.4X10-10 cu cm/molec-sec at 25 deg C (est)
[US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Oct 3, 2006: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm **PEER REVIEWED**

## Chemical Safety & Handling:

**Stability/Shelf Life:**

ABSORBS OXYGEN FROM AIR GIVING RISE TO INACTIVE, COLORLESS OXIDATION PRODUCTS

[Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989., p. 282] **PEER REVIEWED**

**Storage Conditions:**
Store below 40 deg C (104 deg F), preferably between 15 and 30 deg C 59 and 86 deg F), unless otherwise specified by manufacturer.
[Thomson/Micromedex. Drug Information for the Health Care Professional. Volume 1, Greenwood Village, CO. 2006.] **PEER REVIEWED**

**Disposal Methods:**
SRP: At the time of review, criteria for land treatment or burial (sanitary landfill) disposal practices are subject to significant revision. Prior to implementing land disposal of waste residue (including waste sludge), consult with environmental regulatory agencies for guidance on acceptable disposal practices.
**PEER REVIEWED**

## Occupational Exposure Standards:

## Manufacturing/Use Information:

**Major Uses:**
Yellow coloring agent for foods.
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

MEDICATION (See also: Therapeutic Uses)
**PEER REVIEWED**

Therapeutic Category: Vitamine A precursor. Ultraviolet screen.
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

Therapeutic Category (Vet): Vitamin A precursor for all species except cats.
[O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 313] **PEER REVIEWED**

**Manufacturers:**
Atomergic Chemetals Corp., 71 Carolyn Blvd., Farmingdale, NY 11735-1527, (631)694-9000; Prodn site: Farmingdale, NY

[SRI Consulting. 2006 Directory of Chemical Producers-United States. Menlo Park, CA. 2006, p. 717] **PEER REVIEWED**

DSM Nutritional Products, Inc., 45 Waterview Blvd., Parsippany, NJ 07054-1298, (800) 526-0189; Prodn site: Freeport, TX 77541
[SRI Consulting. 2006 Directory of Chemical Producers-United States. Menlo Park, CA. 2006, p. 717] **PEER REVIEWED**

**Methods of Manufacturing:**
beta-Carotene is ... made by a microbial fermentation process from corn and soybean oil.
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 226] **PEER REVIEWED**

**General Manufacturing Information:**
MOST IMPORTANT OF PROVITAMINS A. WIDELY DISTRIBUTED IN PLANT & ANIMAL KINGDOM. IN PLANTS IT OCCURS ALMOST ALWAYS TOGETHER WITH CHLOROPHYLL. ... COMMERCIAL CRYSTALLINE BETA-CAROTENE HAS A VIT A ACTIVITY OF 1.67 MILLION USP UNITS/G. THE IU OF 0.6 UG BETA-CAROTENE IS ALMOST EXACTLY EQUIV TO 0.3 UG VIT A.
[Budavari, S. (ed.). The Merck Index - Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 1989., p. 282] **PEER REVIEWED**

ONE IU OF VITMIN A IS SPECIFIC BIOLOGICAL ACTIVITY OF 0.3 UG OF ALL-TRANS-RETINOL OR 0.6 UG OF BETA-CAROTENE. BECAUSE OF RELATIVELY INEFFICIENT DIETARY UTILIZATION OF BETA-CAROTENE COMPARED WITH RETINOL, NOMENCLATURE IS IN THE TERMS OF RETINOL EQUIVALENTS, WHICH REPRESENTS 1 UG OF ALL-TRANS-RETINOL, 6 UG OF DIETARY BETA-CAROTENE, OR 12 UG OF OTHER PROVITAMIN A CAROTENOIDS.
[Hardman, J.G., L.E. Limbird, P.B., A.G. Gilman. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001., p. 1782] **PEER REVIEWED**

... CONSISTS OF 3 ISOMERS, APPROX 15% ALPHA, 85% BETA, & 0.1% GAMMA. /CAROTENE/
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 226] **PEER REVIEWED**

THEORETICALLY ONE MOLECULE OF BETA-CAROTENE SHOULD YIELD TWO MOLECULES OF VIT A1; HOWEVER, AVAILABILITY OF CAROTENE IN FOODS AS SOURCES OF VIT A FOR HUMANS IS LOW & EXTREMELY VARIABLE. ... UTILIZATION EFFICIENCY OF CAROTENE IS GENERALLY CONSIDERED TO BE 1/6 FOR HUMANS ... .
[Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 939] **PEER REVIEWED**

IN US, AVG ADULT RECEIVES ABOUT HALF OF HIS DAILY INTAKE OF VITAMIN A AS CAROTENOIDS. /CAROTENOIDS/
[Goodman, L.S., and A. Gilman. (eds.) The Pharmacological Basis of Therapeutics. 5th ed. New York: Macmillan Publishing Co., Inc., 1975., p. 1570] **PEER REVIEWED**

Beta carotene is a precursor of vitamin A that is present in green and yellow vegetables, Although beta carotene has 20 cis-trans isomers, it occurs in nature mainly as the all-trans isomer, and the commercially available **synthetic** drug consists of the all-trans isomer. trans-beta-Carotene occurs as red or reddish brown to violet-brown crystals or crystalline powder and is insoluble in water, practically insoluble in alcohol, and sparingly soluble in vegetable oils.
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

**Formulations/Preparations:**
Beta-carotene supplements are available as **synthetic** beta-carotene and natural beta-carotene. **Synthetic** beta-carotene is comprised mainly of all-trans beta-carotene with small amounts of 13-cis beta-carotene and even smaller amounts of 9-cis beta-carotene. Natural beta-carotene is principally derived from the algae Dunaliella salina and is comprised of all-trans beta-carotene and 9-cis beta-carotene. Three mg of beta-carotene is equal to 5,000 UIs. Supplemental intake of beta-carotene ranges from 3-15 mg/day.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

GRADES: ACCORDING TO USP, UNITS OF VIT A, SOLD AS PURE CRYSTALS, AS SOLN IN VARIOUS OILS, AS COLLOIDAL DISPERSION. ALSO 'FOOD CHEMICAL CODEX' /CAROTENE/
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 226] **PEER REVIEWED**

Oral: Capsules: 15 mg, 60 mg (available by nonproprietary name).
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

## Laboratory Methods:

**Clinical Laboratory Methods:**
Simultaneous quantitation and separation of carotenoids and retinol in human milk by HPLC.
[Giuliano AR et al; Methods Enzymol 213 (ISS Carotenoids, Pt. A): 391-9 (1992)] **PEER REVIEWED** PubMed Abstract

Determination of retinol, alpha-tocopherol, and beta-carotene in serum by liquid chromatography.

[MacCrehan WA; Methods Enzymol 189 (Retinoids, Pt. A): 172-81 (1990)] **PEER REVIEWED** PubMed Abstract

**Analytic Laboratory Methods:**
CHROMATOGRAPHY TO DETECT CAROTENOIDS ADDED FOR COLORING PURPOSES, IN MACARONI, NOODLES, FLOUR, SEMOLINA, & EGG YOLK. /CAROTENES/
[Association of Official Analytical Chemists. Official Methods of Analysis. 10th ed. and supplements. Washington, DC: Association of Official Analytical Chemists, 1965. New editions through 13th ed. plus supplements, 1982., p. 13/230 14.156] **PEER REVIEWED**

Method: AOAC 941.15; Procedure: spectrophotometric method; Analyte: carotene; Matrix: fresh plant materials and silages; Detection Limit: not provided.
[Horwitz W, ed.; Official Methods of Analysis of AOAC International 17th ed. (2003). CD-ROM, AOAC International, Gaithersburg, MD] **PEER REVIEWED**

Determination of alpha- and beta-carotene in some raw fruits and vegetables by HPLC.
[Bushway RJ; J Agric Food Chem 34 (3): 409-12 (1986)] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (serum); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm; limit of detection: 10 ng/mL
[Steghens JR et al; J Chromatogr B 694: 71-81 (1997). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.705] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (plasma, serum); procedure: high-performance liquid chromatography with ultraviolet detection at 460 nm
[Kaplan LA et al; Methods Enzymol 189: 155-167 (1990). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.705] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (serum); procedure: isocratic high-performance liquid chromatography with ultraviolet detection at 325 nm, 291 nm, and 450 nm; limit of detection: 50 nM
[Arnaud J et al; J Chromatogr 572: 103-116 (1991). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.707] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (plasma); procedure: high-performance liquid chromatography with ultraviolet detection at 470 nm
[Khachik R et al; Anal Chem 64: 2111-2122 (1992). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.707] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (plasma, serum); procedure: high-performance liquid chromatography with ultraviolet detection at 340 nm, 290 nm, 280 nm, and 450 nm; limit of detection: 100 ng/mL
[Lee BL et al; J Chromatogr 581: 41-47 (1992). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley &

Sons, 2000, p.708] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (serum); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm; limit of detection: 1 ng
[Schafer Elinder L, Walldius G; J Lipid Res 33: 131-137 (1992). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.708] **PEER REVIEWED**

Analyte: beta-carotene; matrix: blood (plasma), tissue (liver, lung); procedure: high-performance liquid chromatography with ultraviolet detection at 445 nm; limit of detection: 10 ng/mL
[Van Vliet T et al; J Chromatogr 553: 179-186 (1991). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.712] **PEER REVIEWED**

Analyte: beta-carotene; matrix: food (cheese); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm; limit of detection: 0.16 ng
[Panfili G et al; Analyst 119: 1161-1165 (1994). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.713] **PEER REVIEWED**

Analyte: beta-carotene; matrix: food (margarine); procedure: high-performance liquid chromatography with ultraviolet detection at 436 nm
[Chase GW Jr et al; J Liq Chromatogr 18: 3129-3138 (1995). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.713] **PEER REVIEWED**

Analyte: beta-carotene; matrix: food (squash, peach, orange juice, palm tree oil); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm; limit of detection: 0.16 ng
[Carvalho CRL et al; J Chromatogr A 697: 289-294 (1995). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.713] **PEER REVIEWED**

Analyte: beta-carotene; matrix: food (squash, broccoli, carrots, collard greens, turnip, kale, mustard greens, zucchini); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm
[Marsili R, Callahan D; J Chromatogr Sci 31: 422-428 (1993). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.715] **PEER REVIEWED**

Analyte: beta-carotene; matrix: food (carrots, tomatoes, pumpkins); procedure: high-performance liquid chromatography with ultraviolet detection at 450 nm
[Jinno K, Lin Y; Chromatographia 41: 311-317 (1995). As cited in: Lunn G; HPLC Methods for Pharmaceutical Analysis. Volumes 2-4. New York, NY: John Wiley & Sons, 2000, p.716] **PEER REVIEWED**

## Special References:

**Special Reports:**
Bendich A; The Safety of beta-Carotene. Nutr Cancer 11 (4): 207-14 (1988). Epidemiological studies have associated low dietary and/or plasma level of carotenoids with higher incidences of certain cancers. This evidence has led the NCI to initiate more than a dozen prospective clinical trials in which supplements of beta-carotene alone, or in combination with other micronutrients, are being taken.

WHO/IPCS; Toxicological Evaluation of Certain Food Additives and Contaminants WHO Food Additives Series 32 (1993)

## Synonyms and Identifiers:

**Related HSDB Records:**
815 [VITAMIN A]

**Synonyms:**
ALL-TRANS-BETA-CAROTENE
**PEER REVIEWED**

Carotaben
**PEER REVIEWED**

BETA,BETA-CAROTENE
**PEER REVIEWED**

BETA-CAROTENE, ALL-TRANS-
**PEER REVIEWED**

CYCLOHEXENE, 1,1'-(3,7,12,16-TETRAMETHYL-1,3,5,7,9,11,13,15,17-OCTADECANONAENE-1,18-DIYL)BIS(2,6,6-TRIMETHYL-, (ALL-E)-
**PEER REVIEWED**

Provatene
**PEER REVIEWED**

PROVITAMIN A
**PEER REVIEWED**

Solatene
**PEER REVIEWED**

SOLATENE (CAPS)
**PEER REVIEWED**

**Associated Chemicals:**
Alpha-carotene;7488-99-5
Gamma-carotene;472-93-5

**Formulations/Preparations:**
Beta-carotene supplements are available as **synthetic** beta-carotene and natural beta-carotene. **Synthetic** beta-carotene is comprised mainly of all-trans beta-carotene with small amounts of 13-cis beta-carotene and even smaller amounts of 9-cis beta-carotene. Natural beta-carotene is principally derived from the algae Dunaliella salina and is comprised of all-trans beta-carotene and 9-cis beta-carotene. Three mg of beta-carotene is equal to 5,000 UIs. Supplemental intake of beta-carotene ranges from 3-15 mg/day.
[Physicians Desk Reference (PDR) for Nutritional Supplements 1st ed, Medical Economics, Thomson Healthcare; Montvale, NJ (2001) p.42] **PEER REVIEWED**

GRADES: ACCORDING TO USP, UNITS OF VIT A, SOLD AS PURE CRYSTALS, AS SOLN IN VARIOUS OILS, AS COLLOIDAL DISPERSION. ALSO 'FOOD CHEMICAL CODEX' /CAROTENE/
[Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Rheinhold Co., 1993, p. 226] **PEER REVIEWED**

Oral: Capsules: 15 mg, 60 mg (available by nonproprietary name).
[McEvoy, G.K. (ed.). American Hospital Formulary Service. AHFS Drug Information. American Society of Health-System Pharmacists, Bethesda, MD. 2006., p. 3556] **PEER REVIEWED**

## Administrative Information:

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Complete Update on 01/20/1993, 1 field added/edited/deleted.
Field update on 12/29/1992, 1 field added/edited/deleted.
Field update on 11/09/1990, 1 field added/edited/deleted.
Complete Update on 10/10/1990, 1 field added/edited/deleted.
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Review

# Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans

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Carotenoids are one of the major food micronutrients in human diets and the overall objective of this review is to re-examine the role of carotenoids in human nutrition. We have emphasized the attention on the following carotenoids present in food and human tissues: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin; we have reported the major food sources and dietary intake of these compounds. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid content and carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures..

**Keywords:** Bioavailability / Carotenoids / Epidemiological studies / Food source / Technology process

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## 1 Introduction

Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in yellow-orange fruits and vegetables and in dark green, leafy vegetables. In plant cells, carotenoids are mainly present in lipid membranes or stored in plasma vacuols [1, 2].

Literature reports on the various aspects of the biosynthesis of carotenoids and the changes in their accumulation in plants through genetic and environmental factors. Food carotenoids have been compiled in several tables and databases, generally including provitamin A carotenoids such as β-carotene and β-cryptoxanthin, as well as others without that provitamin activity, such as lycopene and lutein, and others less studied in relation to human health such as phytoene or phytofluene [1–4].

In human beings, carotenoids can serve several important biological activities. The most widely studied and well-understood nutritional role for carotenoids is their provitamin A activity. Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. Vitamin A, which has many vital systemic functions in humans, can be produced within the body from certain carotenoids, notably β-carotene [5].

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**Abbreviations: FBS,** food balance sheets; **FCTs,** food composition tables; **FFQ,** Food Frequency Questionnaire; **MP,** minimally processed

Carotenoids also potentially play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. Lycopene, the hydrocarbon carotenoid that gives tomatoes their red colour, is particularly effective at quenching the destructive potential of singlet oxygen [6]. Lutein and zeaxanthin and xanthophylls found in corn and in leafy greens such as kale and spinach, are believed to function as protective antioxidants in the macular region of the human retina, protection against cataract formation, coronary heart diseases and stroke [7–9]. Astaxanthin, a xanthophyll found in salmon, shrimp and other seafoods, is another naturally occurring xanthophyll with potent antioxidant properties [10]. Other health benefits of carotenoids that may be related to their antioxidative potential, include enhancement of immune system function [11], protection from sunburn [12] and inhibition of the development of certain types of cancers [13].

In this overview, food sources and intake, effects of food processing and bioavailability have been considered.

## 2 Food sources and intake

### 2.1 Carotenoid content of foods

In developed countries, 80–90% of the carotenoid intake comes from fruit and vegetable consumption. Of the more than 700 naturally occurring carotenoids identified thus far, as many as 50 are present in the human diet and can be absorbed and metabolized by the human body [14]; however only six (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin), representing more than 95% of total blood carotenoids, are present in the blood of people from different countries and have been studied and associated with some health benefits.

The most studied carotenoids are the following six: β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin, which are all important in human nutrition due to their biological activities. 'The Carotenoid Content of US Foods' is a comprehensive database, representative of US food consumption and including raw, processed and cooked forms, as described by Holden *et al*. in 1999 [15]. Similarly, O'Neill *et al*. [3] reported a European database covering the most commonly consumed carotenoid-rich foods in five European countries: UK, Ireland, Spain, France and The Netherlands. This database is a compilation of investigations from the 1990s. In 1995, Hart and Scott [16] investigated the carotenoid content of vegetables and fruits commonly consumed in the UK. Leth *et al*. [17] presented the carotenoid contents of Danish food, and Murkovic *et al*. [18] presented an Austrian Carotenoid Database comprising raw vegetables grown in Austria.

In this paper, only data from recent studies on the above-mentioned six important carotenoids and their content in foods are reported, covering most of the period from about 2000 to March 2007. Foods included are vegetables, fruits and dairy products, representing the main part of carotenoid intake in Europe. Data about exotic fruits imported into Europe are also included. In Table 1, data on the content of carotenoids in raw and in a few processed foods are presented. Contents refer to the edible part of the food and are stated as μg *per* 100 g fresh weight (or volume). In some papers, contents were related to dry weight and those values were converted to fresh weight and included in Table 1 only when the moisture content of the food was documented. Furthermore, zeaxanthin was sometimes included in the reported lutein content, as the two carotenoids are not separated by all employed analytical methods.

The analytical methods are continuously being improved, leading to more specific data on carotenoids. This also results in data on contents of other carotenoids, *e.g*. phytoene and phytofluene, present in tomatoes and tomato products, and violaxanthin present in other vegetables and fruits, *e.g*. melons. Data for these carotenoids are not included in Table 1.

Several factors affect the composition and content of carotenoids in foods, *e.g*. variety, genotype, season, geographic location/climate, stage of maturity and growing conditions.

#### 2.1.1 Genotype effects

The genotype affects the composition and content of carotenoids in different varieties and cultivars of fruit and vegetables. Lenucci *et al*. [40] showed that the content of lycopene and β-carotene varied significantly among 14 cultivars of cherry tomatoes. Likewise, the total carotenoid content ranged from 3700 to 12 200 μg/100 g among 50 cultivars of red-fleshed watermelons from US [45]. Wall [24] studied composition of different cultivars of banana and papaya. The major carotenoids found in bananas were lutein, α-carotene and β-carotene, and the average content of these carotenoids differed up to two-fold among the two cultivars investigated. Among papaya cultivars, lycopene was found in the red-fleshed samples but not in the yellow-fleshed ones, while β-carotene, β-cryptoxanthin and lutein were present in all samples. In conclusion, there is a high variability in the content of carotenoids in foods reported by different authors.

#### 2.1.2 Seasonal, geographical and cultivation variation

The effects of season, geographic location and cultivation practise on carotenoid composition have been investigated in tomato cultivars. Raffo *et al.* [41] harvested greenhouse cherry tomatoes at full ripeness at six different times of the year. No definite seasonal trend nor correlation with solar radiation or temperature was found for total carotenoids (sum of eight carotenoids), nevertheless tomatoes harvested in mid-summer (July) had the lowest average level of lycopene (7061 μg/100 g), whereas tomatoes from March contained 11 969 μg/100 g. Toor *et al*. [46] also studied sea-

**Table 1.** Data for the content of major carotenoids in selected foods (μg/100 g or 100 mL fresh weight/volume)

| Foods | Lutein | Zeaxanthin | β-Cryptoxanthin | α-Carotene | β-Carotene | Lycopene | Reference |
|---|---|---|---|---|---|---|---|
| *Plant origin* | | | | | | | |
| Apricot | 123–188 | n.d.–39 | –[a)] | n.d.[b)]–44 | 585–3800 | 54 | [19–21] |
| Avocado | 213–361 | 8–18 | 21–32 | 19–30 | 48–81 | – | [22] |
| Banana | 86–192 | – | n.d.–5 | 60–156 | 43–131 | n.d.–247 | [23, 24] |
| Basil | 7050 | il[c)] | 89 | n.d. | 4820 | n.d. | [18] |
| Bean, green | 883 | – | – | – | 503 | – | [25] |
| Broccoli | 707–3300 | il | n.d. | n.d. | 291–1750 | n.d. | [17, 18, 25] |
| Cabbage, white | 450 | il | n.d. | n.d. | 410 | n.d. | [18] |
| Carrot | 254–510 | il | n.d. | 2840–4960 | 4350–8840 | n.d. | [17, 18, 26] |
| Chilli, red | n.d. | – | – | – | 6530–15 400 | – | [27] |
| Cornflakes | n.d.–52 | 102–297 | n.d. | n.d. | n.d. | n.d. | [17] |
| Cress | 5610–7540 | – | – | – | 2720–3690 | – | [26, 28] |
| Cucumber | 459–840 | il | n.d. | n.d. | 112–270 | n.d. | [17, 18] |
| Dill | 13 820 | il | 410 | 94 | 5450 | n.d. | [18] |
| Egg plant | 170 | il | n.d. | n.d. | 1110 | n.d. | [18] |
| Endive | 2060–6150 | – | – | – | 1340–4350 | – | [26, 28, 29] |
| Fig | 80 | – | 10 | 20 | 40 | 320 | [30] |
| Grapefruit, red | – | – | – | – | – | 750 | [20] |
| Guava | – | – | 19–118 | n.d. | 102–2669 | 769–1816 | [23] |
| Kale | 4800–11 470 | – | – | – | 1020–7380 | – | [31] |
| Kiwi | – | – | – | – | <20 | <10 | [32] |
| Leek | 3680 | il | n.d. | n.d. | 3190 | n.d. | [18] |
| Lettuce | 1000–4780 | – | – | – | 870–2960 | – | [25, 26, 28, 29] |
| Mango | – | – | 17–317 | n.d. | 109–1201 | <10–724 | [23, 32] |
| Mandarin juice | – | – | 752 | n.d. | 55 | – | [33] |
| Nectarine, peel | – | – | n.d.–31 | – | 5–307 | – | [34] |
| Nectarine, flesh | – | – | n.d.–21 | – | 2–131 | – | [34] |
| Olive oil, extra virgin | 350 | – | n.d. | n.d. | 230 | n.d. | [30] |
| Orange | – | – | 74–141 | n.d. | 171–476 | n.d. | [23] |
| Orange juice | – | – | 16–151 | n.d.–31 | n.d.–98 | – | [33] |
| Papaya | 93–318 | – | n.d.–1034 | n.d. | 81–664 | n.d.–7564 | [23, 24] |
| Parsley | 6400–10 650 | il | n.d. | n.d. | 4440–4680 | n.d. | [17, 18] |
| Pea | 1910 | il | n.d. | n.d. | 520 | n.d. | [18] |
| Peach | – | – | – | – | – | 11 | [20] |
| Peach, peel | – | – | n.d.–36 | – | 11–379 | – | [34] |
| Peach, flesh | – | – | n.d.–16 | – | 4–168 | – | [34] |
| Pepper, green | 92–911 | n.d.–42 | n.d.–110 | n.d.–139 | 2–335 | n.d. | [18, 25, 26, 35] |
| Pepper, orange | 245 | n.d. | 3 | 72 | 400 | – | [35] |
| Pepper, red | 248–8506 | 593–1350 | 248–447 | n.d.–287 | 1441–2390 | – | [35] |
| Pepper, yellow | 419–638 | n.d. | 15–41 | 10–28 | 42–62 | – | [35] |
| Pineapple | | – | 70–124 | n.d. | 139–347 | 265–605 | [23] |
| Pistachio | 770–4900 | – | – | – | n.d.–510 | – | [36, 37] |
| Plum, peel | – | – | 3–39 | – | 217–410 | – | [34] |
| Plum, flesh | – | – | 3–13 | – | 40–188 | – | [34] |
| Potato, sweet | 50 | – | – | – | 7830 | – | [27] |
| Pumpkin | 630 | – | 60 | – | 490 | 500 | [3, 20] |
| Rhubarb | – | – | – | – | – | 120 | [20] |
| Sage | 6350 | il | 87 | n.d. | 2780 | n.d. | [18] |
| Spinach | 5930–7900 | il | n.d. | n.d. | 3100–4810 | n.d. | [18, 38] |
| Tomato | 46–213 | il | n.d. | n.d. | 320–1500 | 850–12 700 | [17–20, 26, 32, 39] |
| Tomato, canned | n.d. | n.d. | n.d. | n.d. | 217–283 | 8480–11820 | [17] |
| Tomato, cherry | n.d.–25 | – | – | – | 300–1100 | 800–12 000 | [32, 40, 41] |
| Tomato, concentrate | – | – | – | – | – | 49 300–94 000 | [20] |
| Tomato juice | 29 | – | – | – | 369 | 1024–11 000 | [20, 42] |
| Tomato ketchup | n.d. | n.d. | n.d. | n.d. | 135–500 | 4710–23 400 | [17, 20, 32, 43] |
| Tomato puree | n.d. | n.d. | n.d. | n.d. | 383–548 | 13 160–26 110 | [17] |
| Tomato sauce, instant | – | – | – | – | – | 5600–39 400 | [20] |
| Tomato soup, instant | – | – | – | – | – | 12 400–19 900 | [20] |
| Watermelon, red | – | – | n.d. | n.d. | 314–777 | 4770–13 523 | [20, 23] |
| Watermelon, yellow | – | – | 59–110 | n.d. | 56–287 | n.d.–109 | [23] |

**Table 1.** Continued

| Foods | Lutein | Zeaxanthin | β-Cryptoxan-thin | α-Carotene | β-Carotene | Lycopene | Reference |
|---|---|---|---|---|---|---|---|
| Wheat flour | 76–116 | n.d. | n.d. | n.d. | n.d. | n.d. | [17] |
| Wheat flour, durum | 164 | n.d. | n.d. | n.d. | n.d. | n.d. | [17] |
| *Animal origin* | | | | | | | |
| Butter | 15–26 | n.d.–2 | 5–8 | n.d.–2 | 296–431 | – | [44] |
| Cheese, ripened | 3 | 0.2 | 0.2 | n.d. | 48 | – | [44] |
| Cheese, young | 4 | 0.2 | 0.1 | n.d. | 62 | – | [44] |
| Egg, yolk | 384–1320 | n.d. | n.d. | n.d. | n.d. | n.d. | [17] |
| Egg | 182 | n.d. | n.d. | n.d. | n.d. | n.d. | [17] |
| Milk, full fat | 0.8–1.4 | n.d.–0.1 | 0.3–0.4 | n.d.–0.1 | 15–19 | – | [44] |
| Milk, semiskimmed | 0.5–0.8 | n.d.–0.1 | n.d.–0.1 | n.d. | 7–9 | – | [44] |

a) –: not included in the reference(s).
b) n.d.: not detected or quantified.
c) il: included in lutein (stated in the reference).

sonal variations in lycopene content of greenhouse tomatoes, and found lowest contents in the summer months (December–February), as temperatures above 30°C were found to inhibit lycopene synthesis more than the slightly positive effect of solar radiation. Sass-Kiss *et al*. [19] found significantly different contents of lycopene in tomatoes from two successive harvest years due to different weather conditions. In addition, processing varieties of tomatoes grown in open fields contained higher amounts of lycopene than table varieties from greenhouses.

Bergquist *et al*. [38] investigated carotenoids in baby spinach cultivated at three different times within two years. Contents of total carotenoids varied about 15% among the three cultivations at commercial harvest time and about 30% after 5 or 9 days storage at 10°C, while the content tended to increase or remained stable during storage because the metabolic patway of some carotenoids continues during the ripness.

In investigation of Setiawan *et al*. [23], three ripe samples from different regions were analysed, and minimum and maximum values found in *e.g.* mango and papaya were respectively: β-cryptoxanthin 17–317 and n.d.–425 μg/100 g, lycopene 49–724 and 4305–7564 μg/100 g, β-carotene 109–1201 and 322–664 μg/100 g.

Kimura and Rodriguez-Amaya [28] compared hydroponic and conventionally grown lettuce, and found a lower (10–30%) carotenoid content (including lutein and β-carotene) in hydroponic cultivated lettuce.

Caldwell and Britz [47] investigated the effect of supplemental UV radiation on the carotenoid composition of greenhouse leaf lettuce. In general, supplemental UV-B increased the carotenoid content of green leaf lettuce and reduced levels in the red-leaf varieties which may be attributed to light-dependent changes in xanthophilly carotenoids content. Furthermore, up to ten-fold cultivar differences were found in levels of carotenoids in plants grown under identical conditions.

Effects of nitrogen rate and form on the accumulation of carotenoid pigments in the leaf tissue of greenhouse-grown kale were investigated by Kopsell *et al.* [31]. Treatment with different amounts of nitrogen at a constant 1:3 ratio of $NH_4$–N and $NO_3$–N showed that concentrations of β-carotene and lutein were not affected by nitrogen rate on a fresh weight basis, however on a dry weight basis the carotenoids increased linearly to increasing nitrogen rate. Increasing $NO_3$–N from 0 to 100%, at a constant nitrogen rate, resulted in increases in both lutein and β-carotene.

Commercially available Spanish orange juices, including one mandarin juice, were investigated by Melendez-Martinez *et al*. [33]. Hulshof *et al*. [44] found an effect of season on the content of β-carotene in milk samples from the Netherlands. β-Carotene was the predominant carotenoid in all the analysed dairy products even if carotenoid levels in dairy products are extremely low and of very little significance to overall intakes. Milk sampled from January to April contained approximately 20% less β-carotene than milk sampled from July to October, probably due to seasonal differences in animal feeding practices. However, no regional differences as a consequence of homogeneous climate were found.

### 2.1.3 Stage of maturity and storage

de Azevedo-Meleiro and Rodriguez-Amaya [29] found large differences in the carotenoid contents between young and mature leaves from the same head of endive, lettuce and New Zealand spinach. In endive and lettuce, the carotenoid concentration of the mature leaves were about two to four times those of the young leaves. In contrast, the mature leaves of New Zealand spinach only contained about 75% that of the young leaves, and the principal carotenoids were β-carotene, lutein, violaxanthin, neoxanthin and lactucaxanthin. The coloured compounds in pistachio nuts from different geographical regions (Greece, Iran, Italy, Turkey), each presenting specific varieties, were studied by Bellomo and Fallico [37]. The level of the main carotenoid, lutein, depended on type of cultivar, cultivation practise and *ripeness* as well as origin of the nuts, the lutein content diminishing with ripening. Among ripe nuts the Italian samples had the highest lutein content.

When storing greenhouse tomatoes at different temperatures for 10 days, Toor and Savage [39], like in earlier observations, found about two-fold more lycopene in tomatoes stored at 15° and 25°C than in refrigerated tomatoes at 7°C (7.5 and 3.2 mg/100 g, respectively).

#### 2.1.4 Potential rich sources

In many developing countries, vitamin A deficiency is widespread, leading to a general need to increase the vitamin A intake of the population, even if the major food source of dietary vitamin A in these area are provitamin A carotenoids. This enhancement of carotenoids might be achieved, *e.g.* by cultivating crops containing higher amounts of provitamin A carotenoids, traditional plant breeding or by genetic engineering [48–50]. Likewise, Western countries focus on fruit and vegetable consumption and the associated health benefits. Carotenoids are among the active components of fruits and vegetables with potential health effects, and enhancement of carotenoid levels might thus be desirable. Examples of investigations into richer sources of carotenoids are outlined below.

Kidmose *et al.* [51] studied carotenoids in different genotypes of spinach. The total carotenoid content varied from 17.76 mg/100 g (in the lightest green genotype) to 22.63 mg/100 g (in the darkest one) with highest β-carotene, lutein and neoxanthin levels. Xu *et al.* [52] analysed the carotenoid composition of peel and juice of ordinary and lycopene-accumulating mutants of orange, pummelo, and grapefruit. Carotenoid profiles of 36 major carotenoids varied with tissue types, citrus species, and mutations. Profiles of peel and juice differed, and content of total carotenoids was much higher in peels.

We summarized the most relevant investigations about the principal food sources of carotenoids. New Zealand spinach are rich in carotenoids, and are one of the most popular leafy vegetables in Brazil and de Azevedo-Meleiro and Rodriguez-Amaya [29] reported levels of about 3800 μg β-carotene, 4800 μg lutein, 2200 μg violaxanthin and 1500 μg neoxanthin *per* 100 g mature leaves. Likewise, Rajyalakshmi *et al.* [53] studied contents of total carotenoid and β-carotene in South Indian forest green leafy vegetables, and found high contents in some varieties. Furtado *et al.* [54] analysed carotenoid content in common Costa Rican vegetables and fruits, and pointed out rich sources. Content of carotenoids in commonly consumed Asian vegetables was studied by Kidmose *et al.* [27]. Many varieties had high contents of β-carotene, lutein and other xanthophylls, *e.g.* drumstick leaves and edible rape turnip leaves. Lako *et al.* [55] reported carotenoid profiles of a wide selection of Fijian fruit and vegetables, and found many rich sources among green leafy vegetables, *e.g.* drumstick leaves as above.

It is also worth noting that the ongoing trend towards globalization is modulating both the availability of foods (*i.e.* exotic fruits, carotenoid-fortified foods), and the social habits in relation to food consumption in some European countries.

### 2.2 Sampling of foods for carotenoid analysis

In the field of nutrition, sampling is generally aimed at taking samples representative of the eating habits of certain consumers, *e.g.* of the population of a nation. Proper sampling is of utmost importance to avoid unintended variability. When designing the sampling plan for a study of carotenoids in vegetables and fruits, it is important to consider many aspects. Thus a sample plan should include conditions that might influence carotenoid composition and content, *i.e.* cultivation conditions like: choice of variety and cultivar, geographical location, season and year, agricultural practices – like nutrients and fertilizers at disposal, and cultivation in open field or in greenhouse – and stage of maturity. Furthermore, harvesting and postharvest handling, storing, possible processing or cooking, should also be taken into account for a sufficient sample description.

### 2.3 Analytical methods

Like the above-mentioned agricultural and sampling aspects, the analytical methods by which the carotenoids are determined influence the levels of the different carotenoids.

The general steps in the analyses of carotenoids include: sample preparation, extraction and saponification, separation, detection and quantification. Errors can be introduced in each of these steps.

Several considerations must be taken into account throughout the analysis to get reliable results, as carotenoids are highly susceptible to isomerization or degradation from light, heat, oxygen, acids, prooxidant metals and active surfaces [56–58]. Otherwise, the carotenoids might to some extent undergo isomerization or degradation.

#### 2.3.1 Sample preparation

Before homogenization, an appropriate portion of the food, *e.g.* vegetables should be trimmed and cleaned and only those parts that are normally eaten should be included in the analyses [18]. The foods might be lyophilized or frozen to avoid changes in the carotenoid concentrations before preparation. These procedures should ensure that representative samples are ready for extraction.

#### 2.3.2 Extraction and saponification

In food analyses, the procedure normally includes extraction of the carotenoids followed by alkaline saponification of the ester forms present in certain foods. In addition, the saponification step removes interfering substances like chlorophylls and unwanted lipids before the final extraction of the carotenoids. Saponification is not necessary for samples without these compounds.

Several extraction procedures have been applied, and have been described in other reviews [57–60]. Numerous organic solvents have been used either alone or in mixtures for liquid-liquid extraction, which is the general procedure. As an alternative to the traditional method, supercritical fluid extraction (SFE) has been applied in some recent investigations [61]. To prevent carotenoid losses during extraction, antioxidants such as butylated hydroxytoluene (BHT) are usually added to the extraction solvent. Moreover, internal standards might be used to assess losses during the extraction [15, 62]. In some studies, an SPE is added as a further purification of carotenoids prior to the determination [17].

#### 2.3.3 Separation, detection and quantification

Traditionally, determination of carotenoids in foods was performed by measuring the total absorption of the extract at a specific wavelength and calculating the amount using β-carotene as standard. This was later improved by separation of carotenes and xanthophylls by open-column chromatography (OCC). The introduction of HPLC equipped with UV and/or PDA detectors made the isolation, detection and quantification of the individual carotenoids possible, thus greatly enhancing the quality of the analytical results. More recently, the application of HPLC coupled with MS (LC-MS) has proven a powerful tool for identification of carotenoids. This technique is very sensitive and might also provide information about structure. By coupling HPLC with NMR the structure of the carotenoids might be completely elucidated.

There are no general HPLC conditions of choice neither for mobile phase nor column [30, 57, 60]. Both normal-phase and RP HPLC can be applied to separate the carotenoids [19, 63, 64]. However, the most frequently used systems are RP [59]. Many different solvents have been applied as gradient or isocratic mobile phases. To prevent oxidation of carotenoids, an antioxidant is often added to the mobile phase. The column selection depends on the requirements for the separation of the individual carotenoids and their isomers. Monomeric C18 columns separate most of the xanthophylls, but not lutein and zeaxanthin, whereas these components can be resolved with polymeric C18 columns [65]. Similarly, the nonpolar carotenoids, *e.g.* α- and β-carotene, are poorly resolved with the monomeric C18 columns and partly separated with the polymeric C18 columns. Since Sander and Wise [65] showed an improved separation of both polar and nonpolar carotenoids including geometric isomers with a polymeric C30 column, this type of column has been used for a variety of food analyses [19, 66, 67].

#### 2.3.4 Quality assurance and standard methods

To get reliable results in analysis of carotenoids it is always advisable to include measures of quality assurance. Preferably, the method should be validated and, *e.g.* sensitivity, selectivity, recovery, repeatability and reproducibility estimated. Scott *et al.* [56] developed a vegetable mix reference material (RM), and the use of standard or in-house RMs is highly recommendable [18] for assuring the analytical quality. Furthermore, purity of the carotenoids should be considered and care taken in the standardization of carotenoid solutions [16].

As reported above, no generally applicable standard method for determination of individual carotenoids in food has been introduced. However, standard methods are available from the Association of Analytical Communities (AOAC) [68] using OCC with spectrophotometric determination of carotenes and xanthophylls, respectively and European Committee for Standardization (CEN) [69] has published a standard method for determination of total β-carotene by HPLC with UV–Vis detection.

### 2.4 Carotenoid intake

It is widely assumed that serum concentrations of carotenoids reflect, at least to some extent, the consumption of carotenoid-containing foods [70]. The influence of diet as a factor of serum carotenoid concentrations has long been known, although both dietary intake and serum concentrations of carotenoids have shown a high variability both within and between subjects in different populations [71–75]. Seasonal variations in individual carotenoid intake, and serum concentrations, have been reported in some European countries (*i.e.* Spain) while not in others (*i.e.* UK, Republic of Ireland, Finland) [3, 73, 74, 76], even when total carotenoid intake may not vary significantly (*i.e.* Spain) [3]. Although fluctuations between seasons may be observed for several carotenoids both in the diet and serum levels [74–77], in Spanish diet, these reach statistical significance only for β-cryptoxanthin (higher in winter) and lycopene (higher in summer); these changes are found to be in accordance with the availability and consumption of the major dietary contributors (*i.e.* citrus fruits and tomato and watermelon, respectively) [76, 77].

A European north–south gradient for the intake of some carotenoids and serum concentrations, both within and between European countries, have been reported [3, 75, 78, 79]. This pattern is consistent with food availability data (*i.e.* fruits and vegetables) among European countries since southern (Mediterranean) countries (*i.e.* Greece, Italy, Portugal, Spain) consume greater amounts of fruits and vegetables than northern countries (*i.e.* UK, Ireland, Scandinavian countries) [80, 81]. In some countries, this geographical trend has been reported for both total and individual carotenoid intake and, overall, it is associated with variations in fruit and vegetables consumption (*i.e.* in UK, low in the

North) and with socioeconomic status and cultural factors. In fact, the specific traditional and cultural factors between the two groups of populations, and in addition the changes in marketing could contribute to the change of life style [78]. Consistently, serum levels also show this distribution trend across north–south axis.

Time trends in carotenoid intake have been scarcely assessed in European countries. Nonetheless, changes in major dietary sources of carotenoids (fruits, vegetables, cereals and recently fortified foods) is known to have occurred in European countries during the last decades [81–83] which is partly explained by changes in socio-economical, demographic and cultural factors. Time variation, on a short-term basis, in carotenoid intake has been assessed in Denmark, where, apparently, intake pattern of carotenoids has not changed from 1995 to 1997 [17]. Similarly, in Spain, using almost the same methodology, a fairly consistent qualitative and quantitative pattern of carotenoid intake from fresh fruits and vegetables was observed on a short-term basis, *i.e.* between 1996 and 2004, although this pattern was different when data were calculated on a longer time scale, *i.e.* 1960–1980 (it could be due to changes in fruit and vegetables consumption of populations) [77].

## 2.5 Methodology

Estimated intakes of carotenoids vary widely both on an individual, regional and national level, and significant seasonal variations in intake of individual carotenoids have been also reported in some countries (*i.e.* Spain) [76, 77]. Carotenoid intake assessment, at both the individual and group level, has been shown to be complicated mainly for the high variability within-subject and between-subject intake, inaccuracies associated with methods of dietary assessment, and inconsistencies in food composition tables (FCTs) and databases [84–86].

The food balance sheets (FBS) [83] present a comprehensive picture of the pattern of a country's food supply during a specified reference period. The FBS shows for each food item – *i.e.* each primary commodity and a number of processed commodities potentially available for human consumption – the sources of supply and its utilization. The total quantity of foodstuffs produced in a country added to the total quantity imported and adjusted to any change in stocks that may have occurred since the beginning of the reference period gives the supply available during that period. On the utilization side a distinction is made between the quantities exported, fed to livestock, used for seed, put to manufacture for food use and nonfood uses, losses during storage and transportation, and food supplies available for human consumption. The *per capita* supply of each such food item available for human consumption is then obtained dividing the respective quantity by the related data on the population actually partaking of it. Data on *per capita* food supplies are expressed in terms of quantity and – by applying appropriate food composition factors for all primary and processed products – also in terms of caloric value and protein and fat content.

**Table 2.** Sources of nutritional data

| Level | Source | Type of data |
|---|---|---|
| Population | Food balance sheets | Ecological; large units |
| Household | House budget survey | Ecological; small units |
| Individual | Nutrition survey | Analytical; individuals |

Source: EURONUT, Report 9, 1987 [87].

**Table 3.** Availability of data for lutein and/plus zeaxanthin content in foods nutritional and epidemiological studies

| Ref. | Type of report | Country (food origin) | Lutein | Lutein + Zeaxanthin |
|---|---|---|---|---|
| [14] | HPLC report | USA | Yes | |
| [89] | HPLC report | Finland | | Yes |
| [90] | HPLC report | Malaysia | Yes | |
| [91] | HPLC report | Spain | Yes | |
| [92] | Database | World wide | | Yes |
| [2] | Database | USA (several) | | Yes |
| [16] | HPLC report | UK | Yes | |
| [93] | Database | Spain | Yes | |
| [94] | HPLC report | USA | Yes | |
| [15][a)] | Database | USA (USA) | | Yes |
| [18] | Database | Austria | | Yes |
| [3] | Database | Europe (several) | | Yes |

a) Zeaxanthin values reported independently for selected foods.
Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Carotenoids content has been calculated applying USDA FCTs.

Sources of nutritional data have been classified at different levels and data obtained are of different type (Table 2) [87].

Regardless of the confidence in the method used for dietary assessment, evaluation of nutrient exposure by dietary means is based on the availability of reliable food composition data. Since the nutritional interest in carotenoids was largely due to their provitamin A activity, traditionally, FCTs and databases have, traditionally, not included values for individual carotenoids in foods, although they have considered vitamin A (retinol equivalents) content. However, the increasing evidence of the potential role of several constituents present in fruits and vegetables (carotenoids) in human health led to a revision of former data and the inclusion of nonprovitamin A carotenoids (*i.e.* lutein) in the new FCTs and databases during the 1990s (Table 3) [88].

## 2.6 Available data of dietary intake

Few studies have been carried out to ascertain the total intakes of carotenoids in the European diet. A European

**Table 4.** Comparison of carotenoid intake (mg/day) in adults in five European countries (data are medians and interquartile ranges)

| | β-Carotene | | Lutein (+ Zeaxanthin) | | Lycopene | | α-Carotene | | β-Cryptoxanthin | | Total carotenoids | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Median | Range | Median | Range | Median | Range | Median | Range | Median | Range | Median | Range |
| Spain (n70) | 2.96 | 1.58–4.41 | 3.25 | 1.75–4.34 | 1.64 | 0.50–2.64 | 0.29 | 0.15–0.51 | 1.36 | 0.74–2.16 | 9.54 | 7.16–14.46 |
| France (n76) | 5.84 | 3.83–8.00 | 2.50 | 1.71–3.91 | 4.75 | 2.14–8.31 | 0.74 | 0.37–1.36 | 0.45 | 0.17–0.88 | 16.06 | 10.3–22.1 |
| UK (n71) | 5.55 | 3.66–6.56 | 1.59 | 1.19–2.37 | 5.01 | 3.2–7.28 | 1.04 | 0.71–1.66 | 0.99 | 0.32–1.64 | 14.38 | 11.77–19.1 |
| Rep of Ireland (n76) | 5.16 | 3.47–7.42 | 1.56 | 1.14–2.1 | 4.43 | 2.73–7.13 | 1.23 | 0.69–1.78 | 0.78 | 0.4–1.44 | 14.53 | 10.37–18.9 |
| The Netherlands (n75) | 4.35 | 2.93–5.7 | 2.01 | 1.42–3.04 | 4.86 | 2.79–7.53 | 0.68 | 0.30–0.90 | 0.97 | 0.50–1.75 | 13.71 | 9.98–17.7 |

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

**Table 5.** Intake (mg/person/day) reported in several European countries

| Ref. | Lutein (+ zeaxanthin) | β-Cryptoxanthin | Lycopene | α-Carotenoid | β-Carotenoid | Dietary method/database | Foods/population assessed (subjects) |
|---|---|---|---|---|---|---|---|
| [73] | 0.92 | – | 1.03 | – | 2.21 | 4 days collection HPLC data | Vegetables UK; *N* = 79 |
| [95] | 0.67 | 0.14 | 0.74 | – | 1.51 | 7 days diary carotenoid database | Total diet UK (EPIC Norfolk cohort) N = 176 controls |
| [78] | – | 0.022–0.033 | – | 0.31–0.34 | 1.47–1.70 | 4 days weighed records (+ eating out) | Total diet UK (National Diet and Nutrition Survey, *N* = 1.478 (<65 years) |
| [96] | 2.45/2.55 (w/m) | 0.21/0.16 (w/m) | 1.30/1.05 (w/m) | 0.69/0.69 (w/m) | 2.90/2.96 (w/m) | Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database | Total diet The Netherlands *N* = 120.693[a] |
| [96] | 1.15 | 0.03 | 0.65 | 0.53 | 1.76 | Dietary questionnaire; energy-adjusted intake Harvard School of Public Health database | Total diet Finland (ATBC study, placebo branch); *N* = 6.771 men |
| [76] | 0.58 | 0.41 | 1.25 | 0.22 | 1.00 | Family Budget Survey HPLC data | Fresh fruits and vegetables Spain; *N* = 72.279 |
| [97] | – | – | – | – | 3.1–5.0 | Two 24 h recalls; CIQUAL database | Total diet Spain; *N* = 2.346 |
| [98] | 1.47 | – | 0.95 | 0.24 | 2.11 | Dietary history questionnaire EPIC database (2nd Edn.) | Total diet Spain; *N* = 354[b] |
| [99] | 0.90 | 0.64 | 2.09 | 0.26 | 1.99 | Dietary history questionnaire EPIC database (2nd Edn.) | Total diet Spain (EPIC cohort), *N* = 41.446 |
| [77] | 0.45 | 0.31 | 1.16 | 0.26 | 1.07 | Family Budget Survey HPLC data | Fresh fruits and vegetables Spain; *N* = 6.000 households |
| [100] | 4.01 | 0.17 | 7.38 | 0.15 | 2.6 | Seven-day dietary diary HPLC data | Total diet Italy (INN-CA Study); *N* = 1.968 |

a) Netherlands Cohort study; 62.412 men, 58.279 women (assessed at baseline).
b) Subjects considered as controls were patients admitted to the hospitals with a variety of diagnosis unrelated to the principal study factors (gastric cancer).

carotenoid food database was published along with the assessment, by a Food Frequency Questionnaire (FFQ) at individual level, of the carotenoid intakes of people groups in a five-country comparative study [3]. Main results are presented in Table 4. However, it should be noticed that the population used in this study was a group in a determined area of each of the five participant countries (*ca*. 80 subjects *per* country). When interpreting the data provided by that study, it should be considered that the levels of intake reported in this study are somewhat consistent with the findings in serum of the same individuals. That is, the relative crude intake and the relative contribution of xanthophylls and carotenes indicate 'true' differences in carotenoid intake (and food sources) among European countries. Participants may not necessarily be representative of the overall population although it was assumed that they followed a typical food intake pattern characteristic of their country. In addition, all subjects filled out a common FFQ.

Table 5 summarizes carotenoids intake in some European countries (UK, Finland, The Netherlands, Spain and Italy) from representative literature. To have an overall view of other countries, we have to take into account the analysis of FBSs. However, both crude data and comparisons should be considered with caution since, as shown, sample size and methodology differ between studies.

Table 6 shows the percentage contribution of individual food items evaluated by FFQ to the total intake of each of the five carotenoids in parentheses.

**Table 6.** Major foods contributing to carotenoid intake in adults in five European countries

| | France (Grenoble) Name (%) | Republic of Ireland (Cork) Name (%) | UK (Coleraine) Name (%) | The Netherlands (Zeist) Name (%) | Spain (Madrid) Name (%) |
|---|---|---|---|---|---|
| β-Carotene | Carrots (38) | Carrots (60) | Carrots (53) | Carrots (42) | Spinach (26) |
| | Spinach (14) | Tomat. prod (13) | Soups (10) | Spinach (12) | Carrots (24) |
| Lutein | Spinach (31) | Peas (19) | Peas (36) | Spinach (30) | Spinach (34) |
| | Lettuce (8) | Broccoli (16) | Broccoli (8) | Broccoli (10) | Lettuce (16) |
| | Eggs (8) | Eggs (10) | Eggs (8) | Peas (9) | Oranges (7) |
| | Mix vegetables (6) | Carrots (9) | Sweetcorn (7) | Chicory (8) | Eggs (7) |
| Lycopene | Tomatoes (25) | Tomatoes canned (23) | Tomatoes (21) | Tomato soup (29) | Tomatoes (55) |
| | Tomatoes canned (16) | Tomato soup (17) | Tomatoes canned (20) | Tomatoes (16) | Tomato puree (42) |
| | Pizza (16) | Pizza (16) | Pizza (15) | Pizza (16) | |
| α-Carotene | Carrots (82) | Carrots (90) | Carrots (88) | Carrots (87) | Carrots (60) |
| | Oranges (6) | Coleslaw (5) | Coleslaw (6) | Oranges (5) | Tangerines (17) |
| β-Cryptoxanthin | Orange juice (50) | Oranges (42) | Orange juice (45) | Tangerines (41) | Tangerines (53) |
| | Oranges (30) | Tangerines (28) | Oranges (26) | Orange juice (33) | Oranges (38) |

Source: Permission *Brit. J. Nutr.*: O'Neill *et al.* 2001 [3].

**Table 7.** Ten top contributors (%) to lutein (+ zeaxanthin) intake in five European countries[a)]

| France ($N = 76$) | Republic of Ireland ($N = 76$) | UK ($N = 71$) | The Netherlands ($N = 75$) | Spain ($N = 70$) |
|---|---|---|---|---|
| Spinach (31) | Peas (19) | Peas (36) | Spinach (30) | Spinach (34) |
| Lettuce (8) | Broccoli (16) | Broccoli (8) | Broccoli (10) | Lettuce (16) |
| Eggs (8) | Eggs (10) | Eggs (8) | Peas (9) | Oranges (7) |
| Mix vegetables (6) | Carrots (9) | Sweetcorn (7) | Chicory (8) | Eggs (7) |
| Cucumber (6) | Tomato (8) | Lettuce (6) | Lettuce (4) | Broccoli (6) |
| Green beans (4) | Oranges (7) | Carrots (4) | Tomato (4) | Peas (6) |
| Courgette (4) | Peppers (6) | Tomato (4) | Oranges (4) | Potatoes (3) |
| Peas (3) | Sweetcorn (4) | Tangerines (4) | Eggs (4) | Tangerines (3) |
| Tomato (3) | Spinach (3) | Celery (4) | Green beans (4) | Peppers (3) |
| Sweetcorn (2) | Lettuce (3) | Spinach (3) | Potatoes (4) | Leeks (2) |
| *Total (%) 75* | *Total (%) 85* | *Total (%) 84* | *Total (%) 81* | *Total (%) 97* |
| Green veg. 56% | Green veg. 47% | Green veg. 57% | Green veg. 65% | Green veg. 67% |

a) Assessed in winter.
Source: Permission *Brit. J. Nutr.*: Granado *et al.* 2003 [88].

Table 7 shows estimations using data obtained in a European multicentre study where dietary intake was estimated using a common FFQ and database of carotenoids in food [88].

As shown, although green vegetables are important contributors to lutein intake in five European groups, relative contribution differs substantially among them. It is also worth noting the relative contribution of nongreen vegetables and fruits and the fact that nongreen foods may account for almost half of the total lutein intake in some groups. More importantly, zeaxanthin, is mostly provided by nongreen vegetables and fruits [76, 88].

In Table 8, a comparison between countries on the relative contribution of each carotenoid to total carotenoids intake calculated from FBS is reported [4, 83].

While several methodological constraints (databases, groups assessed and method for dietary assessment) limit the comparability of crude intakes of carotenoids among groups, an alternative approach to compare groups/populations is to estimate the relative contribution of each carotenoid to the total intake. This approach does not overcome all the constraints regarding the reliability of the data used for comparison but may provide an interesting picture for comparative (ecological) purposes. This approach is based on several facts:

(i) The relative contribution of each carotenoid has some association with its crude intake (g/person/day), and therefore the intake of its major dietary sources, and provides information for each carotenoid (and food sources) within the context of the total diet. For example, intake of β-cryptoxanthin may be similar in two groups but the contribution to total carotenoid intake may be significantly different.

(ii) The above point relates to other nutritional and physiological facts. Carotenoids may interact with each other (synergistic and antagonistic) during absorption, transport, deposition and biological action. Thus, the relative amount of each class and type of carotenoid in the total diet become relevant [101].

**Table 8.** Relative contribution (%) of each carotenoid intake to total carotenoid intake according to FBSs data [83].

| Country | Total intake µg/day[a)] | Lutein (+ zeaxanthin) | β-Cryptoxanthin | Lycopene | α-Carotene | β-Carotene |
|---|---|---|---|---|---|---|
| Germany | 9.368 | 52 | 3 | 8 | 3 | 33 |
| Denmark | 10.092 | 52 | 4 | 7 | 3 | 34 |
| Italy | 15.753 | 45 | 4 | 15 | 3 | 33 |
| Sweden | 7.521 | 48 | 5 | 11 | 3 | 32 |
| UK | 8.654 | 50 | 4 | 9 | 3 | 33 |
| Greece | 20.968 | 40 | 3 | 21 | 4 | 32 |
| France | 13.984 | 50 | 4 | 9 | 3 | 34 |
| The Netherlands | 8.761 | 48 | 5 | 10 | 3 | 33 |
| Spain | 12.789 | 45 | 4 | 14 | 3 | 34 |
| *Europe* | *11.786* | *48* | *4* | *12* | *3* | *33* |

a) Sum of lutein (zeaxanthin), β-cryptoxanthin, lycopene, α-carotene and β-carotene.
Based on data from USDA Food Composition Tables [4].

**Table 9.** Relative contribution (%) of each carotenoid intake to total carotenoid intake[a)]

| Country (ref.) | Carotenoid intake (mg/person/day)[a)] | Foods assessed | Lutein (+ zeaxanthin) | β-Cryptoxanthin | Lycopene | α-Carotene | β-Carotene |
|---|---|---|---|---|---|---|---|
| *Spain* | | | | | | | |
| [76] | 3.5 | Fresh fruits and vegetables | 17 | 12 | 36 | 6 | 29 |
| [3] | 9.54 | Total diet | 37 | 14 | 17 | 3 | 31 |
| [99] | 5.88 | Total diet | 15 | 11 | 36 | 4 | 34 |
| [102] | 3.25 | Fresh fruits and vegetables | 14 | 10 | 36 | 8 | 33 |
| *The Netherlands* | | | | | | | |
| [103] | 6.1 | | | | | | 21 |
| [3] | 13.71 | Total diet | 15 | 7 | 35 | 7 | 32 |
| [96] | 7.55 | Total diet | 32 (M) | 3 | 17 | 9 | 38 |
| | 7.41 | | 34 (W) | 2 | 14 | 9 | 40 |
| *Finland* | | | | | | | |
| [104] | 4.0 | Total diet | 28 | <1 | 20 | 3 | 50 |
| [96] | 4.12 | Total diet | 28 | <1 | 16 | 13 | 43 |
| *France* | | | | | | | |
| [3] | 16.06 | Total diet | 16 | 3 | 30 | 5 | 36 |
| UK | | | | | | | |
| [3] | 14.38 | Total diet | 11 | 7 | 35 | 7 | 39 |
| *Rep. Ireland* | | | | | | | |
| [3] | 14.53 | Total diet | 11 | 5 | 30 | 8 | 36 |
| *Overall range (%)* | – | | *11–37* | *0–14* | *14–36* | *3–13* | *21–50* |

a) Mean or median values; total carotenoid intake = sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene.

(iii) Finally, because of each carotenoid may display different biological functions, actions and associations, relevant both at individual and population level, the relative occurrence of each carotenoid within the total diet may become important when comparing groups within an epidemiological context.

For example, as shown in Table 9 based on the data reported by O'Neill *et al*. [3], using the same dietary method and database, α-carotene and β-carotene show a consistent contribution in the European countries (3–9 and 31–39%, respectively), regardless of the dietary habits and geographical origin of the groups assessed. On the contrary, for lutein and lycopene, a different contribution pattern is observed between Spain (37 and 17%, respectively) and the rest of the European countries (11–16 and 30–35%, respectively). Regarding β-cryptoxanthin, a clearly distinct relevance is observed with Spain showing two- to three-fold more contribution than in others, especially north European countries. All these values are consistently below the mean/median values reported, for example, in Spain (0.3–0.6 mg/day) [3, 76, 77]. Thus, regardless of the method of dietary assessment and used database, sample size and endpoint measured, it is interesting to note that, compared to other dietary carotenoids, β-cryptoxanthin contribute marginally (0–7%) in North European countries (Finland, Denmark, Germany, England, Ireland), whereas in the south (*i.e.* Spain) it accounts for 10–14% (annually) and up to 20% (*i.e.* winter) to total carotenoid intake [3, 76, 77]. While this approach seems to be useful to compare exposure (nutrient intake) in different groups, it also depends on the method of assessment. This fact is highlighted when this is approached using FBSs as shown in Table 8. The apparent lack of variation in the relative contribution among European countries (except for lycopene and lutein) contrasts with the figures obtained in the individual studies performed in the same countries. This may be apparently due to the method used to estimate dietary intake since FBS provide figures on food availability (not consumption) while the individual studies provide information about 'true' nutrient intakes of the individuals although by different methods.

## 3 Effects of food processing on carotenoid stability and/on bioavailability

Carotenoid content and pattern of food material are modified during postharvest storage of plant materials, as well as during processing – at home or industry – and storage of food products. Particularly, thermal processing (*i.e.* blanching, pasteurization, cooking, canning, frying and drying) may decrease carotenoid contents, but at the same time may be beneficial through the disruption of food matrices (*e.g.* cell walls and membranes) and so facilitating the liberation (bound) and solubilization of carotenoids (free and ester forms) resulting in an increased carotenoid bioavailability. Processing operations that reduce the particle size of food material (*e.g.* chopping, grinding, milling or homogenation) or the incorporation of an oil-phase in food formulations (*e.g.* addition of oil to salads, emulsioning), may also enhance carotenoid bioaccessibility [105–109]. Emerging technologies (*e.g.* high pressure-low temperature, pulse electric fields) and several new approaches in food packaging (*e.g.* modified atmospheres, addition of antioxidants and active packages) in addition may modify carotenoid contents of food [110, 111]. Therefore, food processing implies a relevant impact on the nutritional quality of food and the stability of micronutrients in foods during food supply. Thus, food processing has a relevant impact on the dietary patterns of the population.

### 3.1 Postharvest storage

Mayer-Miebach and Spieß [112] reported that the total carotenoid content of *Kintoki* carrots was reduced by about 30% of the initial amount during 8 wks of storage at 1°C with 97% humidity. Lycopene content was reduced to about 60%, while only 20% of the β-carotene content was lost.

Kopas-Lane and Warthesen [113] found that the lutein content in spinach was nearly stable during storage at 4°C for 8 days in the dark, whereas up to 22% was lost when exposed to light.

### 3.2 Thermal processing

The scientific literature shows a wide variability of effects depending on the time/temperature conditions used (Table 10). The effects of important unit operations often used in industry are described below.

#### 3.2.1 Kinetics of thermal degradation/ isomerization

Studies towards the kinetics of thermal degradation and isomerization of carotenoids in food matrices are scarcely found in literature. Dewanto *et al.* [146] showed that the amount of all-trans-lycopene extracted from tomato homogenates, subjected to heat treatment at 88°C increased significantly 1.6-fold after 2 min and 2.7-fold after 15 or 30 min, as compared to non heated homogenates. The total (*Z*)-lycopene content increased by 6, 17 and 35% after 2, 15 and 30 min, respectively. After subjecting *Nutri Red* carrot purees with a 1% oil supplement to 2 h heat treatments at 100, 110, 120, 130 and 140°C, (all-*E*)-lycopene content decreased to 60, 63, 63, 38 and 25% of the initial value, respectively. Oil supplements had no effect on (all-*E*)-lycopene but slightly reduced isomerization. In samples without oil, (9*Z*)-lycopene increased by 10-, 30-, 41-, 43- and 38-fold at 110, 120, 130 and 140°C, respectively. Heat treatment at 70°C degraded only slight amounts of (all-*E*)-lycopene even after a 5 h heating time [121]. In homogenates of a zeaxanthin and lutein containing potato variety, the treatment temperature (25–150°C) had a much more marked effect on the carotenoid pattern than treatment time (0–5 h). The potato variety used for all experiments contained several carotenoids, mainly zeaxanthin (0.2–0.8 mg/100 g) and lutein (0.04–0.16 mg/100 g) [147]. At temperatures above 70°C lutein was totally degraded, while zeaxanthin was stable even for high-temperature and long-time treatments, regenerating 9-*cis*-zeaxanthin.

#### 3.2.2 Blanching/pasteurization

Blanching (70–105°C) and pasteurization (60–85°C) are mild heat treatments for short time periods used to inactivate enzymes and vegetative microorganisms. Data obtained by Aman *et al.* [114], analysing spinach after steam-blanching for 2 min, have shown a decrease of total lutein (17%) and (9*Z*)-lutein contents (7%), while the (13*Z*)-isomer level was unaffected. According to Choe *et al.* [115], the lutein content of spinach was stable during blanching and steaming for 2 and 5 min, respectively. Control samples contained 30.99 mg lutein and 42.86 mg β-car-

**Table 10.** Effect of thermal processing on stability of some nonprovitamin A carotenoids

| Technology | Product | Bioactive compound | Effect | Ref. |
|---|---|---|---|---|
| Blanching | Carrot (*Kintoki*) | (all-*E*)-lycopene | – | [112] |
| | Spinach | (all-*E*)-, (13*Z*)-lutein | – | [114, 115] |
| | | (all-*E*)-, (9*Z*)-lutein | ↓ | [114, 115] |
| Pasteurization | Tomato (puree) | (all-*E*)-lycopene, -lutein | – | [116] |
| | Orange (juice) | (all-*E*)-lutein | ↑ | [117] |
| | | (all-*E*)-zeaxanthin | – | [117] |
| | Orange–carrot (juice mix) | (all-*E*)-lutein | – | [118] |
| | | (all-*E*)-zeaxanthin | ↑ | [118] |
| Cooking | Tomato (homogenates) | (all-*E*)-, *cis*-lycopene | ↑ | [112] |
| | Tomato (juice) | Total lycopene | – | [119] |
| | | *cis*-Lycopene | ↑ | [119] |
| | Tomato (pulp) | (all-*E*)-lycopene | ↓ | [120] |
| | Carrot (*Nutri Red*) with/without oil | (all-*E*)-lycopene (9*Z*)-lycopene | ↓↑ | [121] |
| | Broccoli, spinach, green beans | (all-*E*)-lutein | ↑ | [122] |
| Canning | Tomato (pulp) | (all-*E*)-lycopene | ↑ | [123] |
| | Carrot (*Nutri Red*) with/without oil | (all-*E*)-lycopene (9*Z*) lycopene | ↓↑ | [121] |
| | Kale, corn, spinach, green peas | Total lutein, zeaxanthin | ↑ | [124] |
| | Corn | (all-*E*)-lutein, -zeaxanthin | – | [125] |
| | Sweet corn | (all-*E*)-lutein, -zeaxanthin | ↓ | [114] |
| Osmotic treatment | Carrot (*Nutri Red*) | (all-*E*)-lycopene | ↑ | [126] |
| Hot air drying | Tomato, carrot (*Nutri Red*) | (all-*E*)-lycopene | ↑ | [121, 127, 128] |
| | Tomato | (all-*E*)-lycopene | – | [129, 130] |
| | Tomato (paste) | (all-*E*)-lycopene | ↓ | [131] |
| | Tomato | (all-*E*)-lutein | ↑ | [129, 130, 132] |
| | Potatoes | (all-*E*)-zeaxanthin | ↑ | [133] |
| | Red pepper (whole/cut pods) | (all-*E*)-zeaxanthin | ↑ | [134, 135] |
| | Pepper (whole pods), Paprika | (all-*E*)-zeaxanthin | ↓ | [136, 137] |
| Frying | Potatoes | (all-*E*)-lutein | ↑ | [138] |
| | Carrot (chips) | Total carotenoids | ↓ | [139] |
| Microwave heating | Carrot (*Nutri Red*) (slices) | (all-*E*)-lycopene | – | [12] |
| | Broccoli | (all-*E*)-lutein | ↑ | [124, 140] |
| | Spinach, green beans, broccoli | (all-*E*)-lutein | – | [122] |
| | Sweet potatoes (leaves) | (all-*E*)-lutein | ↓ | [141] |
| | Papaya, broccoli (florets) | Total carotenoids | ↓ | [140, 142] |
| Multistep heat-treated products | Tomato (various commercial products) | (all-*E*)-, *cis*-lycopene | – | [119] |
| | Tomato (paste) | (all-*E*)-lycopene | ↑ | [143] |
| | | | ↓ | [144] |
| | | *cis*-Lycopene | – | [143] |
| | | (all-*E*)-lutein | – | [143] |
| | Orange (juice) | Total carotenoids | ↓ | [145] |

–, No changes; ↑, increase; ↓, decrease.

otene *per* 100 g sample. In an orange–carrot juice mixture, no variations in lutein content were observed after pasteurizing at 98°C for 21s, while about 45% more zeaxanthin was detected due to an enhanced extractability [118]. The same effect was shown after blanching of lycopene containing *Kintoki* carrots at 90°C for 15 min, which raised lycopene content for about 15% [107]. In tomato puree, lycopene and lutein contents were not affected by pasteurization [116].

### 3.2.3 Cooking/canning

A prolonged heating time of 2 h at 100°C caused a partial decrease (18%) of lycopene content in tomato pulp; (*Z*)-isomers were not detected [120]. The amount of lutein extracted from green peas increased by about 10–15% after boiling for 1 h [122]. In sweet corn, canning at 121°C in a rotary retort decreased total lutein and zeaxanthin content by 26 and 29%, respectively, while the amounts of (*Z*)-lutein and (*Z*)-zeaxanthin increased from 12 to 30% and from 7 to 25%, respectively. (13*Z*)-isomers of both lutein and zeaxanthin prevailed as individual stereoisomers [143]. So, from examples above mentioned, the different way of cooking could lead to a decreasing, an increasing or no variations in the content of single carotenoids; in addition, the way of cooking could modify the profile of carotenoid content in relationship with food matrix and stability of specific carotenoids in the foods.

### 3.2.4 Multistep heat treatment

After a commercial hot-break extraction of tomato paste at 90°C for 5–10 min followed by concentration under vacuum at 60–70°C and final sterilization at 121°C for

30 min, the amount of (all-*E*)-lycopene extracted was enhanced about 1.4-fold, while the (*Z*)-lycopene and lutein contents remained unchanged [115]. Also, results obtained by Agarwal *et al*. [119] indicated the stability of (all-*E*)-lycopene under industrial processing conditions: raw tomatoes and various commercial tomato products, after a multistep heat-treatment, were found to contain 5–10% (*Z*)- and 90–95% (all-*E*)-lycopene; no difference was observed.

### 3.2.5 Drying

For hot air drying, whole or chopped plant material is generally exposed to temperatures not exceeding 80°C. Therefore, no significant carotenoid losses or generation of (*Z*)-isomers are expected. However, oxidative losses may occur in some traditional slow drying methods that last over a period of few days. Much higher inlet temperatures are used for spray-drying, thus raising the probability of (*Z*)-isomer generation. Goula and Adamopoulos [131] observed oxidative lycopene losses (up to 32%) during spray-drying (air inlet temperature: 110–140°C) of tomato paste. On the other side, no significant carotenoid losses were observed in tomatoes dried at lower temperature (42°C) [130]. Enhanced carotenoid extractability after hot air drying has been reported by various authors: lycopene [121, 127, 128], lutein [132], zeaxanthin [133].

### 3.2.6 Frying

For frying, the material is cut, blanched, sometimes soaked in an antioxidant solution and, finally fried in fat or oil preheated to temperatures of 150–180°C. Food material is heated rapidly in the surface layers to the temperatures of the frying medium; however temperature does not exceed 100°C in inner layers. Lutein remained stable after frying of eight different potato varieties and a higher extractability of lutein was reported [138].

### 3.2.7 Microwave heating

The main industrial applications of microwave heating are tempering, baking and drying; other uses include blanching and cooking. In papaya, microwave blanching induced small losses of the total carotenoids [142]. Khachik *et al*. [122] studied the effect of microwave cooking on lutein retention and its (*Z*)/(*E*) ratio for different vegetables. Under mild cooking conditions (750 W; spinach: 1.5 min; green beans: 4 min; broccoli: 5 min), the lutein levels and (*Z*)/(*E*) ratios remained unchanged. During microwave cooking (700 W) of sweet potato leaves, (all-*E*)-lutein losses increased with increasing cooking times of up to 56% after 8 min; no (*Z*)-lutein isomers were formed. The (9*Z*)-lutein, contained in the fresh leaves, was completely degraded, and two lutein dehydration products were identified [141]. After microwave vacuum drying with a microwave power program of 400 W continuously, (all-*E*)-lycopene content remained stable in *Nutri Red* carrot slices. However, significant losses of carotenoids were observed, when a combined microwave power programme (600/240 W), by which high temperatures were generated, was used. No (*Z*)/(*E*) isomerization took place [121].

## 3.3 Product storage

The effects of food storage are summarized in Table 11.

### 3.3.1 Frozen storage

Long term frozen storage has been found to cause a reduction of the carotenoid content. For example, for watermelons, a decrease of up to 40% of the lycopene content was observed after 1-year storage at temperature ranges between –20 and –80°C [148]. However, lycopene was stable for three months in diced tomatoes stored at –20 and –30°C [149]. The exclusion of oxygen during frozen storage of tomato products reduces the rate of lycopene degradation [150, 151]. During frozen storage of pizza, the rate of degradation of the lycopene contained in the tomato ingredient is much faster than during frozen storage of the ingredient (tomato dices or purees) [150]. Depending on the packaging method (with/without oxygen exclusion; with/without paper box) up to 70% of lycopene may be destroyed.

### 3.3.2 Cold storage

The cold storage of minimally processed (MP) plant material – generally freshly cut and washed – has been studied by several authors. de Azevedo-Meleiro and Rodriguez-Amaya [29] reported a 19% reduction of the lutein content of MP endive after 5 days storage at 7–9°C. The lycopene content of MP watermelon (75% of the total carotenoids) slightly decreased during storage at 9°C, however stored at 5°C under light the lycopene losses were lower [34].

### 3.3.3 Storage at room temperature

During 1-year storage of commercially canned tomato juice no significant lycopene loss was observed at 25°C either at 37°C [119]. In commercially prepared tomato pulp, puree and paste lycopene remains stable even when stored under conditions of accelerated aging at 30, 40 and 50°C up to 90 days [154]. Light has an effect on the isomerization of lycopene in tomato juice: after 12 wks storage at 25°C in the dark the formation of (9*Z*)- and (13*Z*)-lycopene was favoured, while after the same time at the same temperature but using light storage (13*Z*) and (15*Z*) were the predominant lycopene isomers [159].

During storage of dried tomato products oxidation and isomerization are the main mechanisms of (all-*E*)-lycopene loss. In powders, with a great specific surface exposed to the storage conditions, an increased sensitivity for oxidative lycopene losses can be expected. Isomerization increases with increasing storage time and under illumination conditions; however oxidation increases mainly due to increased storage temperature. The residual moisture of the product

**Table 11.** Effect of storage on stability of some nonprovitamin A carotenoids

| Technology | Product | Bioactive compound | Effect | Ref. |
|---|---|---|---|---|
| Storage <0°C | Watermelon | (all-*E*)-lycopene | ↓ | [148] |
| | Tomato (diced, pulp, puree) | (all-*E*)-lycopene | ↓ | [120, 149, 150] |
| | Pizza | (all-*E*)-lycopene | ↓ | [150, 151] |
| | Green beans | (all-*E*)-lutein | ↓ | [152] |
| | Red grapefruit (juice concentrate) | Total carotenoids | ↓ | [153] |
| Storage 0–10°C | Tomato | (all-*E*)-lycopene | – | [39] |
| | Carrot (*Kintoki*) | Total carotenoids | ↓ | [112] |
| | | (all-*E*)-lycopene | ↓ | [112] |
| | Watermelons | (all-*E*)-lycopene | ↓ | [34] |
| | Spinach | (all-*E*)-lutein | – (*dark*) | [113] |
| | | (all-*E*)-lutein | ↓ (*light*) | [113] |
| | Endive | (all-*E*)-lutein | ↓ | [29] |
| Storage >10°C | Tomato | (all-*E*)-lycopene | ↑ | [39] |
| | Tomato (juice, canned juice, paste, soup, sauce, pulp, puree) | (all-*E*)-lycopene | – | [119, 154, 155] |
| | Tomato (powder) | (all-*E*)-lycopene | ↓ | [156–158] |
| | Tomato (juice) | (all-*E*)-lutein | ↓ | [159] |
| | Red pepper (whole/cut pods, powder) | (all-*E*)-lutein | ↓ | [135] |
| | Carrot (spray dried pulp) | (all-*E*)-lutein | ↓ | [160] |
| | | (9*Z*)-lutein | – | [160] |
| | | (13*Z*)-lutein | ↑ | [160] |

–, No changes; ↑, increase; ↓, decrease.

plays an important role in lycopene stability. Under inert atmosphere (nitrogen) storage, much greater lycopene losses were observed in foam-mat dried tomato powder with a moisture content <1% than in powders with ≈3% moisture content, confirming that the enhancement of oxidative reactions are associated with very low moisture materials [156]. However, in products with higher moisture contents (9–23%), an increase of moisture enhances the oxidative lycopene losses [157]. At very low moisture contents, lipid auto-oxidation is enhanced leading to important lycopene losses. In the intermediate moisture range nonenzymatic browning reactions are favoured, which could provide some protection against carotenoid oxidation.

### 3.4 Summary

It is evident that different processes have different effects on specific carotenoids probably due to: (i) the chemical/stereochemical structure of the carotenoid (*e.g.* carotene, alcohol, epoxide, (*Z*)/(*E*)-isomer), (ii) its integration into a specific food matrices (*e.g.* free or esterified, as crystals or lipid droplets), (iii) the presence of pro-oxidants ($Cu^{2+}$, $Fe^{2+}$) and/or antioxidants (ascorbic acid, vitamin E) therein and (iv) its stability upon heating time and temperature, light as well as oxygen. Therefore, it is difficult to assess a general effect of food processing. In conclusion, the effects of thermal processing and storage on stability and bioavailability of carotenoids depend mainly on the severity of the thermal treatments applied. At lower temperatures (60–100°C), most carotenoids are stable and isomerization is negligible during blanching, pasteurization, cooking, low temperature drying and frying. Due to the disruption of the matrix of plant tissues and the destruction of the integrity of cell walls and membranes, carotenoid extractability is often increased. At temperatures above 100°C, practised for canning and sterilization, total carotenoid contents are decreased, major (*Z*)-isomerization occurs and bioavailability is improved due to enhanced matrix disruption and oil supplements. The fairly high bioavailability rise at processing temperatures above 100°C may be also due to isomerization rather than matrix disruption alone. In contrast, as an effect of oxygen, carotenoids are instable during drying processes as well as during storage of fresh, dry or frozen products. Further studies about processing and storage effects on carotenoids should focus on specific carotenoids in specific vegetables/fruits with the objective of optimizing industrial processes in order to improve the bioaccessibility and bioavailability of carotenoids (see Section 4).

## 4 Bioavailability

Bioavailability is defined as the fraction of a dietary component capable of being absorbed and available for use or storage. This is a crucial point in the assessment of the role of provitamins in human health, both to overcome deficiency and to potentially decrease the risk for several chronic diseases.

### 4.1 Preabsorptive processes and absorption

Studies on absorption of carotenoids started in the early 1960s [161], however the molecular mechanisms involved in their passage through the enterocytes still remain a matter of debate [162]. Bioaccessibility of carotenoids in vegetables is remarkably low and these compounds are characterized by a slow rate of absorption both in animals and humans because their chemical structure deeply interacts with macromolecules within the plant food matrix [162]. As an example, an *in vitro* digestion model system reported that only 1–3% of the β-carotene in raw carrots is accessible for absorption; and the accessibility of lycopene in canned and fresh tomatoes was <1% [163, 164]. Further studies indicated that more than 70% of the carotenoids remained in the final digesta [165].

#### 4.1.1 Storage factor influencing the release of carotenoids from food matrix

A lot of factors can influence the initial release of carotenoids from the food matrix and their subsequent dissolution in lipidic drop in the stomach and duodenum [166]. Release from the food matrix is the initial and important step in the absorption process of carotenoids. Generally they are present in complexes with proteins as in green leaf vegetables or in semicrystalline structure as in carrots and tomatoes. Then they have to be transferred or dissolved in the lipid phase before they are absorbed. Physically altering food by cooking, blending or finely chopping improves release from the food matrix [132, 164]. Furthermore, the gastric hydrolysis of dietary lipids and proteins increases the release of carotenoids from the food matrix, and begins the process of solubilization of carotenoids into mixed lipid micelles in the gut lumen. The transfer of carotenoids from the predominantly aqueous environment to bulk lipid or micelles requires very close proximity of carotenoids to lipid micelles that starts to happen during the gastric digestion [167]. In this phase, the roles of bile salts and pancreatic secretion are critical for the emulsification, and during solubilization of carotenoids in the mixed micelles. Furthermore, Serrano *et al*. [168], showed a significant inverse correlation between small intestine availability of carotenoids (lutein + β-carotene) and content of klason lignin, non-starch polysaccharides and resistant protein in green leafy vegetables that should directly affect the intestinal availability of carotenoids acting as a barrier to the action of digestive enzymes and to the release of carotenoids from the food matrix.

Xanthophylls present in fruits, however, seem to be more efficiently released than β-carotene. *In vitro* studies indicated that, in green vegetables, epoxy-xanthophylls and their ester derivatives present in fruits are transferred more easily into the micellar phase [165, 169]. Furthermore, in the case of dietary ester of zeaxanthin, the partial hydrolysis promoted by carboxyl ester lipase during the small intestinal phase of digestion enhances the bioavailability of this carotenoid [170].

#### 4.1.2 Postharvest factors influencing the carotenoid bioavailability

The effect of food processing on carotenoids bioavailability can be illustrated by comparing the blood response after heating a raw food compared with food that has been heat-treated and/or mechanically homogenized to disrupt the food matrix. Stahl and Sies [171] found that boiling tomato juice with 1% corn oil for 1 h before consumption led to a two-fold increase in lycopene plasma concentrations compared to the consumption of tomato juice not further heated. Porrini *et al*. [172] demonstrated that plasma total lycopene levels were higher after the intake of a commercial tomato puree that had undergone a process of heating and homogenization than after raw tomato consumption, thus demonstrating a significant effect of thermal treatment on food matrix and on absorption. On the same way, van het Hof *et al*. [173] observed that both, heating tomato for 1 h at 100°C and homogenization under high pressure, enhanced the lycopene response in both, triglyceride-rich lipoproteins and plasma, significantly. During sterilization of a *Nutri Red* carrot homogenate with a 1% oil supplement at 130°C for 30 min, the isomeric ratio of (all-*E*)- to total (*Z*)-isomers changed from 90:10 to 50:50. Isomeric ratio of the same homogenate, cooked at 100°C for 30 min without oil supplementation, was not altered. For consumption, oil content of all samples was 1%. Compared to the ingestion of an untreated control (blanched and stored at –50°C), a nine-fold increase with the lycopene content of the chylomicron fraction was found in the sterilized sample; bioavailability of the cooked samples increased by only 2.5-fold. Although no (5*Z*)-lycopene was generated in the homogenates during any of both thermal treatments, this isomer accounted for about 20% of the total lycopene in chylomicrons [174]. A remarkable enrichment of the relative contents of (5*Z*)-lycopene was also observed after ingestion of tomatoes, tomato juice and purée, respectively [175]. In contrast, lycopene uptake from whole cherry tomatoes, ingested either fresh or cooked at 100°C for 15 min without previous mechanical disruption, was not altered [176].

#### 4.1.3 The composition of the meal on bioavailability

Experimental evidence has been accumulated on the role of dietary fat in the absorption and bioconversion of provitamin A carotenoids to vitamin A [14, 177]. The dietary fat intake plays an important role in the plasma responses to β-carotene supplements [178]. Recently, Brown *et al*. [179] showed that use of fat-free or reduced-fat salad dressings limited the absorption of carotenoids, which are abundant in fresh vegetable salads. In a view of these results, the authors suggested the threshold of 3–5 g fat *per* meal reported by Roodenburg *et al.* [180] and adopted as a guideline to promote optimal absorption of β-carotene [181]. In the study by Roodenburg *et al.*, α-carotene and β-carotene were provided in the form of pure supplements dissolved in fat, and not from plant foods. Other investigators used plant sources and found that minimal dietary fat (2.4 g/meal) is sufficient for optimal absorption of provitamin A carotenoids and their bioconversion into vitamin A [9]. The effects of lipid intake on the absorption of carotenoids was confirmed by the observation that the addition of avocado fruit or avocado oil as a lipid source enhances absorption of lycopene and β-carotene and α-carotene, β-carotene and lutein, respectively in humans [182]. Dietary fibre intake is another factor that could regulate carotenoid bioavailability. It is a known fact that fibre decreases the absorption of carotenoids by entrapping them and interacting with bile acids; this leads to an increase of faecal excretion of fats and fat-soluble substances such as carotenoids [183, 184].

The inter-relationship of the different carotenoids present in the food matrix also affects carotenoid absorption. A competitive inhibition, towards the absorption mechanism of a single carotenoid derivative, in fact, may occur at the level of micellar incorporation, intestinal uptake, or lymphatic transport or at one or more of the later steps. It has been proposed that a high-dose intake of carotenoids may antagonize the bioavailability and absorption of other carotenoids. For example, studies on simultaneous ingestion of carotenoids indicate that β-carotene may interfere with absorption of lutein and canthaxanthin, while high doses of simultaneous combination between lycopene and β-carotene decrease bioavailability of both [185, 186]. In contrast, Hoppe *et al*. [187], showed no interaction towards lycopene absorption by β-carotene, β-cryptoxanthin, α-carotene, lutein and zeaxanthin.

#### 4.1.4 Physiological state of the consumer

Parasitism and disease resulting in intestinal dysfunction may have profound effects on carotenoid uptake and bioconversion, but these pathological states have not yet been adequately quantified. For example, in some studies, the lack of observed improvement in vitamin A status in individuals consuming dark green, leafy vegetables may be attributable, at least in part, to concomitant infection with intestinal helminths, *Helicobacter pylori*, or other organisms [188]. Persistent diarrhoea, lipid malabsorption, and deficiencies of vitamin A, protein and zinc also appear to be important factors that impair provitamin A-carotenoid utilization, in addition to their effects on vitamin A metabolism and turnover [166]. Carotenoid-rich fruits and vegetables may indeed provide sufficient vitamin A to meet physiological requirements and even replete body stores under conditions of relatively good health and hygiene. However, debilitating infections and parasitic infestations which are endemic in the tropics and subtropics both compromise carotenoid utilization and increase the individual's requirement for vitamin A. Thus, programs which seek to improve community vitamin A status through food-based interventions will be complemented and strengthened by public health measures which decrease the burden of infection and illness.

Also, age is an other factor that contributes to carotenoid bioavailability [189]. Carroll *et al*. [190] estimated from the analysis FFQ that β-carotene and lycopene are the major dietary carotenoids obtained from a younger and older Irish population. The profile of plasma carotenoid concentrations showed that β-carotene is the major carotenoid in both age groups. Younger groups have higher plasma concentrations of lycopene, β-cryptoxanthin, lutein + zeaxanthin. As described in other European populations these moderate positive associations exist between several plasma carotenoid concentrations and estimated record dietary carotenoids in younger but not in older groups [191].

### 4.2 Methodology to assess bioavailability

Several confounding factors are present in the literature regarding the assessment of carotenoid bioavailability in humans. Generally the pharmacokinetic studies only provided information on relative bioavailability (relative to reference dose or control) and not on the absolute bioavailability of the carotenoids. Moreover, acute studies need to use large doses of carotenoids to elicit a quantifiable change in blood or urine excretion.

Frequently the approaches used in human studies are short-term, single-dose, pharmacokinetic studies or long-term, multiple-dose supplementation assays. In the latter, the information obtained, relative to nutritional status, depletion and/or saturation processes, could be affected by the typology of the protocol used (*i.e*. on samples collections, 'acute', postprandial metabolism or 'chronic') [192]. Furthermore, these studies could be broadly divided into those using large pharmacological doses, which are only partly available due to limitations in the absorption process, and those using more physiological carotenoid doses, either using pure substances, and different matrices, including foods.

Another critical point is the individual response. Based mostly on plasma concentrations observed after carotenoid administration, there is evidence to suggest that there are 'poor' and 'good' absorbers. This fact is frequently observed in single-dose kinetic studies whereas in long-term studies most of the subjects show significant, though highly variable responses. Thus, this discrimination of subjects based on plasma responses has been criticized since a lack of acute plasma response does not necessarily mean absence of absorption.

Studies of bioavailability of carotenoids, however, are difficult for the endogenous presence in plasma and tissues of carotenoids. In most cases, larger doses than those provided by mixed diets need to be supplied in order to observe variations in plasma. To overcome this problem stable isotope-labelled carotenoids are being increasingly used to assess nutrient bioavailability [193]. In this regard, stable isotope labelling can be performed both intrinsically (in growing foods) and extrinsically (single compounds), allowing the study of carotenoid bioavailability (*i.e.* absorption, transport, distribution, storage, excretion, turn-over, …) at dietary levels and regardless of endogenous presence.

These methods, however, have limitations (*i.e.* the perceived health risk and the costs associated with the necessary methodology). Because of these limitations, many studies have been performed using *in vitro* and animal models. Although animal models may provide relevant information with regard to bioavailability in man, no one animal model completely mimics human absorption and metabolism of carotenoids [194]. Extrapolation of these results and their relevance to humans should, therefore, be considered with caution.

*In vitro* models based on human physiology have been developed as simple, inexpensive and reproducible tools to study digestive stability, micellization, intestinal transport and metabolism and to predict the bioavailability of different food components. *In vitro* models have been used in studies on vitamin and carotenoid absorption mechanisms and, recently, models of *in vitro* digestion, micellarization and uptake by cell culture (Caco-2 cells) have been used as a model to assess carotenoid bioavailability from foods [195]. This approach is useful for studying preabsorptive processes and thus food related factors that affect bioavailability. Nonetheless, some type of standardization is needed and a wider use of these protocols will determine whether they are valid in predicting absorbability and/or bioavailability in humans.

Finally, an interesting alternative to estimate carotenoid bioavailability could be the evaluation of compartmental modelling that allows us to describe the absorption, redistribution and disposal of nutrients in the body [196, 197].

### 4.3 Tissue culture experiments for cellular uptake and metabolism

Although the intestinal uptake of carotenoids has been thought to occur by simple diffusion [198], recent studies have reported the existence of protein-mediated transport of carotenoids in enterocytes. Studies in Caco-2 cell monolayers indicate [199–201] that carotenoids and cholesterol could share common mechanistic pathways across the intestinal cell. In fact ezetimibe (EZ), an inhibitor of cholesterol transport as well as cholesterol itself inhibited (in a concentration-dependent manner) β-carotene transport, but did not affect retinol transport. This suggests that β-carotene and cholesterol interact during their transport through Caco-2 cells, and, therefore, nonpolar carotenoids and cholesterol share one (or more) common transporter(s). The scavenger receptor type B1 (SR-BI) was postulated to play a role in intestinal cholesterol [202, 203], and β-carotene absorption. In a similar manner, the putative proteins involved in the facilitated diffusion of carotenoids are identified in the Niemann-Pick C1Like 1(NPC1L1) and the adenosine triphosphate (ATP)-binding cassette (ABC) A1 transporter.

A similar *in vitro* system was proposed to study lutein absorption. Lutein was added to Caco-2 cell culture and the absorption of lutein was measured. The rate of transport of lutein micelles (lutein mixed with phospholipids, lysophospholipids, cholesterol, monoolein, oleic acid and taurocholate) was time- and concentration-dependent and was inhibited by coincubation with anti-SR-BI antibody and BLT1 (a leukotriene receptor). Coincubation with β-carotene, but not lycopene, decreased the lutein absorption rate (approx. 20%) significantly. These results suggest that lutein absorption is, at least partly, protein-mediated and that some lutein is taken up through SR-BI [204]. Although a binding protein specific to lycopene has not yet been verified, *in vivo* studies in rats suggested that one may exist. This could explain the preferential uptake of 14C-lycopene in some tissues [205].

Once the carotenoid is inside the enterocyte its fate depends on its structure. If the carotenoid contains an unsubstituted β-ionone ring with a polyene side-chain of at least 11 carbon atoms, it can be cleaved enzymatically to vitamin A. This central cleavage pathway, which requires molecular oxygen, is catalysed by the enzyme carotenoid 15,15′-monooxygenase, and yields two molecules of (all-*E*)-retinal from (all-*E*) β-carotene. This enzyme apparently cleaves (9*Z*) β-carotene also, yielding a 1:1 mixture of (all-*E*) and (9*Z*) retinal, which can be further oxidized to (9*Z*) retinoic acid. The (9*Z*) and (all-*E*) isomers of β-carotene can also be interconverted [206].

The second pathway of β-carotene metabolism is the eccentric cleavage, which occurs at double bonds other than the central 15,15′-double bond of the polyene chain of β-carotene to produce β-apo-carotenals with different chain lengths. However, given that only trace amounts of apocarotenals are detected in *in vivo* treatment [207] and that they can be formed nonenzymatically from β-carotene auto-oxidation [208], the existence of this pathway has been the subject of debate. The two major sites of β-carotene conversion in humans are the intestine and liver. By direct determination of β-carotene oxygenase activity in human small intestine and liver samples, it was estimated that in a human adult the maximum capacity for β-carotene cleavage by the two tissues would be 12 mg β-carotene *per* day [209]; this amount is much higher than the observed average daily intake of 1.5 mg *per* day in the United States or even the higher daily intake of 6 mg β-carotene/day suggested by some authors as being needed to meet the goal of 90% of vitamin A intake [210].

Very little is known about cellular events that regulate or facilitate the incorporation of carotenoids into lymphatic lipoproteins. Still unsolved is how the flow of hydrophobic carotenoids within the enterocyte is controlled. The poor solubility of carotenoids in aqueous solutions suggests the need for a cytosolic binding protein, but to date no specific binding protein for carotenoids in the intestinal mucosa has been reported. Under normal dietary conditions both the retinyl esters formed from carotenoids in the enterocyte and the intact absorbed carotenoids are incorporated into lymphatic chylomicron [211].

### 4.4 Human studies

The wide presence of carotenoids in foods have attracted the researchers' attention towards human intervention studies. Up till now, many papers have been published in this area and, considering the wide variety of parameters and factors evaluated, it becomes quite difficult to be exhaustive in the description of the so many aspects of carotenoid bioavailability.

In the EPIC study [212], a typical population groups study, the mean of the sum of the six measured carotenoids (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin) varied two-fold between regions in men and women (1.35 μmol/L for men in Malmö *vs*. 2.79 μmol/L for men in Ragusa/Naples; 1.61 μmol/L for women in The Netherlands *vs*. 3.52 μmol/L for women in Ragusa/Naples). Women had higher plasma levels of carotenoids than men, except in the case of lycopene. This is in agreement with data reported earlier [74, 213]. Mean carotenoid levels in plasma, in population groups of several regions, showed broader distributions: Italian regions, Athens and UK vegetarians had the highest lycopene and lutein levels while β-carotene and α-carotene were highest among UK vegetarians and β-cryptoxanthin levels were higher in the Spanish regions [212].

Supplementation studies represent another way to test the carotenoids bioavailability in humans; within a multicentre study, serum responses to carotenoid supplementation (lutein, lycopene or α-carotene + β-carotene) were assessed in a randomized, placebo-controlled intervention study [214]. The trial involved 400 apparently healthy men and women (40 men, 40 women/region) from five European regions (France, Northern Ireland, Republic of Ireland, The Netherlands and Spain) and it was conducted using identical time protocols (16 months), capsule preparations and very similar doses (approx. 15 mg carotenoids), allowing relative comparisons between each carotenoid treatment. In addition, the centralization, randomization and quality control of analysis eliminate interlaboratory analytical bias and improve reliability of the results. Carotenoid supplementation was set at dietary achievable levels and then, the supplement of α- and β-carotene supplied an amount equivalent to that contained in 100 g cooked carrots; lutein amount was similar to that present in 200 g cooked spinach and lycopene was equivalent to that provided by 600 g raw tomato or 100 g tomato paste. Data from this study showed that supplementation with α + β-carotene (carotene-rich palm-oil) resulted in a 14- and 5-fold increase in serum levels respectively. Supplementation with lutein (from marigold extracts) elevated serum lutein (about five-fold), zeaxanthin (about double) and ketocarotenoids (not supplied), whereas lycopene supplementation (derived from tomato paste) resulted in a two-fold increase in serum lycopene. Isomer distribution of β-carotene and lycopene in serum remains constant regardless of the isomer composition in the capsules. In Spanish volunteers, additional data [215] showed that serum response to carotenoid supplementation reached a plateau after 4 wks of supplementation whereas no significant side-effects (except carotenodermia) nor changes in biochemical or haematological indices were observed. The presence of a chromatographic peak (tentatively identified as lutein monopalmitate) was only detected in subjects with relatively high serum lutein levels (>1.05 μmol/L). This peak may be indicative of a ceiling effect on saturation of the transport capacity of lutein, which may be re-esterified *in vivo* when it is supplied in excess of normal dietary intake [214, 215].

A lot of human epidemiological studies suggest a protective effect of diets rich in carotenoids, composed mainly of fruit and vegetables, against cancers at various sites. In contrast, intervention studies with higher concentrations of synthetic β-carotene more available than that in fruit and vegetables, have failed to provide the expected protection [216–221]. In addition, β-carotene is an important antioxidant in our daily diet which might be significant for health promoting even if its role for disease prevention is still not clear. Concerning lycopene, a correlation between lycopene derived from tomato products supplementation and risk of prostate cancer, was reported by Basu and Imrhan [222] in a recent review of 20 studies, even if future investigations are required to clarify the lycopene role and its action mechanism.

These results suggest that at present there is still insufficient evidence to advocate the consumption of isolated carotenoids for prevention of several chronic diseases [79, 223–227]. In fact, data collected with the same methodology, comparable and representing a large number of population are required to quantify the intake of carotenoids and to represent the consumption of the population.

## 5 Concluding remarks

Carotenoids are a wide variety of molecules present in the human diet so our review is extensive and covers different aspects. The main dietary sources of carotenoids were reviewed. We have tried to summarize positive and negative effects of food processing, storage, cooking on carotenoid bioavailability. In particular, we have evidenced the possibility to improve carotenoids bioavailability in accordance with changes and variations of technology procedures.

We focused our attention on several factors influencing carotenoid accumulation and bioavailability and on the potential health properties and possible biological role of these phytochemicals in human physiology.

The metabolism, absorption and excretion of carotenoids have been studied extensively *in vitro*, in animal models and in humans.

Although a lot of literature data are available for the design and interpretation of intervention studies [228, 229], further investigations are required to understand the absorption and metabolism pathways and the action mechanism of carotenoids in humans. From this point of view, this paper could be a useful updated knowledge for both expert and not expert readers. It also highlights the need for further research with appropriate approaches (*i.e*. dietary intake evaluation, development and update of a carotenoid database for different countries).

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