ORIGINAL SUBMISSION

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Division of
Biotechnology and
GRAS Notice Review

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5 100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS notice for palm carotenoids

Dear Sir/Madam:

Pursuant to the proposed rule outlined at *Federal Register,* Vol. 62, No. 74, p. 18936 - 18937, April 17, 1997 (Sec. 170.36 of Part 21 of the *Code* of *Federal Regulations*), Malaysian Palm Oil Board (MPOB) hereby submits this notification that the use of palm carotenoids as ingredients in foods is exempt from the premarket approval requirements of the Food, Drug, and Cosmetic Act because MPOB has determined that such use is generally recognized as safe (GRAS) based on scientific procedures.

Three copies of the GRAS Exemption Claim, additional information documents, and an electronic copy of the documents are enclosed.

Sincerely,

Susan S. Cho, Ph.D.
Consultant to MPOB

GRAS exemption claim for palm carotenoids

We hereby claim that the use of the substances described below (palm carotenoids from the Malaysian Palm Oil Board (MPOB)) are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined such use is Generally Recognized As Safe (GRAS).

On behalf of Malaysian Palm Oil Board (MPOB), NutraSource (an independent consulting firm) assembled a panel of experts highly qualified by scientific training and experience to evaluate the safety of the intended uses of palm carotenoids. The panel included Dr. Susan Cho at NutraSource (Clarksville, MD), Dr. Michael Falk at Life Science Research Organization (LSRO, Bethesda, MD), and Dr. George Fahey at the University of Illinois (Urbana, IL). Following independent critical evaluation of the available data and information, the panel has determined that the use of palm carotenoids (that are manufactured by an industry member of MPOB, such as Carotech and Carotino) described in the enclosed notification is GRAS based on scientific procedures.

After reviewing the available data, the Expert Panel also concluded in its December 2009 statement that the intended use of MPOB palm carotenoids (to be used as an ingredient in breakfast cereals, juice drinks, energy drinks, dairy fruit drinks, instant soup, low fat dressings, meal replacements, meatless meat products, nutrient bars, salty snacks and crackers, and yogurt as well as in medical foods), is safe and GRAS for the general population. Under the intended use, estimated daily median intakes in the U.S. population aged 1 y and above are 0.87, 0.20, and 1.75 mg and 90th percentile daily intakes are 4.78, 1.14, and 14.8 mg for β-carotene, α-carotene, and lycopene, respectively.

This determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

1. **Name and address of the notifier:**
   Rosidah Radzian
   Malaysian Palm Oil Board TAS America
   Malaysian Embassy Building
   3516 International Ct., NW.
   Washington, D.C. 20008

2. **Common or trade name:** Palm mixed carotene complex, palm carotene concentrate, palm carotene concentrate, or palm mixed carotene.
   Trade Names: Carotech products - Caromin® 10%, Caromin® 20%, Caromin® 25%, Caromin® 30%, Carosol™ 3%, Alphabeads® 7.5%, Carosem™ 1%, and Carosem™ 5%; Carotino products - Carotino CC-05, Carotino CC-10, Carotino CC-20, and Carotino CC-30.

3. **Product description:** Palm carotene concentrate contains a full spectrum carotenoid complex with high concentrations of β-carotene and α-carotene as well as small amounts

3. **Product description:** Palm carotene concentrate contains a full spectrum carotenoid complex with high concentrations of β-carotene and α-carotene as well as small amounts of γ-carotene and lycopene. It is important to note that the carotenoid profile of crude palm oil is similar to that of carrot, with α-carotene and β-carotene constituting more than 90% of the total carotenoids. The industry members of Malaysian Palm Oil Board (MPOB), Carotech and Carotino, formulate their palm carotenoids into 5%, 10%, 20%, 25%, and 30% carotenoid oil suspensions (in palm oil), 7.5% beadlets, 3% powder, and 1% and 5% emulsion products prior to marketing.

4. **Applicable conditions of use of the notified substance**

   a. Foods in which he substance to be used:

   Palm carotenoids are intended for use, as a dietary source of carotenoids in foods such as breakfast cereals, juice drinks, energy drinks, soups, dressings, fats and oils, meal replacements, meatless meat products, nutrient bars, salty snacks and crackers, yogurt, egg and imitation egg products, and medical foods.

   b. Levels of use in such foods

   Intended use of palm carotenoids—recommended concentrations, mg/kg.

Food category	Total carotenoids	β-carotene	α-carotene	-carotene	Lycopene
Fats and oils, margarine	15.58		5.15	0.36	0.06
Canned green peas, canned mangoes, mayonnaise	15.58	10	5.15	0.36	0.06
Meatless bullions and consomme	31.16	20	10.3	0.72	0.12
Pickled cucumber	31.16	20	10.3	0.72	0.12
Baked goods, bread, rolls, cakes	2.93	1.88	0.969	0.0676	0.0112
Meatless soups, soup mixes	2.93	1.88	0.969	0.0676	0.0112
Baking mixes, cookies, crackers, salty snacks	2.93	1.88	0.969	0.0676	0.0112
Grain products, such as pastas or rice dishes	2.93	1.88	0.969	0.0676	0.0112
Ready-to-eat cereals	2.93	1.88	0.969	0.0676	0.0112
Frozen dairy desserts and mixes, ice cream and mixes	2.93	1.88	0.969	0.0676	0.0112
Processed fruits, juices, juice drinks, punches	2.93	1.88	0.969	0.0676	0.0112
Meatless meat products (soy-based imitation meat)	2.93	1.88	0.969	0.0676	0.0112
Processed vegetables, juices	2.93	1.88	0.969	0.0676	0.0112
Sweet sauces, toppings, syrups	2.93	1.88	0.969	0.0676	0.0112
Gelatins, puddings, fillings	2.93	1.88	0.969	0.0676	0.0112
Meatless gravies, sauces	2.93	1.88	0.969	0.0676	0.0112
Imitation egg products	2.93	1.88	0.969	0.0676	0.0112
Soft drink-orange drink type only	1.00	1.88	33.1	2.3	0.4
Granola bars, cereal bars	2.93	1.88	0.969	0.0676	0.0112
Meal replacements shakes, mixes, functional beverages	2.93 mg/kg liquid	1.88	0.969	0.0676	0.0112
Medicinal foods		180 mg/d			

Intended use - Maximum concentrations of palm carotenoids, mg/kg.

Food category	Total carotenoids	β-carotene	α-carotene	- carotene	Lycopene
Fats and oils, margarine	38.9	25	12.875	0.9	0.15
Canned green peas, canned mangoes, mayonnaise	155.8	100	51.5	3.6	0.6
Meatless bullions and consommé	311.6	200	103	7.2	1.2
Pickled cucumber	467.4	300	154.5	10.8	1.8
Baked goods, bread, rolls, cakes	15.58	10	5.15	0.36	0.06
Meatless soups, soup mixes	15.58	10	5.15	0.36	0.06
Baking mixes, cookies, crackers, salty snacks	15.58	10	5.15	0.36	0.06
Grain products, such as pastas or rice dishes	15.58	10	5.15	0.36	0.06
Ready-to-eat cereals	15.58	10	5.15	0.36	0.06
Frozen dairy desserts and mixes, ice cream and mixes	15.58	10	5.15	0.36	0.06
Processed fruits, juices, juice drinks, punches	15.58	10	5.15	0.36	0.06
Meatless meat products (soy-based imitation meat)	15.58	10	5.15	0.36	0.06
Processed vegetables, juices	15.58	10	5.15	0.36	0.06
Sweet sauces, toppings, syrups	15.58	10	5.15	0.36	0.06
Gelatins, puddings, fillings	15.58	10	5.15	0.36	0.06
Meatless gravies, sauces	15.58	10	5.15	0.36	0.06
Egg substitute products	15.58	10	5.15	0.36	0.06
Soft drink-orange drink type only	15.58	10	5.15	0.36	0.06
Granola bars, cereal bars	15.58	10	5.15	0.36	0.06
Meal replacements shakes, mixes, functional beverages	15.58	10	5.15	0.36	0.06

c. Purposes for which the substance is used: As a food ingredient for the population consuming one or more of the food products listed above, except for heavy smokers, heavy alcohol drinkers and workers exposed to asbestos.

5. **Exposure estimates**

The first set of estimates is based on the assumption that the products in each food category will be used at the recommended intake levels under the intended use. The median intakes would be 2.1, 0.78, and 1.75 mg/d for β-carotene, α-carotene, and lycopene in the U.S. population aged 1 y and above. The 90th percentile intakes were estimated to be 6.6, 2.2, and 14.9 mg/d for β-carotene, α-carotene, and lycopene, respectively (Table 7). The second set of estimates is based on the assumption that all the products in each food category will be used at the maximum intake levels under the intended use although it is far from a realistic situation. Even if all of the products are used at the maximum levels, the median intakes would be 7.6, 3.5, and 1.8 mg/d for β-carotene, α-carotene, and lycopene in the U.S. population aged 1 y and above. The 90th percentile intakes were estimated to be 18.2, 8.3, and 14.9 mg/d for β-carotene, α-carotene, and lycopene, respectively (Table 8). It is estimated that the intended use would

increase the γ-carotene intake levels by 0.04 mg/d (the level of increase observed for lycopene) since the concentrations of γ-carotene and lycopene are comparable in palm mixed carotene.

Medical foods (180 mg/d) have not been included in the exposure estimate of the general population since those foods will be used by a small segment of a special population.

6. **Basis of GRAS determination**: Through scientific procedures.

7. **Review and copying statement**: The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of notifier, or will be sent to FDA upon request.

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carotene, and lycopene, respectively (Table 8). It is estimated that the intended use would increase the γ-carotene intake levels by 0.04 mg/d (the level of increase observed for lycopene) since the concentrations of γ-carotene and lycopene are comparable in palm mixed carotene.

Medical foods (180 mg/d) have not been included in the exposure estimate of the general population since those foods will be used by a small segment of a special population.

6. **Basis of GRAS determination**: Through scientific procedures.

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I. Identity of substance

A. Common or trade name: Palm mixed carotene complex, palm carotene concentrate, palm carotene concentrate, or palm mixed carotene.
Trade Names: Carotech products - Caromin® 10%, Caromin® 20%, Caromin® 25%, Caromin® 30%, Carosol™ 3%, Alphabeads® 7.5%, Carosem™ 1%, and Carosem™ 5%;
Carotino products - Carotino CC-05, Carotino CC-10, Carotino CC-20, and Carotino CC-30.

B. Product description: Palm carotene concentrate contains a full spectrum carotenoid complex with high concentrations of β-carotene and α-carotene as well as small amounts of γ-carotene and lycopene. It is important to note that the carotenoid profile of crude palm oil is similar to that of carrot, with α-carotene and β-carotene constituting more than 90% of the total carotenoids. The industry members of Malaysian Palm Oil Board (MPOB), Carotech and Carotino, formulate their palm carotenoids into 5%, 10%, 20%, 25%, and 30% carotenoid oil suspensions (in palm oil), 7.5% beadlets, 3% powder, and 1% and 5% emulsion products prior to marketing.

C. Standards of identity: We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity that is located in Title 21 of the Code of Federal Regulations.

D. Background

There are more than 600 carotenoids known to exist in nature. Carotenoids are yellow, orange, and red pigments that are widely distributed in plants (IOM 2000, 2001). About 50 of the naturally occurring carotenoids can potentially yield vitamin A and thus are referred to as provitamin A carotenoids. β-Carotene is the most abundant and most efficient provitamin A in foods. Carotenoids are fat-soluble pigments found primarily in fruits and vegetables. Carotenoids play a primary role as powerful antioxidants to counter the ravages of free radical-mediated chronic diseases, such as cardiovascular diseases (CVD), cancer, aging, and inflammation (Voutilainen et al. 2006).

Palm carotene concentrate contains a wholesome, full spectrum carotenoid complex with high concentrations of β-carotene and α-carotene as well as small amounts of γ-carotene and lycopene. It is important to note that the carotenoid profile of crude palm oil is similar to that of carrot, with α-carotene and β-carotene constituting more than 90% of the total carotenoids (Table 1). Carotenoid-rich oil can be obtained from the pressed fiber of the oil palm fruit. Extracted oil contains 4,000-6,000 ppm of carotenes.

Table 1. Carotenoid composition (%)

	Palm oil - *E. guineensis pisifera*	Palm oil – *E. oleifera*	palm carotene concentrate	Carrot
β-carotene	54.39	54.08	66.7	64.2
α-carotene	33.11	40.38	32.5	32.9
Lycopene	4.50	0.07	0.52	0
γ-carotene	0.48	0.08	0.52	NA
Zeacarotene	1.2	1.0		
Lutein + zeaxanthin				2.9

From Choo 1994, Ong and Tee 1992, and Holden et al. 1999

Palm oil is a semi-solid fat at ordinary room temperature due to the presence of a mixture of solid saturated triglycrerides and high melting point oleoglycerides and mono-linoleoglycerides dispersion throughout the liquid dioleoglycerides. Fractionation extends the uses of palm oil; the products obtained are a liquid oil (olein, 70-80%) and a solid fat (stearin, 20-30%). Palm oil is considered a GRAS ingredient based on its historical use prior to 1958 in the U.S.

E. Chemistry and structure of β-carotenoids

Palm mixed carotenoids are several carotenoids extracted from palm oil, mostly β-carotene and α-carotene with small quantities of lycopene and other carotenoids. Caromin® contains both the cis- and trans-isomers (the ratio of cis:trans=35:65) of β-carotene compared to synthetic β-carotene that typically provides only the trans-isomer. Chemical formulas, molecular weights (MW), and Chemical Abstracts Service (CAS) numbers of carotenoids are summarized in Table 2 and Figure 1.

Table 2. Chemical formulas, molecular weights (MW), and CAS numbers of carotenoids

Compound	Semi-systematic Names	MW	CAS No.
α-Carotene	β, ε- Carotene	536.88	7488-99-5
β-Carotene	β, β- Carotene	536.88	7235-40-7
γ-Carotene	β, Ψ- Carotene	536.88	472-93-5
Lycopene	Ψ, Ψ- Carotene	536.88	502-65-8

Empirical molecular Formula $C_{40}H_{56}$

Carotenoids can lose some of their activity in foods during storage due to the action of enzymes and exposure to light and oxygen. Dehydration of vegetables and fruits may greatly reduce the biological activity of carotenoids. On the other hand, carotenoid stability is retained in frozen foods.

F. Manufacturing process

Industry members of MPOB, Carotech and Carotino, describe their manufacturing process as follows: The starting raw material is crude palm oil, an edible vegetable oil extracted from the mesocarp of the oil palm. Crude palm oil is naturally reddish in color because it contains a high amount of natural mixed carotenoids. Crude palm oil contains the highest natural concentration

of mixed carotenoids and palm tocotrienol-rich fraction (TRF) in nature (500 parts per million (ppm) carotenoids and 800 ppm TRF). In addition to these two phytonutrients, there are other minor components naturally found in palm oil such as sterols, squalene, and Co-Q10. These phytonutrients (minor components) make up about 1% in total of the oil. The remaining 99% is the oil (triglycerides).

A patented process (US Patent No. 5,157,132) extracts and concentrates this 1% of minor components in the fruits of oil palm. In the process, crude palm oil is converted to methyl esters and glycerol through trans-esterification. The minor components such as the mixed carotenoids, TRF, squalene, sterol, etc are not involved in this reaction and are not changed chemically or structurally. The ratio, chemical structure, and characteristics remain the same in the starting natural crude palm oil. The reaction process only targets and involves the oil part (triglycerides). The remaining phytonutrients are washed counter-currently with hot water and are subjected to molecular distillation. In the molecular distillation process, under extreme vacuum and low temperature, all the methyl esters are distilled out to leave a concentrated residue of carotenoids. The concentrated carotenoids then are subjected again to molecular distillation (varying vacuum and temperature) to recover various concentrations of mixed carotenoids (Caromin® 20%, 25%, and 30%) (US Patent No. 5,157,132 and US 7,141,712 B2).

Caromin® liquids are the starting material for the formulation of palm Caromin® beadlets. Gelatin, corn starch, sucrose (GRAS; 21CFR184.1854), and ascorbyl palmitate (GRAS; 21CFR184.3149) are added to Caromin® liquids. The mixture is spray-dried into dry beadlets and powders.

G. Specifications

Tables 3-1 to Table 3-12 list specifications of palm carotenoids. In general, specifications have the following common characteristics: (1) Any number in the product name indicates the concentration (as a %) of total carotenoids; for example, Caromin® 30% means the product contains 30% carotenoids and 70% (100%-30%) of other components. Carotino CC-05 means the product contains 5% carotenoids and 95% (100%-5%) of other components. (2) The maximum moisture concentration is 0.5-1% in a liquid form of palm Caromin®. (3) Caromins are available in many forms: liquid (oil suspension), powders, beadlets, and emulsion. (4) Among components other than carotenoids, the liquid form contains mostly palm oil. The beadlet form contains a mixture of gelatin, corn starch, sucrose, and ascorbyl palmitate in addition to small amounts of palm oil. The powder form contains modified starch, ascorbic acid, natural tocopherol, silicon dioxide, plant-based emulsifier. The emulsion form contains glycerine, rosemary extract, mixed tocopherols, polysorbate 80, sorbitan monooleate, ascorbyl palmitate, water, and vegetable oil. Physical functionality of each carotenoid form determines the composition of the ingredients other than palm carotenoids For example, the emulsion form requires emulsifiers such as polysorbate 80 and sorbitan monooleate.

Unless otherwise noted, the palm carotenoids have the following common specifications: pH value of 6-7.5, moisture <1.0 %, lead <0.5 ppm, standard plate count <1000 CFU/g, yeast and mold <100 CFU/g, and *E. coli* and Salmonella-negative. Caromins contain approximately

0.3% tocopherols. Caromins have a shelf life of 36 mo in their unopened original container when they are kept in cool, dry areas away from heat or sunlight.

Table 3-1. Specifications of Carotech palm carotenoids - Caromin® 10% (oil suspension)

Composition	%
α-carotene, %	3.3
β - carotene, %	6.5
γ- carotene, %	0.35
Lycopene, %	0.2
Total carotenoids, %	>10.0
Palm oil, %	<90.0

Table 3-2. Specifications of Carotech palm carotenoids - Caromin® 20% (oil suspension)

Composition	%
α-carotene, %	6.5
β - carotene, %	13.0
γ- carotene, %	0.7
Lycopene, %	0.4
Total carotenoids, %	>20.0
Palm oil, %	<80.0

Table 3-3. Specifications of Carotech palm carotenoids - Caromin® 25% (oil suspension)

Composition	%
α-carotene, %	8.0
β - carotene, %	15.8
γ- carotene, %	0.9
Lycopene, %	0.5
Total carotenoids, %	>25.0
Palm oil, %	<75.0

Table 3-4. Specifications of Carotech palm carotenoids - Caromin® 30% (oil suspension)

Composition	%
α-carotene, %	9.7
β - carotene, %	18.9
γ- carotene, %	1.1
Lycopene, %	0.6
Total carotenoids, %	>30.0
Palm oil, %	<70.0

Table 3-5. Specifications of Carotech palm carotenoids - Alphabeads® 7.5% (beadlets)

Composition	%
α-carotene, %	2.4
β-carotene, %	5.0
γ- carotene, %	0.25
Lycopene, %	0.17
Total carotenoids, %	7.5
Palm oil, %	25
Gelatin, %	29
Sucrose, %	10
Ascorbyl palmitate, %	2.6
Natural tocopherol, %	1.5
Corn starch, %	16.4
Moisture, %	<8.0

Table 3-6. Specifications of Carotech palm carotenoids – Carosol™ 3.0% (powder)

Composition	%
α-carotene, %	0.98
β-carotene, %	1.89
γ- carotene, %	0.1
Lycopene, %	0.07
Total carotenoids, %	3.05
Palm oil, %	13.0
Plant-based emulsifier, %	17.9
Modified starch, %	61.5
Ascorbic acid, %	2
Silicon oxide, 2%	2
Natural tocopherol, %	0.5

Table 3-7. Specifications of Carotech's palm carotenoids – Carosem™ 1.0% (emulsion)

Composition	%
α-carotene, %	0.33
β-carotene, %	0.68
γ- carotene, %	0.005
Lycopene, %	0.005
Total carotenoids, %	1.02
Palm oil, %	21.98
Glycerine, %	35.0
Water, %	25.0
Polysorbate, %	7.5
Sorbitan monooleate, %	7.5
Tocopherol, %	1.0
Ascorbyl palmitate, %	1.0

Table 3-8. Specifications of Carotech's palm carotenoids – Carosem™ 5.0% (emulsion)

Composition	%
α-carotene, %	1.65
β-carotene, %	3.50
γ- carotene, %	0.025
Lycopene, %	0.025
Total carotenoids, %	5.20
Palm oil, %	28.8
Glycerine, %	20.0
Water, %	25.0
Polysorbate 80, %	10.0
Sorbitan monooleate, %	10.0
Rosemary extract, %	0.5
Tocopherol, %	0.5

Table 3-9. Specifications of Carotino CC-05 (oil suspension)

Composition	%
Total carotenoids, %	>5.0
Palm oil, %	<95.0

Table 3-10. Specifications of Carotino CC-10 (oil suspension)

Composition	%
Total carotenoids, %	>10.0
Palm oil, %	<90.0

Table 3-11. Specifications of Carotino CC-20 (oil suspension)

Composition	%
Total carotenoids, %	>20.0
Palm oil, %	<80.0

Table 3-12. Specifications of Carotino CC-30 (oil suspension)

Composition	%
Total carotenoids, %	>30.0
Palm oil, %	<70.0

H. Analytical methods

High performance liquid chromatography employing a C30 column with photo diode array detector (wavelength absorbance at maximum between 450 and 500 nm) can be used for the analysis of carotenoids. A multitude of cis-isomers of α-, β-, and γ-carotene were separated. β-carotene (Sigma C-9750 or Calbiochem 217538) or α:β-carotene (1:2; Sigma C4646) can be used as standards (Hao et al. 2005; Mortensen 2009).

II. Natural occurrence and exposure to carotenoids

A. Food sources of carotenoids

Vegetable oils such as palm oil are the richest source of mixed carotene in nature (Voutilainen et al. 2006). Carotenoids are rich in yellow/orange vegetables and fruits and dark green leafy vegetables: yellow/orange vegetables – carrots, sweet potatoes, pumpkins, winter squash; yellow/orange fruits – apricots, cantaloupes, papayas, mangoes, carambolas, nectarines, peaches; dark green leafy vegetables – spinach, broccoli, endive, kale, chicory, escarole, watercress and beet leaves, turnips, mustard, dandelion ; other good vegetable and fruit sources – summer squash, asparagus, peas, sour cherries, prune plums. The main sources of some carotenoids are presented in Table 4.

Table 4. Main sources of carotenoids

β-Carotene	α-Carotene	Lycopene
Palm oil	Palm oil	Tomato and tomato products
Carrots	Carrots	Watermelon
Apricots	Collard greens	Pink grapefruit
Mangoes	Pumpkin	Papaya
Red pepper	Corn	Guava
Kale	Yellow pepper	Rose hip
Spinach	Cloudberry	
Broccoli		

Adopted from Voutilainen et al. 2006

B. Current consumer intake levels

No recommended dietary allowances for carotenoids exist in the U.S. or in Europe. The amount of carotenoids in the diet is difficult to estimate, partly because the methods used in preparation of food composition tables are not sufficiently specific or sensitive.

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES 2005-2006) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics, and the Nutrition Coordinating Center, estimates of the first survey day (d) average intakes of vitamin A and carotenoids were calculated from the food code list and the survey database of diet recalls. The

NHANES provides the most current food consumption data available for the American population. The NHANES was conducted between 2005-2006 with non-institutionalized individuals in the U.S. The most-up-to date carotenoid intake status for the U.S. population ages 1 y and older are presented in Table 5 (NHANES 2005-2006). The current median intakes of β-carotene, α-carotene, and lycopene in the U.S. are estimated to be 0.57, 0.039, and 1.75 mg/d for the population aged 1 y and above. The 90th percentile intakes of β-carotene, α-carotene, and lycopene were estimated to be 4.45, 0.98, and 14.8 mg/d, respectively. The NHANES and other dietary survey datasets are not designed to calculate the consumption levels of γ-carotene due to its insignificant contribution to the American diet.

Table 5. Current daily carotenoid intakes (before the intended use), mg/d (NHANES 2005-2006)

Nutrient	Age (y)	N	Median	SE	90th Pctl	SE
β-carotene, mg	1+	8792	0.57	0.013	4.45	0.17
β-carotene, mg/kg BW	1+	8720	0.009	0.000	0.070	0.003
α-carotene, mg	1+	8792	0.038	0.001	0.98	0.048
α-carotene, mg/kg BW	1+	8720	0.0006	0.000	0.015	0.001
Lycopene, mg	1+	8792	1.75	0.079	14.8	0.38
Lycopene, mg/kg BW	1+	8720	0.026	1.1	0.238	0.007
Total Carotenoids, mg	1+	8792	4.82	0.15	20.5	0.49
Total Carotenoids, mg/kg BW	1+	8720	0.074	2.1	0.34	0.008

Pctl=percentile; Kg BW=Kg body weight

C. Intended use

Table 6 presents primary applications of carotenoids that include breakfast cereals, juice drinks, energy drinks, soups, dressings, fats and oils, meal replacements, meatless meat products, nutrient bars, salty snacks and crackers, yogurt, egg and imitation egg products, and medical foods. In addition, carotenoids can be used in medical foods (treating erythropoietic protoporphyria) to provide 180 mg β-carotene/d.

Table 6a. Intended use of palm carotenoids—recommended concentrations, mg/kg.

Food category	Total carotenoids	β-carotene	α-carotene	γ-carotene	Lycopene
Fats and oils, margarine	15.58	10	5.15	0.36	0.06
Canned green peas, canned mangoes, mayonnaise	15.58	10	5.15	0.36	0.06
Meatless bullions and consomme	31.16	20	10.3	0.72	0.12
Pickled cucumber	31.16	20	10.3	0.72	0.12
Baked goods, bread, rolls, cakes	2.93	1.88	0.969	0.0676	0.0112
Meatless soups, soup mixes	2.93	1.88	0.969	0.0676	0.0112
Baking mixes, cookies, crackers, salty snacks	2.93	1.88	0.969	0.0676	0.0112
Grain products, such as pastas or rice dishes	2.93	1.88	0.969	0.0676	0.0112
Ready-to-eat cereals	2.93	1.88	0.969	0.0676	0.0112
Frozen dairy desserts and mixes, ice cream and mixes	2.93	1.88	0.969	0.0676	0.0112
Processed fruits, juices, juice drinks, punches	2.93	1.88	0.969	0.0676	0.0112
Meatless meat products (soy-based imitation meat)	2.93	1.88	0.969	0.0676	0.0112
Processed vegetables, juices	2.93	1.88	0.969	0.0676	0.0112
Sweet sauces, toppings, syrups	2.93	1.88	0.969	0.0676	0.0112
Gelatins, puddings, fillings	2.93	1.88	0.969	0.0676	0.0112
Meatless gravies, sauces	2.93	1.88	0.969	0.0676	0.0112
Imitation egg products	2.93	1.88	0.969	0.0676	0.0112
Soft drink-orange drink type only	1.00	1.88	33.1	2.3	0.4
Granola bars, cereal bars	2.93	1.88	0.969	0.0676	0.0112
Meal replacements shakes, mixes, functional beverages	2.93 mg/kg liquid	1.88	0.969	0.0676	0.0112
Medicinal foods		180 mg/d			

Table 6b. Intended use - Maximum concentrations of palm carotenoids, mg/kg.

Food category	Total carotenoids	β-carotene	α-carotene	γ-carotene	Lycopene
Fats and oils, margarine*	38.9	25	12.875	0.9	0.15
Canned green peas, canned mangoes, mayonnaise*	155.8	100	51.5	3.6	0.6
Meatless bullions and consommé*	311.6	200	103	7.2	1.2
Pickled cucumber*	467.4	300	154.5	10.8	1.8
Baked goods, bread, rolls, cakes	15.58	10	5.15	0.36	0.06
Meatless soups, soup mixes	15.58	10	5.15	0.36	0.06
Baking mixes, cookies, crackers, salty snacks	15.58	10	5.15	0.36	0.06
Grain products, such as pastas or rice dishes	15.58	10	5.15	0.36	0.06
Ready-to-eat cereals	15.58	10	5.15	0.36	0.06
Frozen dairy desserts and mixes, ice cream and mixes	15.58	10	5.15	0.36	0.06
Processed fruits, juices, juice drinks, punches	15.58	10	5.15	0.36	0.06
Meatless meat products (soy-based imitation meat)	15.58	10	5.15	0.36	0.06
Processed vegetables, juices	15.58	10	5.15	0.36	0.06
Sweet sauces, toppings, syrups	15.58	10	5.15	0.36	0.06
Gelatins, puddings, fillings	15.58	10	5.15	0.36	0.06
Meatless gravies, sauces	15.58	10	5.15	0.36	0.06
Egg substitute products	15.58	10	5.15	0.36	0.06
Soft drink-orange drink type only	15.58	10	5.15	0.36	0.06
Granola bars, cereal bars	15.58	10	5.15	0.36	0.06
Meal replacements shakes, mixes, functional beverages	15.58	10	5.15	0.36	0.06

*FAO/WHO has permitted the use of β-carotene that can be used singly or in combination with other colors such as annatto, β-apo-8'-carotenal, or β-apo-8'-carotenoic acid methyl and ethyl esters. The maximum levels of these foods have been adopted from the FAO/WHO standards.

D. Estimated daily intake of palm carotenoids from GRAS uses

Using food intake data reported in the 2005-2006 NHANES, exposure levels to MPOB palm carotenoids that will result from the intended uses were estimated. Results of the exposure estimates under the intended use of palm carotenoids in foods for the U.S. population ages 1 y and older are presented in Tables 7 and 8. The first set of estimates is based on the assumption that the products in each food category will be used at the recommended intake levels under the intended use. The median intakes would be 2.1, 0.78, and 1.75 mg/d for β-carotene, α-carotene,

and lycopene in the U.S. population aged 1 y and above. The 90th percentile intakes were estimated to be 6.6, 2.2, and 14.9 mg/d for β-carotene, α-carotene, and lycopene, respectively (Table 7). The second set of estimates is based on the assumption that all the products in each food category will be used at the maximum intake levels under the intended use although it is far from a realistic situation. Even if all of the products are used at the maximum levels, the median intakes would be 7.6, 3.5, and 1.8 mg/d for β-carotene, α-carotene, and lycopene in the U.S. population aged 1 y and above. The 90th percentile intakes were estimated to be 18.2, 8.3, and 14.9 mg/d for β-carotene, α-carotene, and lycopene, respectively (Table 8). It is estimated that the intended use would increase the γ-carotene intake levels by 0.04 mg/d (the level of increase observed for lycopene) since the the concentrations of γ-carotene and lycopene are comparable in palm mixed carotene.

Medical foods (180 mg/d) have not been included in the exposure estimate of the general population since those foods will be used by a small segment of a special population.

Table 7. Estimated exposure to carotenoids under the intended use assuming that all the products are used at the recommneded levels of the intended use* (NHANES 2005-2006)

Nutrient	Age (y)	N	Median	SE	90th Pctl	SE
β-Carotene, mg	1+	8792	2.13	0.034	6.61	0.15
β-Carotene, mg/kg BW	1+	8720	0.032	0.0005	0.116	0.003
α-Carotene, mg	1+	8792	0.78	0.012	2.16	0.044
α-Carotene, mg/kg BW	1+	8720	0.012	0.0002	0.041	0.001
Lycopene, mg	1+	8792	1.75	0.084	14.85	0.38
Lycopene, mg/kg BW	1+	8720	0.027	0.001	0.238	0.007
Total Carotenoids, mg	1+	8792	7.26	0.137	23.80	0.47
Total Carotenoids, mg/kg BW	1+	8720	0.137	0.002	0.40	0.008

*Exposure estimates have not included medical food users.

Table 8. Estimated exposure to carotenoids under the intended use assuming that all the products are used at the maximum levels of the intended use* (NHANES 2005-2006)

Nutrient	Age (y)	N	Median	SE	90th Pctl	SE
β-Carotene, mg	1+	8792	7.56	0.11	18.26	0.27
β-Carotene, mg/kg BW	1+	8720	0.116	0.002	0.341	0.007
α-Carotene, mg	1+	8792	3.49	0.040	8.26	0.136
α-Carotene, mg/kg BW	1+	8720	0.053	0.001	0.159	0.004
Lycopene, mg	1+	8792	1.79	0.088	14.87	0.378
Lycopene, mg/kg BW	1+	8720	0.027	0.001	0.238	0.007
Total Carotenoids, mg	1+	8792	16.52	0.241	40.03	0.590
Total Carotenoids, mg/kg BW	1+	8720	0.253	0.003	0.731	0.014

*Exposure estimates have not included medical food users.

III. Basis for GRAS determination

A. Current regulatory status

It should be noted that β-carotene is already listed as a GRAS ingredient under the 21 CFR Part 184 (LSRO 1979). β-Carotene is approved by FDA as a food color under 21 CFR 73.95. Also, the Food and Agriculture Organization (FAO)/WHO has approved β-carotene as a permissible food colorant in 1992 (JECFA, 1992) and has specified the maximum concentrations of added β-carotene for the following foods: edible fats and oils including margarine, 25 mg/kg; pickled cucumbers, 300 mg/kg singly or in combination with other colors; and canned green peas, canned mango, and mayonnaise, 100 mg/kg singly or in combination with other colors. The colors are permitted for the purpose of restoring colors lost during processing and for the purpose of standardizing color as long as color does not deceive or mislead the consumer by concealing damage or inferiority or by making the product appear to be greater than actual value.
The FDA had no question on GRAS notification of BASF synthetic lycopene (GRN 000119) and lycopene from *Blakeslea trispora* (GRN 000173).

Carotenoids are included in the *"Everything" Added to Food in the United States* (EAFUS) list. The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.

This GRAS determination for carotenoids is based upon scientific procedures in accordance with section 201(s) (21 U.S.C. §321(s)) of the Federal Food, Drug, and Cosmetic Act (21U.S.C.§301 et. seq.) ("the Act", 21 CFR 170.30). In general, materials of natural biological origin that have no significant detrimental effect and no known health hazard are eligible for GRAS status (FDA 1997, 2008). Considering that carotenoids are of biological origin, that carotenoids exist naturally in many foods that have long histories of human consumption, and that carotenoids do not represent a known health hazard, carotenoids are considered as GRAS.

B. Intended technical effects

The palm carotenoids can be used as a food ingredient as a source of a "natural carotenoid". β-Carotene also can be used in a medical foods at the level of 180 mg/d (IOM 2000).

<u>Carotenes as pro-vitamin A</u>

Carotenes, in particular β-carotene, have long been known for their pro-vitamin A activity, as they can be transformed into vitamin A in vivo. In addition, α-carotene and γ-carotene, which are present in crude palm oil, have a similar activity. Traditionally, vitamin A activity of β-carotene has been expressed in International Units (IU; 1 IU = 0.60 μg of all-trans β -carotene). However, this conversion factor does not take into account the poor bioavailability of carotenoids in humans. The carotene:retinol equivalency ratio (μg:μg) of a low dose (less than 2 mg) of purified β-carotene in oil is approximately 2:1 (i.e., 2 μg of β-carotene in oil yields 1 μg of retinol) (Table 10; IOM 2000). This ratio was derived from the relative amount of β-carotene required to correct abnormal dark adaptation in vitamin A-deficient individuals. It is estimated

that 6 μg of β-carotene from a mixed diet is nutritionally equivalent to 1 μg of β-carotene in oil (IOM 2000). Therefore, the retinol activity equivalency (μg RAE) ratio for β-carotene from food is estimated to be 12:1 (6 x 2:1). One RAE for dietary provitamin A carotenoids other than β-carotene is set at 24 μg on the basis of the observation that the vitamin A activity of α- and γ–carotene is approximately half of that for β-carotene (Table 9; IOM 2000).

Table 9. Absorption and bioconversion of ingested provitamin A carotenoids to retinol based on RAE ratio (IOM 2000).

Consumed	**Absorbed**	**Bioconverted**
Dietary or supplemental Vitamin A (1μg) ➜	Retinol ➜	Retinol (1μg)
Supplemental β-carotene (2 μg) ➜	β-carotene ➜	
Dietary β-carotene (12 μg)➜	β-carotene ➜	
Dietary α-carotene, γ-carotene, or β-cryptoxanthin (24 μg) ➜	α-carotene, γ-carotene or β-cryptoxanthin ➜	

Carotenoids as antioxidants

β-Carotene, α-carotene, and lycopene are effective single oxygen quenchers, which can induce pre-cancerous changes in cells. Singlet oxygen is capable of triggering free radical chain reactions (Choo et al. 1992). Carotenoids have protective properties against free radical damage that is believed to be responsible for numerous degenerative diseases such as atherosclerosis and cancer (Voutilainen et al. 2006). In fact, α- and β-carotenes as well as lycopene have anti-cancer properties, with α-carotene 10 times as potent as β-carotene as an anti-cancer agent (Murakoshi et al. 1989). In a study involving an in vitro induction of lipid peroxidation in liver homogenates by an azo-initiator of peroxyl radicals, an inverse correlation between tissue carotenoid level and accumulation of lipid peroxidation products was observed, with α-carotene > lycopene > β-carotene, suggesting that α-carotene is more potent as an antioxidant than lycopene, and lycopene more potent than β-carotene (Serbinova et al. 1992).

C. Review of safety data

1. Bioavailability and metabolic fate of carotenoids

Bioavailability refers to the proportion of β-carotene that can be absorbed, transported, and utilized by the body once it has been consumed. Carotenoids also are solubilized in micelles in the intestinal lumen from which they are absorbed into duodenal mucosal cells by a passive diffusion mechanism. Approximately 9-22% of a single dose of 45 μg to 39 mg β-carotene is known to be absorbed (IOM 2001). Bioavailability of carotenoids is influenced by a number of

factors such as the amount of dietary carotenoids, food processing, and the presence of fat. The absorption efficiency decreases as the amount of dietary carotenoids increases (IOM 2001). The amount of dietary fat required to ensure carotenoid absorption is low (approximately 3-5 g per meal). Carotenoids from dietary supplements are better absorbed than carotenoids from foods (IOM 2001).

Absorbed β-carotene is principally converted to vitamin A by the enzyme, β-carotene-15, 15-dioxygenase, in intestinal absorptive cells. The central cleavage of β-carotene by this enzyme will, in theory, result in two molecules of retinal. β-Carotene also can be cleaved to yield β-apocarotenals that can be further degraded to retinal or retinoic acid (IOM 2001). The predominant form of vitamin A in human lymph, whether originating from ingested vitamin A or provitamin A carotenoids, is retinyl ester (retinol esterified with long-chain fatty acids, typically palmitate and stearate) (IOM 2001). Along with exogenous lipids, the newly synthesized retinyl esters and non-hydrolyzed carotenoids are transported from the intestine to the liver in chylomicrons and chylomicron remnants. Retinol is metabolized in the liver to numerous products, some of which are conjugated with glucuronic acid or taurine for excretion in bile. The portion of excreted vitamin A metabolites in bile increases as the liver vitamin A exceeds a critical concentration. This increased excretion has been suggested as a protective mechanism for reducing the risk of excess storage of vitamin A (IOM 2001).

The vitamin A activities of palm oil carotenes are evident from a rabbit study using palm carotene concentrate (Yap et al. 1997). Rabbits fed diets enriched with palm carotenes (309 mg/kg diet) had the various carotenes present in varying amounts in their plasma and organ tissues, mainly in liver and adrenal glands. Most of the supplemented α- and β-carotenes were metabolized to retinol and retinyl esters, which were mainly stored in the liver and, to a lesser extent, pancreas. The contents of retinol and retinyl esters in the livers of rabbits fed diets supplemented with palm carotenes were, respectively, three and 30 times higher than those in rabbits fed diets without palm carotenes. The major retinyl esters present in the organ tissues were retinyl palmitate, oleate, and linoleate, whereas only retinyl palmitate and stearate were found to be predominant in plasma. ε-Retinyl esters, which are derived from α-carotene, also were found in the pancreas. For rabbits fed diets enriched with palm carotenes but depleted of palm vitamin E, relatively small amounts of carotenes and retinyl esters were detected (only in the plasma and liver), indicating that vitamin E substantially affects the disposition of dietary carotenes and their metabolites in plasma and tissues of rabbits.

Van het Hof et al. (1999) compared palm oil carotenoids with synthetic β-carotene for their ability to increase plasma concentrations of carotenoids of 69 healthy adult volunteers. Four days of supplementation with natural palm oil carotenoids (7.6 mg/d of α-carotene, 11.9 mg/d of all-trans-β-carotene, 7.5 mg/d of cis-β-carotene) or synthetic β-carotene (23.8 mg/d of all-trans-β-carotene, 4.4 mg/d of cis-β-carotene), added to a mixed meal, resulted in significant increases in plasma concentrations of the supplemental carotenoids as compared to consumption of a low-carotenoid meal (i.e., 7.2-fold increase in α-carotene and 3.5-fold increase in all-trans- β-carotene following palm oil carotenoids; 6.9-fold increase in all-trans-β-carotene following synthetic β-carotene). As the carotenoid content differed between the treatments, the relative plasma responses were calculated per milligram of β-carotene intake. The relative plasma responses

were similar for the two supplements, suggesting that the presence of α-carotene does not affect the bioavailability of β-carotene from palm oil.

Lycopene, like other carotenoids, is known to be poorly absorbed after oral (gavage) administration (Jonker et al.2003). Mathews-Roth et al. (1990) reported the distribution of [^{14}C]-labeled lycopene (specific activity 101 uCi/mg) in rats and monkeys dosed by gavage with 20 and 50 uCi, respectively. After administration of ^{14}C lycopene, peak accumulation of radioactivity in plasma occurred between 4 and 8 h in rats and between 8 and 48 h in monkeys. In rats, the liver contained the largest amount of radioactive pigment. In monkeys, with the exception of one stomach sample, liver was the main depot organ for lycopene. The other organs tested accumulated various amounts of pigment. No labeled metabolic products of lycopene were found.

A number of previously conducted studies have shown that lycopene is detected in the plasma and tissues of rats following incorporation into the daily diet (Jonker et al. 2003). While the extent of absorption may be greater in humans, it is known that the lycopene molecule is absorbed and distributed similarly in rats and humans. Thus, the rat can be regarded as a useful and appropriate animal model for assessing the potential toxicity of lycopene in humans (Boileau et al. 2000; Bramley, 2000; Carughi and Hooper, 1994; Ferreira et al. 2000; Kaplan et al. 1990; Mayne et al. 1999; Nierenberg and Nann, 1992; Schierle et al. 1997; Schmitz et al. 1991; Stahl et al. 1992; Zhao et al. 1998).

2. Safety/toxicity of palm carotenoids —Preclinical studies

There is no evidence that β-carotene or other carotenoids are teratogenic, mutagenic, or carcinogenic in long-term bioassays in experimental animals (Diplock 1995; Heywood et al. 1985; IOM 2001; LSRO 1979; Woutersen et al. 1999).

1) Palm carotenoids

a. Subchronic toxicity studies

The palm carotenoid concentrate prepared by molecular distillation has been subjected to a toxicological study (Choo et al. 1992). Four groups of Sprague-Dawley rats (12 rats per group) were fed a semi-purified diet supplement with 0.2% Caromin® 25% (or 5000 mg carotenoids/kg BW/d) and a control diet for 16 wk (Table 10). Histopathological examinations showed the major organs, such as the heart, lungs, adrenals, kidneys, liver, and spleen, to be normal for all groups, suggesting that palm oil carotenoid concentrate is not harmful. No extensive or significant amounts of fat were deposited in the heart and coronary vessels, and the aorta was normal in all groups. Administration of methyl esters and ethyl esters of carotenoids at 5 g/kg BW/d also did not show any abnormalities. The authors concluded that the carotenoid concentrate and the other experimental diets do not have toxicological effects on the major organs of male rats. Thus, the NOAEL of Caromin® 25% is considered to be 5000 mg/kg BW/d, which provides a mixture of 833, 406, 6.5, and 6.5 mg/kg BW/d of β-carotene, α-carotene, γ–carotene, and lycopene, respectively, from a palm oil source.

Another study with male and female Sprague Dawley rats reported that an oral dose of 2 g Caromin® 25%/kg BW resulted in no deaths, no clinical abnormalities, and no macroscopic abnormalities (Carotech 2008; Table 10).

Table 10. Toxicity studies of palm carotenoids

Species	Route	Dose	Duration	Result	Reference
Subchronic toxicity studies					
Rat	Diet	5 g/kg BW/d (or 833 mg β-carotene, 406 mg α-carotene, 6.5 mg γ–carotene, and 6.5 mg lycopene from a palm oil source.	16 wk	No abnormalities	Choo et al. 1994
Rat	Oral	2 g/kg BW/d	Single dose	No abnormalities	Carotech 2008

b. Mutagenicity study

Masuda et al. (1995) examined the mutagenicity of palm fruit carotene using the reverse mutation test with bacteria, the chromosomal aberration test with mammalian cells, and the micronucleus test in mice. Carotene induced neither reverse mutation in *Salmonella typhimurium* TA98, TA1537, TA100, TA1535, and (or) *E. coli* WP2uvrA, nor structural and numerical (polyploidy) chromosomal aberrations in the Chinese hamster fibroblast cell line (CHL). In addition, no increase in micronucleated polychromatic erythrocytes was elicited in the micronucleus test in CD-1(ICR) male mice. It was concluded that palm carotene had no mutagenic activity in these in vitro and in vivo tests.

Tan et al. (1992) studied the effect of palm carotenes (PC) and synthetic carotene (SC) on the development of papilloma (chemical-induced skin cancer) and lymphoma (viral-induced lymph cancer) in genetically recessive HRS/J female hairless mice. The order of this subcutaneous lymphoma (SL) inhibition phenomenon by PC in the feed was concentration-dependent; 0.3% PC > 0.1% PC > 0.05% PC groups. The onset of SL in 0.3% SC group was sooner than the 0.3% PC group. All the PC groups completely inhibited epithelial papilloma formation after the mice were 7 mo old. An inhibition by PC appeared to be mediated by SL regression in a concentration-dependent fashion. The 0.3% PC group showed the strongest anti-tumorigenic effect (and the least toxic effect with respect to mortality). It was reported that palm oil carotene concentrate (Caromin®) may prevent the development of hormone-dependent breast cancer in a study investigating the effect of retinoic acid and palm oil concentrate on estrone sulphatase and estradiol-17β hydroxysteroid dehydrogenase activities in MCF-7 and MDA-MB-231 breast cancer cell lines (Carotech 2008).

2) β-Carotene (mostly adopted from LSRO 1979 and Woutersen et al. 1999)

There is no evidence that β-carotene is genotoxic, reprotoxic, or teratogenic (IOM 2001). No signs of organ toxicity have been found in subacute, subchronic, or chronic oral toxicity studies in experimental animals receiving doses of up to 1000 mg β-carotene/kg BW/d via the diet (IOM 2000; Woutersen et al. 1999). Thus, the NOAEL of β-carotene is considered to be 1000 mg/kg BW/d.

a. Acute toxicity studies of β-carotene

Table 11 summarizes acute toxicity studies on β-carotene.

Table 11. Acute toxicity studies of β-carotene

Species	Route	Dose	Duration	Result	Reference
Mice	Oral	10 g /kg BW/d, cis- or trans-β-carotene	3 d	No deaths among the 40 mice receiving trans-β-carotene and only one among the 40 dosed with cis-β-carotene	Banziger and Hane 1967
Guinea pig	i.p. injection	300 or 600 mg/kg BW, natural carotene	Single dose; observed 4 d	Edema and inflammation, but no necrosis, at the injection sites; fatty yellow material was observed in the peritoneum	Wells and Hedenburg 1916
Female rat	i.m. injection	6 g/ kg	Single dose, observed 3-48 d	Inflammation within the injection area; no apparent ill-effects	Zbinden and Studer 1958
Rat	Oral	800 mg/kg BW/d, carotene, mainly β-carotene	10 d	No abnormalities	Lewis and Reti 1935
Rat	Oral	520 mg/kg BW/d	7 d	No abnormalities except 40% reduction in prothrombin and kaolin-activated partial thromboplastin time indices	Takahashi 1995
Beagle dog	Oral	250 mg - 8 g/kg BW	6 d	No toxic manifestations	Bagdon et al. 1960

i.p.= intraperitoneally; i.m.=intramuscularly

a-1) Mouse study

CF-1 mice of both sexes weighing 17 to 25 g were orally given four samples of cis- and trans-β-carotene (10 g carotene/kg BW; Banziger and Hane, 1967). The mice were observed for 72 h. There were no deaths among the 40 mice receiving trans-β-carotene and only one among the 40 dosed with cis-β-carotene.

a-2) Guinea pig study

Guinea pigs were injected intraperitoneally with 100 or 200 mg “natural carotene” (about 300 or 600 mg/kg BW) dissolved in olive oil (Wells and Hedenburg 1916). A slight rise in body

temperature was observed 2 to 6 h after injection. Control animals receiving olive oil alone showed a similar rise. The urine of the animals receiving carotene was of a deep reddish-brown color. The guinea pigs were killed after 4 d. At necropsy, considerable amounts of fatty yellow material were observed in the peritoneum, but there were no signs of inflammation or other local changes. Twenty milligrams carotene in olive oil (about 60 mg/kg) also were injected intradermally into two guinea pigs. Edema and inflammation, but no necrosis, were noted at the injection sites.

a-3) Rat studies

Zbinden and Studer (1958) injected intramuscularly 10 female rats with 1 g β-carotene (about 6 g/kg) in oil. No apparent ill effects were observed and necropsies 3 to 48 d later revealed only a spreading inflammation within the injection area.

Lewis and Reti (1935) orally administered approximately 800 mg/kg BW/d of "crystallized carotene" (presumably β-carotene) to young rats for 10 d. During the latter period of the experiment, about 40% of the administered carotene was found in feces. Significant amounts were detected in the liver and adrenals. Growth of rats was normal and no signs of toxicity were apparent. The number of the animals tested was not stated. The authors stated, but offered no documentation, that rats tolerated well, "high" doses of carotene injected intravenously and intraperitoneally.

From a rat study using approximately 520 mg/kg BW/d for 7 d, Takahashi (1995) reported no abnormalities except red-colored feces and a 40% reduction in prothrombin and kaolin-activated partial thromboplastin time indices. At autopsy, small hemorrhages in the lungs were found in 1/6 rats. This study focused on evaluation of hemorrhage toxicity.

a-4) Beagle dog study

Bagdon et al. (1960) administered β-carotene to two young beagle dogs with an initial dose of 250 mg /kg BW. The dose was doubled on successive days until a single dose of 8 g/kg BW was given on the sixth day. No toxic manifestations were detected at any time.

b. Subacute toxicity studies of β-carotene (Table 12)

Table 12. Subacute toxicity studies of β-carotene

Species	Route	Dose	Duration	Result	Reference
Rat	Oral	250, 375, or 500 mg/kg BW/d	4 wk	No abnormalities except red discolored feces	Merkle1980
Male rat	Stomach tube	1 g/d, 5 d/wk	4 wk	No abnormalities except slight yellowish discolorations of body fat and liver	Schärer et al. 1961
Rat	Oral	80 mg/kg BW	34 d	No abnormalities	Davies and Moore 1934
Dog	Oral	2 g /kg BW/d	3 wk	No abnormalities except for a slight diarrhea	Bagdon et al. 1960
Dog	Oral	1, 10, or 100 mg/kg/d, 5 d/wk	13 wk	No abnormalities except some deposition in kidney and liver	Bagdon et al. 1960

b-1) Rat studies

Merkle (1980) studied oral toxicity of male and female Sprague-Dawley rats by feeding 250, 375, or 500 mg β-carotene/kg BW/d as a feed admixture for 4 wk. Treatment-related abnormalities in clinical signs, hematology, clinical chemistry, and urinalysis were not observed. At autopsy, the only abnormality observed was red discolored feces, which disappeared within 2 wk after treatment. Relative liver and kidney weights in both males and females of the top-dose group were significantly increased. This difference with controls disappeared within 2 wk after treatment.

Schärer et al. (1961) administered 1 g β-carotene by stomach tube to young male rats 5 d weekly for 4 wk. The animals were killed at 1, 8, or 15 d after completion of the carotene administration. Weight gains and liver function tests were normal. Necropsies revealed slight yellowish discolorations of body fat, but all organs were otherwise normal. Yellow pigment deposits were noted in the liver, but not in the kidneys or other organs.

Davies and Moore (1934) reported no harmful effects from a study of rats fed 8 mg carotene daily in the diet (about 80 mg/kg BW) for 34 d. It was estimated that only about 1% was absorbed.

b-2) Dog study

Two dogs were given 2 g β-carotene/kg BW/d by mouth for 21 d (Bagdon et al. 1960). The dogs tolerated this dosage without ill-effect, except for a slight diarrhea. These investigators then administered 1, 10, or 100 mg per kg to dogs orally 5 d weekly for 13 wk. No signs of toxicity were observed at any of the dose levels. The hematological values remained normal. At necropsy, the livers of the carotene-fed animals showed deposition of “material” in the Kupffer cells. A few kidney sections were slightly hyperemic and many of the collecting tubules contained small amounts of amorphous deposits in the lumen. No changes were apparent in the heart, spleen, adrenals, gastrointestinal tract, gall bladder, pancreas, gonads, bone marrow, thyroid, pituitary, central nervous system, or bladder.

c. Subchronic studies (Table 13)

Table 13. Subchronic toxicity studies of β-carotene

Species	Route	Dose	Duration	Result	Reference
Male rat	Oral	100 or 500 mg/kg BW/d, 5 d weekly	34 wk	No abnormalities	Schärer and Studer 1962
Rat	Oral	250, 500, or 1000 mg/kg BW/d	90 d	No abnormalities except orange-yellow adipose tissue and/or liver	Buser1995
Male rat	Oral	Up to 3127 mg/kg BW/d	90 d	No abnormalities except reddish discoloration of the GI tract. NOAEL - 3127 mg/kg BW/d	Nabae et al. 2005
Female rat	Oral	Up to 3362 mg/kg BW/d	90 d	No abnormalities except reddish discoloration of the GI tract. NOAEL - 3362 mg/kg BW/d	Nabae et al. 2005
Rat	Oral	0.63, 1.25, 2.5, or 5% in diet (β-carotene from dunaliella alga)	90 d	NOAELs -1.25% (696 mg/kg BW/d) for males and 5% (2879 mg/kg BW/d) for females (based on growth suppression). Increased serum levels of AsT, AIT, AIP, Ca, and total cholesterol in the 1.25-5% groups.	Kuroiwa et al. 2006
Rat	Oral	1 g/ kg	100 d	No abnormalities	Zbinden and Studer 1958
Rat	i.m.	25 mg/kg BW/d; 5 d weekly	17 wk	No abnormalities except cyst at the injection site; fatty infiltration in the liver and kidney	Zbinden and Studer 1958
Rabbit	Oral	500 mg/kg BW/d; 5 d weekly	11 wk	No abnormalities	Zbinden and Studer 1958
Male adult ferret	Oral	2.4 mg β-carotene/kg/d	6 mo	Localized proliferation of alveolar cells (type II pneumocytes), alveolar macrophages, and keratinized squamous epithelium	Wang et al. 1999

i.m.=intramuscularly; AsT=aspartate aminotransferase; AIT= alanine aminotransferase, AIP=alkaline phosphatase

c-1) Rat studies

Schärer and Studer (1962) studied subchronic toxicity of β-carotene with young male rats given 100 or 500 mg/kg BW 5 d weekly for 34 wk. The carotene was administered as a 20% solution in arachis oil. Growth, general condition, blood metabolites, kidney, and liver function tests, and organ histology were normal.

Buser (1995) conducted a 90 d oral toxicity study with Wistar rats fed 250, 500, or 1000 mg β-carotene/kg BW/d as a feed admixture for 90 d. There were no treatment-related abnormalities in clinical signs (except for orange/red feces), hematology, clinical chemistry, or organ weights. With the exception of orange-yellow adipose tissue and/or liver in a number of females in all dose groups, no gross lesions related to β-carotene treatment were observed. The

discoloration disappeared after a non-treatment period. Histopathological examination of a large number of organs and tissues did not reveal any treatment-related abnormalities.

From a 90 d oral toxicity study of β-carotene derived from *Blakeslea trispora*, a natural food colorant, Nabae et al. (2005) concluded that the NOAELwas a dietary concentration of at least 5% (3127 mg/kg BW/d for males, 3362 mg/kg BW/d for females) for β-carotene derived from *B. trispora* under the present experimental conditions. F344 rats fed the agent at dietary concentrations of 0, 0.2, 1.0, and 5.0% for 90 d (10 males and 10 females/group). There were no treatment-related adverse effects with regard to body weight, food and water consumption, urinalysis, ophthalmology, hematology, serum biochemistry, or organ weight. On clinical observation, red coloring of fur was noted in both sexes of the 1% and 5% group rats, with red feces observed in all treated group animals, and necropsy revealed all rats of the treated groups had reddish coloration of the contents of the gastrointestinal tract due to the pigmentation and, thus, lacked toxicological significance. On histopathological examination, sporadic spontaneous lesions known to occur in this strain of rats were the only findings, with no specific relation to the test substance.

Kuroiwa et al. (2006) conducted a 90 d subchronic toxicity study of Dunaliella carotene in F344 rats at dose levels of 0 (control), 0.63, 1.25, 2.5, and 5% in a powdered basal diet. Dunaliella carotene from dunaliella alga has β-carotene as its main constituent. No mortality or treatment-related clinical signs were observed throughout the experimental period in any of the groups. Body weight gain was slightly but significantly reduced from week 5 to the end of the experiment in 2.5 and 5% males. Increased blood platelet (PLT) concentrations were observed in 1.25 and 5% males, and 2.5 and 5% females. Significant elevations in serum total cholesterol were observed in all treated rats. Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were increased in rats at higher dosage levels (AST and ALP in the 2.5 and 5% groups; ALT in the 1.25, 2.5, and 5% groups). Other hematology and serum biochemistry data did not show any effects of β-carotene. Organ weight measurement and histopathological observation revealed no toxicological changes. Increases in serum Ca were observed in the lowest group in both sexes. Based on growth suppression, no-observed-effect level (NOAELs) were estimated to be 1.25% (696 mg/kg/d) for males and 5% (2879 mg/kg/d) for females.

Zbinden and Studer (1958a) investigated subchronic toxicity of β-carotene in young rats of both sexes by feeding 1 g β-carotene/kg BW/d for 100 d. The weight gain of the male rats lagged slightly behind that of the controls. No significant changes were detected in the blood or in any of the organs examined. Other rats received 4.5 mg β-carotene (about 25 mg/kg BW) intramuscularly twice daily, 5 d/wk for 17 wk. The animals grew normally and their general condition was good. Large cysts containing oil and β-carotene were present in all animals at the site of injection. Fatty infiltration of the Kupffer cells of the livers and partial epithelial desquamation of the convoluted tubules in the kidneys were noted. No pathological changes were apparent in the lungs, spleen, epidermis, bones, or marrow.

Bagdon (1960) studied the effects of either crystalline β-carotene or a mixture containing 25% “degradation products” (not further identified) at a 1% concentration in the diet (about 1

g/kg BW/d) in 40 Sprague-Dawley rats of both sexes for 13 wk. No untoward effects were observed in either group on growth, general condition, food consumption, or hemopoietic tissues. Gross and microscopic findings were not unusual. The only significant observation was a decreased incidence of fatty infiltration in Kupffer cells of animals receiving the mixture of β-carotene and degradation products compared with Kupffer cells of animals receiving crystalline β-carotene alone.

c-2) Rabbit study

Zbinden and Studer (1958b) evaluated subchronic toxicity of β-carotene in rabbits by feeding 500 mg /kg BW/d five times weekly for 11 wk. No significant changes were noted in their general condition and the tissues appeared normal at necropsy.

c-3) Ferret study

Male adult ferrets (1.0 to 1.2 kg) were fed a basal diet containing either low β-carotene (0.16 mg/kg/d) or the basal diet supplemented with β-carotene (2.4 mg β-carotene/kg BW/d) for 6 mo (Wang et al. 1999). Large increases in the concentration of β-carotene in both plasma (up to 21-fold) and lung tissue (up to 300-fold) were found in the ferrets given a basal diet supplemented with β-carotene for 6 mo. Localized proliferation of alveolar cells (type II pneumocytes), alveolar macrophages, and keratinized squamous epithelium were observed in all ferrets given the β-carotene supplement.

d. Chronic toxicity and carcinogenicity studies of β-carotene

Table 14. Chronic toxicity and carcinogenicity studies of β-carotene

Species	Route	Dose	Duration	Result	Reference
Dog	oral	50, 100, or 250 mg/kg BW/d	104 wk	No abnormalities except perisinusoidal fat storage cells and pale orange foci on the surface of the liver.	Buser 1983a
Rat	oral	100, 250, 500, or 1000 mg/kg BW/d water-soluble beadlets	114-116 wk	No abnormalities except yellow-orange fur and adipose tissue. Reduced weight gain in the 500 and 1000 mg groups.	Matschiner 1973
Rat	oral	100, 250, 500, or 1000 mg/kg BW/d; water-soluble beadlets	104 wk	No abnormalities except orange stomach contents and adipose tissue; a yellow fur or skin.	Buser 1983b
Rat	oral	50 to 100 mg/kg BW/d	110 wk	No abnormalities except slight peripheral fatty infiltration in the liver	Bagdon 1960

d-1) Dog study

Buser (1983) evaluated chronic toxicity of β-carotene in dogs by feeding with doses of 50, 100, or 250 mg/kg BW/d as a feed admixture for 104 wk (Table 14). Two of eight dogs/sex in

each group were autopsied after 52 wk of treatment. Three of the six remaining animals in treated groups were maintained for a non-treatment period (week 88 to 104). Three animals per sex and per group were treated for up to 104 wk. There were no adverse effects on food and water consumption, hematology, clinical chemistry, urinalysis, ophthalmoscopy, and organ weights. Macroscopic examination revealed irregular pale orange foci on the surface of the liver of most dogs maintained on the β-carotene diets, but not on those of controls. Histopathological examination after 104 wk of treatment revealed perisinusoidal fat storage cells (Ito cells) in the dogs fed β-carotene. The number of these cells did not increase dose-dependently, but the cells did not disappear after a non-treatment period of 17 wk. In the absence of any degenerative changes in the liver, the presence of these cells is considered to indicate storage of vitamin A rather than to reflect a toxic effect.

d-2) Rat studies

In a combined chronic toxicity/carcinogenicity study conducted by Matschiner (1973), four groups of 60 male and 60 female Sprague-Dawley CD rats were fed β-carotene in the diet at concentrations of 100, 250, 500, or 1000 mg β-carotene/kg BW/d for 114 wk (females) or 116 wk (males). In the 1000 mg/kg BW/d group, body weight gain was lower than in placebo controls. Between wk 27 and 104, reduced weight gain was also seen at lower doses. However, this effect on body weight gain was not accompanied by any sign of organ toxicity. Ophthalmoscopic examinations revealed no treatment-related effects, nor did hematology, blood chemistry, or urinalysis. Macroscopic observations at autopsy revealed yellow-orange fur and adipose tissue in a number of animals of the 1000 and 500 mg/kg groups and only a few of the lower-dose groups. Microscopic evaluation of a wide range of tissues revealed no signs of systemic toxicity and no unusual types of tumors or increased incidence of tumors.

In a carcinogenicity study (Buser 1983b), groups of 100 male and 100 female CD mice were fed diets containing β-carotene at concentrations of 100, 250, 500, or 1000 mg β-carotene/kg BW/d as water-soluble (WS) beadlets for life for 104 wk. Red feces were observed in all animals that received β-carotene. A number of β-carotene-treated mice had orange stomach contents and adipose tissue, a yellow fur or skin, and vacuolated cells lining the sinusoids of the liver. The tumor profile was similar among the groups.

Bagdon (1960) tested chronic toxicity of β-carotene with young Wistar rats (15 male and 15 female) fed a diet containing 0.1% β-carotene for 110 wk (1960). The rats consumed an estimated 50 to 100 mg β-carotene/kg BW/d (~80,000 to 160,000 IU) during this period. The growth rate and the food consumption of the β-carotene-fed rats did not differ significantly from control animals. Hemoglobin, erythrocytes, and total and differential leucocyte counts during the 70th experimental week were normal. Four animals from each group were sacrificed after 1 y and all surviving animals after 110 wk, at which time their tissues were examined for pathologic changes. The livers of both β-carotene-treated and control rats occasionally showed slight peripheral fatty infiltration, but no other changes. The Kupffer cells of the β-carotene group were filled with a fatty, fluorescing material that was thought to be vitamin A. There were no histopathologic changes in any of the other tissues examined and no signs of hypervitaminosis A. No tumors were reported.

e. Mutagenicity of β-carotene (Adopted from Krinsky and Johnson 2005; LSRO 1979; Mathews-Roth 1988; Woutensen et al. 1999)

β-Carotene has been found to be neither mutagenic in the Ames test (up to 4151mg per plate) nor in the mouse bone-marrow micronucleus test at doses up to 234 mg/kg BW (Albertini et al. 1992; Gallandre et al. 1979; Gocke 1994; Heywood et al. 1985; Schüpbach et al. 1979). β-Carotene exhibited no mutagenic activity in microbial assays with *Salmonella typhimurium*, strains TA-1535, -1537, -1538, -98, and -100 and *Saccharomyces cerevisiae* strains DR with and without activation by tissue homogenates and supernatants from mouse, rat, and monkey (Brusick 1977; LSRO 1979). Arriaga-Alba et al. (2000) reported that β-carotene (30%), used by pharmaceutical laboratories as dietary complements, was not toxic or mutagenic for the *S. typhimurium* TA102 strain at a dose equivalent of 1500 IU.

The development of strains of *Salmonella typhimurium* that could be mutated by compounds or factors associated with oxidant stress (Ames et al. 1973) has enabled investigators to study the protective or antimutagenic effects of a wide variety of compounds. The ability of carotenoids to prevent bacterial mutagenesis in *S. typhimurium* has been reviewed earlier (Krinsky 1993).

β-Carotene was negative in differential toxicity assays in *Bacillus subtilis* and *Escherichia coli*, either in the absence or presence of metabolic systems. β-carotene did not affect the frequency of sister chromatid exchanges in organ cultures of Balb/c mouse mammary gland, chromosomal aberrations, or micronuclei in Chinese hamster ovary cells and of micronuclei in metabolically competent human hepatoma cells (Mathews-Roth et al. 1988; Woutersen et al. 1999). Arriaga-Alba et al. (2000) reported that the mutagenic effect of nalidixic acid and norfloxacin against *E. coli* hisG48 strains was inhibited with 1500 IU of β-carotene. The antimutagenic effect of β-carotene against mutations induced by pipemidic acid was observed even with 150 IU of β-carotene. Arriaga-Alba et al. (2000) also reported that β-carotene inhibited quinolone-induced mutagenesis in sensitive strains of *S. typhimurium* and in the presence of the S9 fraction of rat liver.

Santamaria et al. (1984) demonstrated the protective effects of β-carotene against the mutagenic potential of 8-methoxypsoralen and ultraviolet light type A (UV-A, 340–400 nm). Cyclophosphamide-initiated mutagenesis also can be blocked by addition of β-carotene to this organism (Belisario et al. 1985), and several other carotenoids have been evaluated using aflatoxin B1 to induce mutagenesis. When either 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) or benzo(a)pyrene (B(a)P) were used to initiate mutagenesis, β-carotene, canthaxanthin, β-apo-80-carotenal and methyl β-apo-80-carotenoate all demonstrated a dose-dependent decrease in mutagenicity (Azuine et al. 1992).

However, not all of these studies were supportive of a role for carotenoids as antimutagens. Terwell and van der Hoeven (1985) were unable to demonstrate any antimutagenicity for β-carotene when benzo(a)pyrene or cigarette smoke condensate was used as the mutagen. A slight antimutagenic effect of β-carotene was reported by these authors when an aqueous extract of fried beef was tested as a mutagen (Ong et al. 1989).

f. Studies on the effects of β-carotene on genotoxicity or malignant transformation (Adopted from Krinsky 1993; Krinsky and Johnson 2005; LSRO 1979; Mathews-Roth 1988; Woutensen et al. 1999)

Genotoxicity can cover a variety of insults to the genome, which can be expressed as DNA damage, formation of micronucleated cells, sister chromatid exchanges (SCE), chromosomal aberrations, translocations, mutagenesis, or even death of the cell. The data suggest that β-carotene administration prevented genetic damage caused by mutagens both in bacterial and cell culture systems (Agarwal et al. 1993; Konopacka and Rzeszowska-Wolny 2001; Manoharan and Banerjee 1985; Mukherjee et al. 1991; Pung et al. 1988; Salvadori et al. 1992; Schwartz et al. 1990; Stich and Dunn, 1986; Stich et al. 1990; Weitzman et al. 1985; Zhang et al. 1991, 1992).

Mukherjee et al. (1991) reported that β-carotene at a concentration of 27 mg/kg induced a significant increase in chromosomal aberrations, whereas 2.7 mg/kg β-carotene did not. However, the same research group reported in a subsequent paper (Agarwal et al. 1993) that β-carotene did not significantly induce chromosomal aberrations at doses of 0.27 and 27 mg/kg. Salvadori et al. (1992) also found no clastogenic effect of β-carotene given five times at 200 mg/kg.

When human lymphocytes were exposed to 2 Gy of c-rays, extensive genotoxicity occurred, as evaluated by the formation of micronucleated cells. The addition of 1–5 ug/ml β-carotene, either before or within 1 h of treatment, resulted in a significant reduction in the number of micronuclei in the irradiated cells (Konopacka and Rzeszowska-Wolny 2001).

Weitzman et al. (1985) used either phorbol myristate acetate-stimulated PMNs, or xanthine oxidase and hypoxanthine to generate reactive oxygen species directly to induce increased SCE in CHO cells. When 10–50 uM β-carotene was added to the cell culture system, a significant protection against the generation of SCE was observed.

When either methylmethanesulfonate or 4-nitroquinoline-1-oxide were added to CHO cells growing in culture, a variety of genotoxic symptoms appeared, including chromosomal aberrations, translocations, or the development of micronuclei (Stich and Dunn, 1986). The addition of β-carotene to these cultures resulted in a dose-dependent inhibition of genotoxicity, and since the authors were not able to detect retinol in these cells, they concluded that the inhibitory effects of β-carotene were not associated with its conversion to retinol. The CHO cells were not protected by β-carotene against other genotoxic compounds such as gallic acid, tannic acid, an aqueous extract of the areca nut, or hydrogen peroxide. In a related study, Stich et al. (1990) measured the chromosome instability of C127 cells transformed with bovine papillomavirus. A number of antioxidants, including β-carotene, canthaxanthin, retinoic acid, retinol, ascorbate, and ellagic acid, when added to the cultures at physiological concentrations (β-carotene and canthaxanthin at 0.5 uM), were able to decrease the papillomavirus-induced chromosome instability.

Manoharan and Banerjee (1985) have used mouse mammary cell organ cultures treated with chemical carcinogens such as dimethylbenzanthracene (DMBA) to induce SCE. When β-carotene was added to the medium during the 24 h initiation stage, there was a significant

decrease in the number of SCE. They also noted a similar protective effect when two other carcinogens, N-nitrosodiethylamine and methylnitrosourea, were studied. Other studies on the effects of β-carotene on cells and cellular systems are summarized in Table 15.

Table 15. Effects of β-carotene on cells and cellular systems

Cells	Action measured	Initiated by	Observed effects	Reference
Mammary, mouse	SCE	DMBA/MNU/DENA	Inhibition	Manoharan and Banerjee 1985
CHO	SCE	PMNL or HX/XO	Reduces SCE	Weitzman et al. 1985
CHO	Transformation	Methylmethanesulfonate or 4NQO	Inhibited	Stich and Dunn 1986
C3H/10T1/2	Transformation	X-rays or MCA	Inhibition	Pung et al. 1988
C3H/10T1/2	Gap junctional communication	Spontaneous	Increased	Zhang et al. 1991
C3H/10T1/2	Connexin43 expression	Spontaneous	Upregulated	Zhang et al. 1992
Human squamous normal keratinocytes	Growth	Spontaneous	Inhibited	Schwartz et al. 1990
C127	Chromosome instability	Bovine papillomavirus	Inhibited	Stich et al. 1990

CHO=Chinese Ovary Hamster; DENA=diethylnitrosamine ; DMBA=dimethylbenzanthracene; HX=hypoxanthine; MCA=methylcholanthrene; MNU=methylnitrosourea; 4NQO= 4-nitroquinoline 1-oxide; PMNL= polymorphonuclear leukocytes; SCE=sister chromatid exchanges, XO=santhine oxidase

g. Reproduction toxicity and developmental toxicity studies (from Woutersen et al. 1999)

The data suggest that large doses of pure β-carotene do not cause embryotoxicity in rodents. In addition, studies of individuals with congenitally high concentrations of plasma carotenoids and babies born carotenemic because of their mothers' intake of large amounts of carotenoid-containing foods during pregnancy revealed no abnormalities attributable to the carotenoid molecule.

In a three generation study (Heywood et al. 1985), maintaining rats throughout three generations (for each generation, two litters were obtained) on β-carotene-containing diets at dose levels of 100, 250, 500, or 1000 mg/kg BW/d did not affect reproductive function such as mating performance, pregnancy rate and duration, litter size, pup mortality, litter and pup weight, or development of offspring. In this study, the fecal pellets were orange/red and the fur and extremities were yellow/orange in β-carotene treated animals.

Bagdon et al. (1960) reported that administration of a 0.1% β-carotene diet (about 50 to 100 mg/kg/d) to both male and female Wistar rats for four successive generations resulted in no signs of hypervitaminosis A or other toxic manifestations. First and second generation rats were bred during the 16th experimental week, and the third generation animals during the 40th wk. Number of litters, day to parturition, litter size, and weight at birth, were normal throughout three successive reproductive cycles.

Komatsu et al. (1971) studied the teratogenic effects of β-carotene given to pregnant Wistar rats. The basal diet was stated to be nutritionally complete although no fat source was indicated. Three groups of rats received, respectively, 180, 360, or 1800 mg β-carotene/kg BW by mouth from the 9th to 12th day of pregnancy. The report does not make clear whether these quantities were administered as single or multiple doses during this period. Resorptions occurred in the vitamin A-treated group and among those receiving 180 and 3600 mg/kg β-carotene, but not in rats receiving the intermediate dosage (360 mg/kg). Deformities of the 14th rib and shortening of various bones were observed in the fetuses of the vitamin A-treated animals, but not in the control or carotene-treated group. Incomplete sternebral ossification was reported in all three carotene groups. The significance of this observation is puzzling since 16.7% of the control group also was affected and the incidence was higher (80%) among fetuses whose mothers received 180 mg carotene/kg BW than among those (55.8%) whose mothers had received 20 times this amount.

Another study (Kistler et al. 1981) showed that consumption of dietary β-carotene (from d 7 to d 16 of gestation) in daily doses of 250, 500, or 1000 mg/kg BW did not have embryotoxic or teratogenic effects in rats. A slight reduction in maternal BW gain was observed in the 1000 mg/kg dose group. Doses of 100, 200, or 400 mg β-carotene/kg BW were neither embryotoxic nor teratogenic in rabbits when given orally from d 7 to 19 of gestation (Heywood et al. 1985).

To test the effect of β-carotene on fertility, Shärer and Studer (1962) administered 100 or 500 mg/kg by stomach tube 5 d per wk for 34 wk to young male Wistar rats. Upon attaining weights of about 250 g in 10 wk, the rats were mated with virgin females. All the carotene-treated males proved fertile and did not differ from controls in the number of females impregnated or in the number of offspring produced. No adverse effects were found in blood counts, kidney or liver function, organ weight, or in the histology of a variety of tissues and organs.

h. Carcinogenicity studies of β-carotene (adopted from Woutersen et al. 1999).

In numerous experimental studies, a wide variety of carcinogens known to be capable of inducing epithelial tumors in various organs such as skin, colon, pancreas, urinary bladder, oral cavity, stomach, small intestine, salivary glands, and respiratory tract were administered in combination with synthetic β-carotene either simultaneously or sequentially by different routes to mice, rats, and/or hamsters (Alam and Alam 1986; Beems et al. 1987; Colacchio et al. 1986; Imaida et al. 1990; Nishino et al. 1995; Yun et al. 1995). In nearly all studies in animal models of carcinogenesis, β-carotene appeared to be cancer-protective, or to be ineffective in preventing cancer, except for a few studies suggesting a cancer-enhancing effect (IARC 1998; Woutersen 1999).

In almost all studies on skin carcinogenesis (over 20 studies in mice) and buccal pouch carcinogenesis in hamsters (about 20 studies), β-carotene appeared to be cancer preventive (IARC 1988). The exceptions were the studies in which β-carotene was ineffective against skin cancer, in which case β-carotene was administered only before initiation (Mathews-Roth and Krinsky 1987), in the late stage of carcinogenesis (Steinel and Baker 1990), or after tumour development only (Shklar et al. 1989).

Most of the many studies on liver carcinogenesis (about 20 studies in rats) found β-carotene to have cancer-preventive effects (IARC 1998). The exceptions were two studies in rats (Astorg et al. 1996, 1997) and one study in mice (Murakoshi et al. 1992).

β-Carotene showed a tendency to inhibit colon carcinogenesis. Protective effects were reported in two studies in mice (Basu et al. 1988; Temple and Basu 1987) and in 4 studies in rats (Alabaster 1991, 1992; Komaki et al. 1996; Shivapurkar et al. 1995; Yamamoto et al. 1994). Three studies in rats reported no effect.

β-Carotene showed inconsistent results in pancreas carcinogenesis. Protective effects were reported in 2 studies in rats (Appel et al. 1991; Appel and Woutersen 1996) and in one study in hamsters (Woutersen and van Garderen-Hoetmer 1988). One study in rats (Woutersen and van Garderen-Hoetmer 1988) and one study in hamsters (Appel et al. 1996) reported no effect.

The cancer-preventive effect of β-carotene on urinary bladder carcinogenesis was not consistent: protective effects were shown in two studies in mice (Hicks et al. 1984; Mathews-Roth et al. 1991) and in one study in rats (Pedrick et al. 1990) and no effect in one mouse study (Mathews-Roth et al 1991).

The cancer-preventive effect of β-carotene on stomach/small intestine/salivary gland carcinogenesis appeared to be generally ineffective in rats (Alam et al. 1988; Azuineet al. 1992; Jones et al. 1989; Yamamoto 1994), although some preventive activity was reported in one rat study (Alam and Alam 1987).

Respiratory tract carcinogenesis studies showed inconsistent effects of β-carotene on cancer-protective effects. No effects were found in three studies in hamsters (Beems 1987; Moon 1994; Wolterbeek et al. 1995) and in two studies in mice (Nishino 1995; Yun et al. 1995). A hamster study (Furukawa et al. 1999) reported protective effects. However, Beems (1987) found a slight enhancing effect of β-carotene on benzo[*a*]pyrene-induced benign tumours (epidermoid papillomas) in trachea, bronchi, and larynx. However, the incidence and severity of pre-neoplastic changes in the respiratory tract (dysplasia in the larynx, metaplasia in the trachea and bronchi, and alveolar bronchiolization in the lungs) were not influenced by β-carotene. The animals were given the β-carotene-supplemented diet throughout the study and before the first intratracheal instillation of benzo[*a*]pyrene.

Furukawa et al. (1999) reported that the β-carotene treatments significantly reduced both the incidence and multiplicity of hyperplasia of the upper respiratory tract in a dose-dependent manner in Syrian hamsters treated with diethylnitrosamine (DEN) and cigarette smoke. In this study, a total of 120 male 5-wk-old hamsters were divided into 4 groups, each consisting of 30 animals. After a single subcutaneous (s.c.) injection of 100 mg/kg DEN, hamsters in groups 1-4 were administered diets supplemented with β-carotene at doses of 0.25, 0.05, 0.005 or 0% during experimental weeks 1 to 13, and simultaneously exposed to cigarette smoke (9 min twice a day, 5 d/wk). In all groups, epithelial hyperplasia and/or papillomas were induced in the larynx and trachea. The data indicate that β-carotene inhibits tumorigenesis, even at the high dose of 0.25%, under the present experimental conditions.

3) α-Carotene

No data are available on acute, subacute, subchronic, or chronic toxicity of α-carotene. However, a few studies indicate that α-carotene is neither mutagenic nor carcinogenic. In fact, it was demonstrated that α-carotene was more effective than β-carotene in inhibiting chemically-induced skin tumor progression (Nishino et al. 2000) and liver and lung carcinogenesis (Murakoshi et al.1992). Although no NOAEL value is available for α-carotene, available data indicate that α-carotene is as safe as β-carotene.

a. Mutagenicity of α-Carotene

Rauscher et al. (1998) extracted carotenoids from the water-insoluble residues of some carotenoid-rich fruits and vegetables and tested for inhibition of mutagenicities induced by aflatoxin B1 (AFB1), benzo[a]pyrene (BaP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and cyclophosphamide (CP) in histidine-deficient strains of *Salmonella typhimurium*. The antimutagenicity was mainly associated with the fractions of the hydrocarbon carotenoids (α- and β-carotene, lycopene), and also the carotenolesters (oranges). In the case of BaP, antimutagenic activity, quantified by dose-response curves, was exhibited by 8'-apo- β-carotenal, α- and β-carotene, β-cryptoxanthin, lutein, retinal, and retinol (ID50-values: 20-100 nmol ml/1 top agar, 50-70% maximum inhibition at 1 umol ml/1 top agar), while the maximum inhibition by torularhodin did not exceed 40%. Closely similar results were obtained with AFB1. The bacterial mutagenicity of CP was strongly reduced by α- and β-carotene, canthaxanthin, and retinol (ID50-values: 67-112 nmol ml/1 top agar, 50-63% maximum inhibition at 1 umol ml/1 top agar). In the case of IQ, the carotenoids exhibited weak antimutagenic potency (7-43%, ID50-values of retinal and retinol: 160 and 189 nmol ml/1 top agar, 60% and 55% inhibition, respectively). The mutagenic activity of the proximal mutagen of IQ, N-OH-IQ, in *S. typhimurium* TA 98NR was not significantly reduced by any carotenoid tested.

b. α-Carotene and DNA damage

Collins et al. (1998) employed the comet assay (single cell alkaline gel electrophoresis) to measure strand breaks, oxidized pyrimidines, and altered purines in the DNA of lymphocytes from volunteers supplemented with α- and β-carotene, lutein, lycopene, or placebo. There was a significant negative correlation between basal concentrations of total serum carotenoids and oxidized pyrimidines. A similar correlation was seen between individual carotenoids (notably

lutein and β-carotene) and oxidized pyrimidines. However, carotenoid supplementation did not have a significant effect on endogenous oxidative damage.

c. Carcinogenicity of α-Carotene

α-Carotene showed higher activity than β-carotene in suppressing tumorigenesis in skin, lung, liver, and colon (Murakoshi et al. 1992; Narisawa et al. 1996; Nishino et al. 2000).

In a skin tumorigenesis experiment, a two-stage mouse skin carcinogenesis model was used (Nishino et al. 2000). From one week after initiation by 100 μg of 7,12-dimethylbenz[a]anthracene (DMBA), 1.0 μg of 12-otetradecanoylphorbol-13-acetate (TPA) was applied twice a week. α- or β- Carotene (200 nmol) was applied with each TPA application. A greater potency of α-carotene than β-carotene was observed. The percentage of tumor-bearing mice in the control group was 68.8%, whereas the percentages of tumor-bearing mice in the groups treated with β- and α-carotene were 31.3 and 25.0%, respectively. The average number of tumors per mouse in the control group was 3.73, whereas the α-carotene-treated group had 0.27 tumors per mouse ($P < 0.01$). β-Carotene treatment also decreased the average number of tumors per mouse (2.94 tumors per mouse), but the difference from the control group was not significant.

Murakoshi et al. (1992) compared the inhibitory effect of natural α-carotene, obtained from palm oil, with that of β-carotene on spontaneous liver carcinogenesis in C3H/He male mice. Male C3H/He mice, which have a high incidence of spontaneous liver tumor development, were treated for 40 wk with β- or α-carotene (at a concentration of 0.05%, mixed as an emulsion into drinking water), or vehicle as a control. The mean number of hepatomas per mouse was significantly decreased by α-carotene supplementation *(per os* administration in drinking water at a concentration of 0.05%, *ad libitum)* as compared with that in the control group ($P < 0.001$); the control group developed 6.31 tumors per mouse, whereas the α-carotene-treated group had 3.00 tumors per mouse ($P < 0.001$). On the other hand, the β-carotene treated group did not show a significant difference from the control group, although a tendency toward a decrease was observed (4.71 tumors per mouse).

Narisawa et al. (1996) evaluated short-term suppressive effects of β- or α-carotene on N-methylnitrosourea (MNU)-induced colon carcinogenesis in Sprague-Dawley rats. Rats received three intrarectal doses of N-methylnitrosourea in week 1, and a daily gavage of de-escalated doses of carotenoids during week 2 and 5. β- or *α*-Carotene (6 mg, suspended in 0.2 ml of corn oil, intragastric gavage daily), or vehicle as control, were administered during experimental weeks 2 and 5. Lycopene and α-carotene inhibited the development of ACF quantitated at week 6, but β-carotene did not. The mean number of colonic ACF in the control group was 62.7, whereas the β- or α-carotene-treated group had 56.1 and 42.4 ($P < 0.05$), respectively. Palm carotene inhibited the development of ACF without showing statistical significance (palm carotene, 51.3 vs. control, 69.3). Thus, the greater potency of α-carotene than β-carotene was observed also in this experimental model.

<u>α -Carotene and lung cancer</u> (adopted from Veeramachaneni and Wang 2009)

There are very few studies that have examined the effects of α-carotene on lung cancer. In carcinogen-initiated mice, α-carotene was found to be more effective in decreasing mean number of lung tumors/mouse compared to β-carotene (Murakoshi et al. 1992). The greater potency of α-carotene than β-carotene in the suppression of tumor promotion was confirmed by other two-stage carcinogenesis experiments; i.e., 4-nitroquinoline 1-oxide (4NQO)-initiated and glycerol-promoted ddY mouse lung carcinogenesis model (Murakoshi 1992). 4NQO (10 mg/kg BW) was given by a single s.c. injection on the first experimental day. Glycerol (10% in drinking water) was given from experimental week 5 to 30 continuously. β- or α-Carotene (at a concentration of 0.05%), or vehicle as a control, was mixed as an emulsion into drinking water during the promotion stage. α-Carotene, but not β-carotene, reduced the number of lung tumors per mouse to about 30% of that in the control group ($P < 0.001$). The average number of tumors per mouse in the control group was 4.06, whereas the α-carotene-treated group had 1.33 tumors per mouse ($P < 0.001$). β-carotene treatment did not show any difference from the control group. Furthermore, α-carotene supplementation reduced the area of lesions (hyperplasia and adenomas) in the lungs of mice injected with multiple carcinogens (Tsuda et al. 1996). To conclude, while there is evidence both *in vitro* and *in vivo* to support a protective effect of carotenoids against lung cancer in experimental studies, the mechanisms underlying these effects need to be further investigated.

Inhibitory effects of α-carotene on proliferation of the human neuroblastoma cell line GOTO

α-Carotene inhibited the proliferation of the human neuroblastoma cell line GOTO in a dose- and time-dependent manner (Murakoshi et al. 1989). In addition, it was about 10 times more inhibitory than β-carotene. Northern blot analysis indicated that α-carotene caused maximum suppression of the level of the N-myc messenger RNA of GOTO cells. This suppression occurred within 18 h of α-carotene treatment, after which the level of the N-myc messenger RNA gradually recovered to the basal level. Analysis by flow cytometry indicated that when GOTO cells were exposed to α-carotene, they were arrested in the G0-G1 phase of their cell cycle. However, as the level of the N-myc messenger RNA was recovering, these cells resumed normal cycling. These results suggest that not only β-carotene, but also α-carotene, may play an important role in cancer prevention (Murakoshi et al. 1992).

Antioxidant activity of α-carotene

Farombi and Britton (1999) investigated the antioxidant effectiveness of palm α-carotene in comparison with β-carotene in an organic solution containing egg yolk phosphatidycholine (EYPC) in the presence of lipid soluble 2,2[1]-azobis (2,4-dimethyl valeronitrile) (AMVN)-generated peroxyl radicals by measuring the formation of phosphatidyl choline hydroperoxide (PCOOH) and thiobarbituric acid-reacting substances (TBARS). Although all the carotenoids tested at 1 mol % relative to EYPC retarded ($P < 0.05$) the chain propagation reaction of PCOOH formation, α-carotene had the highest activity but this was less than α-tocopherol. α-Tocopherol, α-carotene, and β-carotene reduced PCOOH accumulation by 78, 65, and 40%, respectively. α-Carotene significantly suppressed TBARS formation by 68%, while β-carotene elicited 50% reductions. These results suggest that α-carotene may better attenuate peroxyl radical-dependent lipid peroxidation than β-carotene in organic solution.

4) **Lycopene** (mostly adopted from BASF 2002; McClain et al. 2003; HPM 2005;Veeramachaneni and Wang 2009)

Lycopene appeared neither genotoxic nor reprotoxic or teratogenic. No signs of organ toxicity have been found in subacute, subchronic, or chronic oral toxicity studies in experimental animals receiving doses of up to 1000 mg lycopene/kg BW/d via the diet (BASF 2002; HYM 2005; Jonker et al. 2003). Thus, the NOAEL of β-carotene is considered to be 1000 mg/kg BW/d.

a. Acute toxicity of lycopene

Acute toxicity of lycopene was found to be mild in mice. Subcutaneous injection of 3 g/kg BW produced only a transient decrease in ‘‘body tone’’. Oral and i.p. administration of this dose was without effect (Milani et al. 1970).

Niederhauser et al. (1996) conducted a 4 wk study in HanIbm Wistar rats (6/sex/group) to assess and characterize the toxic potential of Lycopene 10% WS beadlet formulation containing 2% of the impurity apo-120-lycopenal as compared to a Lycopene 10% WS beadlet formulation containing <0.1% of this impurity. The following criteria were evaluated during the study: clinical signs, body weight, feed consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, macro- and microscopic examination of a complete list of tissues and organs, plasma concentrations of lycopene, apo-120-lycopenal, and vitamin A. The only treatment-related changes observed in the lycopene groups were a red discoloration of the feces and a brown-orange discoloration of the liver in most animals receiving either lycopene alone or lycopene supplemented with apo-120-lycopenal. It was concluded that lycopene beadlet formulation administered orally (dietary admix) to rats for one month at a dose of 1000 mg/kg BW/d was tolerated without any clinically significant adverse effect. The apo-120-lycopenal impurity at the concentration of 2% in the formulation did not result in any clinically significant additional findings.

As shown in Table 16, other subacute toxicity studies showed that lycopene at doses up to 1000 mg/kg BW/d resulted in no abnormalities except red discoloration of the feces, brown-orange discoloration of the liver, and brown-yellow fine granulated pigment deposits in the hepatocytes (Breinholt et al. 2000; Gradelet et al. 1996; Jewell and O’Brien, 1999; McClain and Bausch, 2003). From these studies, the NOAEL of lycopene is considered to be 1000 mg/kg BW/d.

Table 16. Acute and subacute toxicity studies of lycopene **(**No significant toxicological effects except discoloration and minor biochemical changes)

Species	Route	Dose of lycopene	Duration	Result	Reference
Mice	s.i.	3 g/kg BW	Single dose	No abnormalities except a transient decrease in body tone	Milani et al. 1970
Mice	Oral or i.p.	3 g/kg BW	Single dose	No abnormalities	Milani et al. 1970
Rat	Oral	1000 mg/kg BW/d	4 wk	No abnormalities	Niederhauser et al. 1996
Rat	Oral	0, 1, 5, 50, or 100 mg/kg BW/d	14 d	Increased activity of liver enzymes BROD and EROD	Breinholt et al. 2000
Rat	Feed	45 mg/kg BW/d	15 d	Decreased activity of liver enzyme NDMAD	Gradelet et al. 1996
Rat	Feed	45 mg/kg BW/d	16 d	Decreased activity of lung enzyme BROD	Jewell and O'Brien, 1999
Rat	Feed	1,000 mg/kg BW/d (lycopene) + <0.01, 0.3, or 2% apo-12'-lycopenal (impurity)	4 wk	Red discoloration of the feces, brown-orange discoloration of the liver, brown-yellow fine granulated pigment deposits in the hepatocytes.	McClain and Bausch, 2003

s.i.=subcutaneous injection; i.p.=intraperitoneal injection; BROD = benzyloxyresorufin-O-dealkylase; EROD = ethoxyresorufin O-dealkylase; NDMAD = nitrosodimethylamine N-demethylase

b. Subchronic and chronic toxicity studies of lycopene

b-1) Rat studies

Most of subchronic studies on lycopene used the rat as an animal model. A 100 d feeding study was conducted in young rats treated at doses of 1000 mg/kg BW/d of crystalline β-carotene or lycopene or 7,70-dihydro- β-carotene mixed with a small amount of food (Zbinden and Studer (1958). The test substance mixtures were normally completely consumed, but treatment affected the appetite, especially in the second half of the study, during which the growth rate in males was slightly decreased in comparison to the controls. Except for isolated spontaneous deaths in the first week of the study (β-carotene 4/20, lycopene 2/20, bixin 3/20, controls 1/40), all rats remained in good health. No adverse clinical signs were observed. White and red blood cell status (8th and 14th week) were within normal limits. Liver, kidney, adrenal gland, spleen, pancreas, testis, ovary, skin, bone and bone marrow were histologically normal. Signs of tissue storage of the carotenoids were not detected by microscopic examination.

Buser and Urwyler (1996) conducted a 14 week oral toxicity study in Han-Ibm Wistar rats with Lycopene 10% WS beadlet formulation as a dietary admix at concentrations to provide 50, 150, and 500 mg/kg BW/d lycopene. A control group received the beadlet formulation without lycopene and another control group received the powdered diet. There were 26 rats/sex/group with 6 rats/sex assigned to recovery groups for a 5 week treatment-free period. Lycopene was well tolerated at doses up to 500 mg/kg BW/d. After 93 d of treatment, slight hematological

variations (generally less than 10%) were observed sporadically. Due to the small magnitude of change and the lack of dose response relationship, these were considered not related to treatment. Statistically significant, relatively small increases in AST and ALT were observed in male, but not female rats in the 50, 150, and 500 mg/kg BW/d groups after 93 d of treatment (AST, 2-fold at the low-dose and 3-fold at the mid- and high-dose; ALT no change at the low-dose and 2-fold at the mid-and high-dose). In view of the relatively small magnitude of change and the absence of histopathological findings in the liver, these changes were considered not toxicologically significant. It is concluded that a daily intake of up to 500 mg/kg BW/d lycopene over a period of 14 wk is considered not to induce toxicologically significant changes in rats.

Mellert et al. (2002) conducted a 13 wk subacute toxicity study in rats with synthetic lycopene products (oral gavage using water as the vehicle). In this study, the NOAEL was found to be the highest dose level evaluated, i.e., 300 mg lycopene/kg BW/d.

b-2) Dog study

Zbinden and Studer (1958) fed one dog with 100 mg/kg BW/d of crystalline lycopene in gelatin capsules for 6 mo. In 192 doses, the animal received a total of 272 g lycopene without displaying any signs of intolerance. Body weight increased from 13 to 15 kg, and blood status was normal when checked on four occasions. At the end of the study, laboratory findings were within the normal range. The testing included: serum bilirubin, bromsulphthalein test, non-protein nitrogen, blood sedimentation rate, urine (sugar and protein), urobilinogen, and sediment. At necropsy, all the organs were macroscopically normal although there was marked accumulation of lycopene in the liver.

Table 17 includes other subchronic and chronic toxicity studies of lycopene, which indicate that the NOAEL of lycopene was 1000 mg/kg BW/d (Black, 1998; Boileau et al. 2000; Jonker et al. 2003; Mellert et al. 2002; Nagasawa et al. 1995; Zhao et al. 1998; Zbinden and Studer, 1958).

Table 17. Subchronic and chronic toxicity studies of lycopene **(**No significant toxicological effects except discoloration and minor biochemical changes)

Species	Route	Dose	Duration	Result	Reference
Subchronic toxicity studies					
Male rat	Diet	0, 1, 5, 5.7, or 57.5 mg/kg BW/d	8 wk	No abnormalities	Boileau et al. 2000
Rat	Oral	2.4, 6.0, 12, 24, or 60 mg/kg BW/d	10 wk	No abnormalities	Zhao et al. 1998
Rat	Gavage	50, 150, or 300 mg/kg BW/d	13 wk	No abnormalities except red discoloration of the feces, jejunum, and cecum	Mellert et al. 2002
Rat	Diet	0.25, 0.5, or 1.0% (145 to 616 mg/kg BW/d)	90 d	No abnormalities	Jonker et al. 2003
Rat	Oral	50, 150, or 500 mg/kg BW/d	14 wk	No abnormalities	Buser and Urwyler 1996
Rat	Diet	1,000 mg/kg BW/d	100 d	No abnormalities	Zbinden and Studer 1958
Chronic toxicity studies					
Dog	Capsule	100 mg/kg BW/d	192d	Pigment deposition in the liver and kidney	Zbinden and Studer 1958
Rat	Diet	10 to 20 mg/kg BW/d	200 d	Slight accumulation of pigments in the liver	Zbinden and Studer 1958
Female mice	Diet	90 mg/kg BW/d	28 wk	No abnormalities	Black 1998
Mice	Diet	0.07 mg/kg BW/d	10 mo	No abnormalities	Nagasawa et al. 1995

c. Geneotoxicity studies of lycopene

Naturally occurring and synthetic formulations of lycopene showed negative results in various mutagenicity and genotoxicity test systems: *in vitro* with bacterial (*Salmonella typhimurium* and *Escherichia coli* strains) and mammalian (mouse lymphoma cells and human lymphocytes) test systems, *in vivo* with mice, rats, and humans, and in *in vitro* assays (He and Campbell, 1990; Jonker et al. 2003; McClain and Bausch 2003; Mure and Rossma 2001; Rauscher et al. 1998; Thompson 1994). Similarly, oral exposure to naturally occurring lycopene did not induce chromosomal damage in mouse bone marrow cells (Rauscher et al. 1998) or DNA damage in human lymphocytes (Collins et al. 1998; Pool-Zobel et al. 1997; Riso et al. 1999). There was no evidence of clastogenicity or DNA damage in mouse peripheral blood or rat hepatocytes, respectively, following oral dosing with synthetic lycopene formulations (McClain and Bausch 2003).

d. **Reproduction toxicity and developmental toxicity studies**

In studies investigating the reproductive toxicity of synthetic lycopene formulations, no evidence of maternal, reproductive, or developmental toxicity was reported in rats or rabbits

following exposure throughout gestation, at doses up to 1000 mg lycopene/kg BW/d (rats) or 200 mg lycopene/kg BW/d (rabbits) (Christian et al. 2003; McClain and Bausch 2003; Zbinden and Studer 1958).

In a two-generation reproduction study in rats (1000 ppm crystalline lycopene in the diet), there were no relevant differences between treated and control animals from the first generation (Zbinden and Studer 1958). The duration of gestation was normal, and no malformations were seen. The F1 generation was treated for 200 d, after which 4 treated and 4 control animals of the first generation were sacrificed. In the lycopene-treated animals, the females had marked accumulation and the males had slight accumulation of a brown, relatively rough granular pigment in the liver, which was not observed after paraffin embedding and clearing. No other histopathologic liver changes were observed. No histopathological abnormalities were noted in other organs.

A teratology study was conducted in mated Wistar female rats with WS beadlet formulations of lycopene and lycopene plus added apo-120-lycopenal (Eckhardt 1996). The apo-120-lycopenal is present as an impurity in lycopene bulk product and was tested by adding a 10-fold excess of apo-120-lycopenal (20 mg/1000 mg of lycopene). Animals (14/group) were treated orally (dietary admix) at daily dose levels of 0 (beadlet control), 1000 mg/kg of lycopene, and 1000 mg/kg of lycopene plus added apo-120-lycopenal (20 mg/kg BW) from d 6 to 18 of gestation and C-sectioned on day 21 of gestation. No signs of maternal toxicity or teratogenic effects were observed and the reproductive and developmental criteria were normal except for a very small increase in the incidence of extra-thoracic (14th) ribs observed in the two lycopene groups as compared to the controls. The incidence was 14.7% in the control group, 26.1% in the lycopene group, and 16.8% in the lycopene with added apo-120-lycopenal. Extra-thoracic ribs are considered to be a skeletal variation, which is fetal-toxic, but not a teratogenic effect. It was concluded that no teratogenic effects were noted in rats with 1000 mg/kg BW/d lycopene or lycopene with added apo-120-lycopenal beadlet formulations.

Developmental (embryo-fetal) toxicity studies of synthetic crystalline lycopene products revealed no developmental toxicity in rats at maximum feasible doses as high as 3000 mg/kg BW/d, or in rabbits at maximum feasible doses as high as 2000 mg/kg BW/d (BASF 2002).

e. Mutagenicity of lycopene

Several studies indicate that lycopene is not mutagenic or clastogenic. To test the mutagenicity of lycopene, Gocke (1996) conducted the Ames test with 7 tester strains including TA100 and TA97. It was found that pure crystalline lycopene was devoid of mutagenic activity. To explore the possibility of a degradation product of lycopene, a batch of pure crystalline lycopene was exposed to air and light and tested in TA97 and TA100, which were found to have mutagenic activity. The mutagenic activity was reduced in the presence of an antioxidant, α-tocopherol; however, α-tocopherol had little or no effect on the pre-formed mutagenic degradation products. Lycopene in a beadlet formulation (10% WS) was tested and found to be devoid of mutagenic activity. Stressed material also was tested (45 ^{o}C stored for 1, 2, 4, and 8 wk) in strains TA100 and TA97 and no mutagenic effects were observed.

Muster et al. (1996a) evaluated mutagenicity in mouse lymphoma cells with lycopene 10% WS beadlet formulation for genotoxic activity by examining its ability to induce mutations at the tk locus in mouse lymphoma cells. No increase in the mutant frequency was observed with lycopene beadlet formulation as compared to controls, with or without S9 metabolic activation.

Miller et al. (1996a) tested a lycopene 10% WS beadlet formulation for structural and numerical chromosome aberrations in human peripheral blood lymphocytes, with and without S9 metabolic activating system. The incidence of cells with structural or numerical aberrations was not significantly increased as compared to controls. It was concluded that Lycopene 10% WS formulation did not cause clastogenic or aneuplodic effects.

Muster et al. (1996b) tested a lycopene 10% WS beadlet formulation for its ability to induce unscheduled DNA synthesis in the hepatocytes of rats dosed orally with fresh and aged formulations. The aged material was stored for 8 wk at 45 ^{o}C with oxygen, then at 4 ^{o}C protected from light and under nitrogen. Neither the fresh nor aged formulations showed increased DNA repair activity. It was concluded the lycopene 10% WS beadlet formulations, fresh or aged, did not produce DNA damage in the hepatocytes of treated rats.

In a study of Miller et al. (1996b), the mutagenic and clastogenic potential of lycopene 10% S beadlet formulation was tested in the mouse micronucleus test in bone marrow. Fresh and aged beadlet formulations were tested. The beadlet formulation was aged by storage for 8 wk at 45 ^{o}C in a closed brown bottle exposed to oxygen, then for 8 d at 4 ^{o}C under nitrogen . Neither the fresh nor aged formulations caused any chromosome damage or effects on spindle formation. It was concluded that lycopene 10% WS formulations, fresh or aged, did not induce clastogenic or spindle damaging effects in mouse bone marrow.

Muster et al. (1999) also reported that fresh or aged lycopene-containing soft drinks did not induce clastogenic or spindle-damaging effects in peripheral blood of treated mice. In this study, lycopene 10% WS beadlet formulation was evaluated in the mouse micronucleus test for mutagenic and clastogenic effects. Fresh (50 ppm) and aged (25 and 50 ppm) soft drink preparations were administered to mice for 14 d as drinking water. The material was aged for 28 d at 45 ^{o}C in the dark. No toxicologic effects were observed in mice treated for 14 d.

f. Carcinogenicity

Relatively fewer studies have examined the effects of lycopene supplementation on lung cancer. *In vitro* work using lung cancer cell lines demonstrated that lycopene prevented cell proliferation and growth, DNA damage, and suppressed IGF-1 stimulated growth (Levy et al. 1995; Muzandu et al. 2005). However, lycopene supplementation at different doses in rats and mice initiated with a carcinogen resulted in inconsistent data: no effect on tumor incidence (Hecht et al. 1999), decreased tumor incidence and multiplicity in male mice but not in female mice (Kim et al. 1997), or increased lung mutagenesis in mice (Guttenplan et al. 2001). In these studies, it is unknown whether the concentrations of lycopene reached biologically significant levels because none of these studies reported tissue/plasma levels of lycopene.

Male ferrets exposed to cigarette smoke and supplemented with lycopene had reduced lung squamous metaplasia, reduced proliferating cell nuclear antigen staining (PCNA), and reduced phosphorylated Bcl-2 associated death domain (p-BAD) expression (Veeramachaneni and Wang 2009). Lycopene treatment also restored caspase 3 expression levels and increased insulin like growth factor-binding protein 3 (IGFBP-3) levels in ferrets (Liu et al. 2003).

5) γ-Carotene

There are no separate studies on the basic toxicity in rats or other species that one would expect to exist in the scientific literature. In a subchronic toxicity study of rats (16 wk), the palm carotenoid concentrate providing 6.5 mg/kg BW/d of γ–carotene did not result in any adverse effects (Choo et al. 1992). Because γ-carotene is included in 'carotenoids' as a very minor component and has a chemical structure that is very similar to other carotenoids, one would expect that γ–carotene is as safe as other carotenoids. A number of human and animal studies that examine the effect of 'carotenoids' complex on the human or animal may have included γ-carotene as a minor component. For example, studies on palm carotenoids have already captured the effects of γ-carotene when reporting the effects of mixed carotenes. As discussed above, palm carotenoids demonstrated no toxicity. It is emphasized that palm carotenoids contains only 0.5% of γ-carotene and that the γ-carotene contents of Caromin® 10 and Caromin® 30 are 0.05 and 0.15%, respectively.

6) Palm oil

Table 18 summarizes the toxicity studies on palm oil. A subacute toxicity of palm oil extract (containing 80% T3) in young rats and mice demonstrated that up to 2500 mg/kg BW was safe (Oo et al. 1992). Also, repeatedly heated refined and crude palm oils showed no mutagenicity (Manorama et al. 1989). All the experiments were conducted under GLP.

Table 18. Toxicity studies of palm oil

Species	Route	Test material	Duration	Result	Reference
Rat	Oral	Palm oil extract, 2500 mg/kg BW/d	30 d	No abnormalities	Oo et al. 1992
Rat	Feed	5% red palm oil in diet	42 d	No abnormalities	Watkins et al. 2001
S. typhimurium TA100 and TA98	W/ & w/o metabolic activation	Heated (8-24 h) refined and crude palm oils	48 h	No mutagenic activity	Manorama et al. 1989

3. **Safety/toxicity of carotenoids -Clinical studies (**adopted from BASF 2002; HYM 2005; Jonker et al. 2003; LSRO 1979; McClain and Bausch 2003; Veeramachaneni and Wang 2009; Woutersen et al. 1999)

Although carotenoids are not essential for human health, they have biological actions that may be important in maintaining health and preventing the appearance of serious diseases such

as cancer and cardiovascular disorders (IOM 2001; Voutilainen et al. 2006; Woutersen et al. 1999). The IOM report (2001) on β-carotene and carotenoids states the following: **"**No adverse effects other than carotenodermia have been reported from the consumption of β-carotene or other carotenoids in food. Carotenodermia is a harmless but clearly documented biological effect of high carotenoid intake. It is characterized by a yellowish discoloration of the skin that results from an elevation of carotene concentrations.

β-Carotene is used therapeutically at extremely high doses (approximately 180 mg/d) for the treatment of erythropoietic protoporphyria, a photosensitivity disorder (IOM 2000, 2001). No toxic side effects have been observed at these doses. In addition, long-term supplementation with β-carotene to persons with adequate vitamin A status does not increase the concentration of serum retinol (Nierenberg et al. 1997).

To date, no human clinical studies reported any adverse effects of carotenoids in the general population. The four major primary prevention studies completed did not provide evidence of a significant protective effect of β-carotene on risk of cancer, except for the Linxian study that found a protective effect on stomach cancer. Two clinical trials reported an increase in lung cancer and heart disease associated with pharmacological dosages (20-30 mg/d) of supplemental β–carotene in current smokers, in heavy alcohol drinkers, or in asbestos workers (Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study Group, 1994; Omenn et al. 1996a, 1996b). It was speculated that the conditions in the lungs of heavy smokers may tip the β-carotene antioxidant/prooxidant balance toward a prooxidant state. A number of prooxidants are known to stimulate tumor promotion (Cerutti 1985). Accordingly, the β-carotene radicals that may have been induced by cigarette smoke in lung tissue might have mutagenic or carcinogenic potential, thus increasing the risk of lung cancer. Similarly, the enhanced lung carcinogenesis in asbestos workers was explained by the oxidative potential of asbestos. Asbestos fibers contain iron, a powerful catalyst for oxidation reactions, which might, along with β-carotene, result in enhanced formation of carcinogenic products. Carcinogenesis is a multistep process taking years involving oncogene activation and suppressor gene deactivation. β-Carotene may affect various stages of carcinogenesis. For instance, a possible beneficial effect of β-carotene at the cancer initiation stage has been demonstrated in several animal models.

However, two major interventional studies (Physicians' Health Study and the Linxian study) did not report any adverse effects of supplemental β- carotene (15 mg/d or 50 mg/2d; Hennekens et al. 1996; Blot et al. 1993). Also a meta analysis of 26 cohort studies showed no correlation between α- and β-carotene intakes and lung cancer and an inverse correlation between lycopene intakes and lung cancer incidence (Gallicchio et al. 2008).

Supplemental β-carotene had no significant effect on the incidence of other major cancers (prostate, bladder, colon or rectum, stomach (ATBC Cancer Prevention Study Group 1994; Omenn et al. 1996 a, 1996b).

No observational studies linked β-carotene with increased risk of CVD. Overall, carotenoids have either protective or no effects on CVD in the general population.

Based on the totality of scientific data, IOM (2000, 2001) concluded as follows: "Because of the inconsistent data on adverse effects of β-carotene, a UL cannot be established at this time. ULs are not established for other carotenoids due to a lack of suitable data." Upper Tolerable Intake Level (UL) is defined as a safe upper level for consumption over a lifetime or the highest level of a daily nutrient intake that will most likely present no risk of adverse health effects in almost all individuals in the general population (IOM 2001).

a. Carotenoid intake and lung cancer

Observational studies

From a meta analysis of 6 randomized clinical trials examining the efficacy of β-carotene supplements and 25 prospective observational studies assessing the associations between carotenoids and lung cancer, Gallicchio et al. (2008) concluded that β-carotene supplementation is not associated with the risk of developing lung cancer. Findings from prospective cohort studies suggest inverse associations between carotenoids and lung cancer; however, the decreases in risk are generally small and not statistically significant. In this study, the pooled relative risk (RR) for the studies comparing β-carotene supplements with placebo was 1.10 (95% confidence limits (CI): 0.89, 1.36; P = 0.39). Among the observational studies that adjusted for smoking, the pooled RRs comparing highest and lowest categories of total carotenoid intake and of total carotenoid serum concentrations were 0.79 (95% CI: 0.71, 0.87; P < 0.001) and 0.70 (95% CI: 0.44, 1.11; P = 0.14), respectively. For β-carotene, highest compared with lowest pooled RRs were 0.92 (95% CI: 0.83, 1.01; P =0.09) for dietary intake and 0.84 (95% CI: 0.66, 1.07; P = 0.15) for serum concentrations. The RR comparing highest and lowest categories of intake was 0.89 for α-carotene; for serum concentrations, the RR was 0.71 for lycopene. No observational studies reported adverse effects of other carotenoids, including α-carotene and lycopene.

Interventional studies

Table 19 summarizes human intervention studies on dietary intake of carotenoids and lung cancer.

Table 19. Human intervention studies on dietary intake of carotenoids and lung cancer.

Dose/length of β-carotene Supplementation	Subjects/Design	Results	Ref.
15 mg/d, 5 y	Linxian study: 29584 men and women aged 40-69 y in the general population of Linxian, China, 30% smokers. Factorial design of combination of β-carotene, vitamin E, and selenium supplements	Significantly lower overall mortality (RR 0.91; 95% CI: 0.84, 0.99) and cancer incidence (RR 0.87; 95% CI: 0.75, 1.00) in the group receiving β-carotene (plus vitamin E and selenium). Also associated with reduced risk of lung cancer death (RR 0.55; 95% CI: 0.26, 1.14)	Blot et al. 1993
20 mg/d; 5-8 y (median 6.1y)	ATBC study: 29133 male smokers aged 50-69 y in Finland, 100% smokers; Factorial design (2 x 2, β-carotene and α-tocopherol)	Significantly increased lung cancer incidence in subjects administered β-carotene (RR 1.16; 95% CI: 1.02, 1.33). Increased overall mortality (8%) also associated with β-carotene supplementation. Adverse effect stronger in subjects smoking > 20 cigarettes/d (RR 1.25; 95% CI: 1.07, 1.46) vs. 5-19 cigarettes/d (RR 0.97; 95% CI: 0.76, 1.23), and in those with higher alcohol intake (≥11 g/d) (RR 1.35; 95% CI: 1.01, 1.81) vs. lower intake (RR 1.03; 95% CI: 0.85, 1.24)	ATBC Prevention Study Group 1994; Albanes et al. 1996
30 mg/d + retinol palmitate (25000 IU/d) or placebo; 4 y	CARET study: 18314 male and female smokers, former smokers, and workers exposed to asbestos in USA, 60% smokers.	Significantly increased lung cancer incidence in subjects administered β-carotene (plus retinol) (RR 1.28; 95% CI: 1.04, 1.57). Increased overall mortality (17%) also associated with β-carotene supplementation. Higher risk for lung cancer in association with β-carotene in the highest vs. lowest quartile of alcohol intake (RR 1.99; 95% CI: 1.28, 3.09), and current smokers had higher risk for lung cancer than former smokers.	Omenn et al. 1996a, 1996b
50 mg every other day; 12 y	PHS study: 22071 male physicians aged 40 to 84 y in USA, 11% smokers; Factorial design (2 x 2, β-carotene and aspirin)	No difference in the incidence of total cancer or lung cancer or overall mortality	Hennekens et al. 1996

The ATBC Cancer Prevention Study showed a significantly higher incidence of lung cancer (RR 1.18; 95 % CI:1.03,1.36) and total mortality (RR 1.08; 95 % CI: 1.01, 1.16) in current smokers supplemented with 20 mg/d β-carotene (with or without 50 mg of α-tocopherol) for 5 to 8 y (ATBC Prevention Study Group 1994). In this ATBC trial, men who consumed more

than 11 g/d of alcohol (approximately one drink per day) showed an adverse response to β-carotene supplementation (Albanes et al. 1996).

In addition, the Carotene and Retinol Efficacy Trial (CARET), a multicenter lung cancer prevention trial of a nutrient combination versus placebo in asbestos workers and smokers (Omenn et al. 1996a, 1996b), reported more lung cancer cases in the supplemented group. The nutrient combination used in CARET included supplemental β-carotene (30 mg/d) plus retinol (25000 IU/d). In the CARET study, adverse effects were associated with the individuals in the highest quartile of alcohol intake (Omenn et al. 1996a). However, it should be noted that CARET used a nutrient combination, without a factorial design, and it is not clear whether the reported effects were attributable to β-carotene, retinol, or a combination of both nutrients.

In contrast, the Physicians' Health Study (PHS) of supplemental β-carotene versus placebo in 22071 male U.S. physicians reported no significant effect of 12 y of supplementation with β-carotene (50 mg on alternate days) on cancer or total mortality (Hennekens et al. 1996). With regard to lung cancer, there was no indication of excess lung cancer in the β-carotene-supplemented individuals, even among smokers who took the supplements for up to 12 y. Also β-carotene supplementation (50 mg on alternate days) had no significant benefit or harm on cancer or CVD during more than 12 y of treatment and follow-up.

One additional trial, which was not designed as a lung cancer prevention trial, produced results that are of relevance to the topic of lung cancer prevention (Blot et al. 1993). The trial tested the efficacy of four different nutrient combinations in inhibiting the development of esophageal and gastric cancers in 30000 men and women aged 40 to 69 y living in Linxian County, China (Blot et al. 1993). One of the nutrient supplements was a combination of β-carotene, selenium, and vitamin E. After a 5-y intervention period, those who were given this combination had a 13% reduction in cancer deaths (RR 0.87; 95 % CI: 0.75, 1.00), a 9% reduction in total deaths (RR 0.91; 95 % CI: 0.84, 0.99), a 4% reduction in esophageal cancer deaths (RR 0.96; 95 % CI: 0.78,1.18), and a 21% reduction in gastric cancer deaths (RR 0.79; 95 % CI: 0.64, 0.99) (Blot et al. 1994). For lung cancer, this trial had limited statistical power, with only 31 total lung cancer deaths. However, the relative risk of death from lung cancer was 0.55 (95 % CI: 0.26,1.14) among those receiving the combination of β-carotene, α-tocopherol, and selenium. The smoking prevalence, including individuals who had ever smoked cigarettes for 6 or more months, was 30%.

It appears that the effect of antioxidants on cancer may depend on the doses (nutritional versus pharmacological), baseline antioxidant status (different between gender and/or nutritional status) and health status of subjects (healthy versus cancer high-risk subjects). The dosages of β-carotene used in the ATBC and CARET studies were 20 to 30 mg/d for 2-8 y; 20-30 mg β-carotene in a supplement form correspond to 120 -180 mg of dietary β-carotene (1 mg β-carotene in a supplement form corresponds to 6 mg β-carotene in the diet; IOM 2000). The ATBC study and CARET specifically selected populations at high risk for lung cancer development, namely, heavy smokers and asbestos-exposed workers, respectively. These subjects had been exposed to high loads of carcinogens for many years before the onset of the trials; in the ATBC study, subjects had smoked an average of one pack of cigarettes per day for 36 y. Subgroup

analysis by Albanes et al. (1997) indicated that adverse effects in the ATBC study were restricted to persons who smoked one pack of cigarettes or more per day. Cigarette smoke has been invoked as a prooxidant. It has been suggested that in the presence of high concentrations of β-carotene, cigarette smoke might lead to oxidative destruction of β -carotene, resulting in the formation of oxidized metabolites or breakdown products of β-carotene that may induce oxidative stress and carcinogenesis (Hurst et al. 2005; Krinsky and Johnson 2005; Siems et al. 2009). This effect was not observed with high doses of lycopene (Liu et al. 2003). Therefore, it is probable that these populations already had initiated cancer cells at the beginning of the studies, and that the critical early phase in cancer development was not investigated. A possible beneficial effect of β-carotene at the cancer initiation stage, such as seen in certain animal studies, could not be evaluated in this special population. In contrast, the PHS, which is the longest completed nutrient intervention trial to date, was designed to evaluate the effect of β-carotene on overall cancer incidence and mortality in a population not at particular risk.

b. Carotenoids and CVD health

Observational studies

Epidemiologic studies have shown that a high intake of carotenoids is associated with a reduced risk of CVD, although the results of these studies are somewhat conflicting. The studies were categorized into two groups according to the source of carotenoids measured: dietary intake or serum or plasma concentrations of carotenoids (Voutilainen et al. 2006). Dietary intakes of α-carotene were inversely associated with the risk of CVD or atherosclerosis. Serum levels of α-carotene showed inconsistent responses between total serum carotenoid concentrations and the risk of CVD. No studies showed adverse effects of α-carotene and lycopene.

Table 20 summarizes human observational studies on dietary intake of carotenoids and CVD/atherosclerosis. No observational studies linked β-carotene with increased risk of CVD. Overall, carotenoids have either protective or no effects on CVD.

Table 20. Human observational studies on dietary intake of carotenoids and CVD/atherosclerosis

Source of carotenoids	Design	Name of the study/Population	Result	Reference
α- and β-Carotene, lutein plus zeaxanthin, lycopene, and ß-cryptoxanthin	Prospective, 12 y FU 73286 F	Nurses Health Study, American	Inverse significant associations between the highest quintiles of intake of β-carotene and α-carotene and risk of CVD	Osganian 2003
α- and β-Carotene, lutein, and lycopene	Prospective, 8 yr FU 4338 M	HPS, American	α- and β-carotene and lycopene had no association with a risk of stroke	Ascherio et al. 1999
Carotenoids with provitamin A activity	Prospective, 14 yr FU 5133 M+F	Finnish Mobile Clinic Study, Finnish	Nonsignificant inverse association between dietary intake of carotenoids with provitamin A activity and the risk of coronary mortality in women	Knekt et al. 1994
Carotenoids with provitamin A activity	Cross-sectional 12773 M+F	ARIC Study, American	Those in the highest quintile of carotenoid consumption had a lower prevalence of plaques	Hirvonen et al. 1999
β-Carotene	Prospective, 4 y 4802	The Rotterdan Study, Dutch	Significantly decreased risk of MI in highest β-carotene intake quartile	Klipstein-Grobusch et al. 1999
β-Carotene	Case-control 433 + 869 F	Italian	The risk of nonfatal AMI in women was inversely related to intake of β-carotene–containing foods	Tavani et al. 1997
β-Carotene	Prospective, 30 y 1843 M+F	Western Electric Study, American	A modest decrease in the risk of stroke with higher intake of β-carotene	Daviglus et al. 1997

Adopted from Voutilainen et al 2006. MI=myocardial infarction; AMI= acute myocardial infarction; FU=follow up; M=male; F=female; HPS=The Health Professionals Follow-up Study; the ARIC study= the Atherosclerosis Risk in Communities study

Osganian et al. (2003) reported that the association between the specific carotenoids and coronary artery disease (CAD) risk did not vary significantly according to smoking status. In a prospective study with 73286 female nurses using a semi-quantitative food-frequency questionnaire, they identified 998 incident cases of CAD during the 12-y follow-up (803590 person-y). After adjustment for age, smoking, and other CAD risk factors, there were inverse associations between the highest quintiles of α-carotene and β-carotene intakes and the risk of CAD, but not with lycopene intakes.

Other prospective and case-control studies have shown an association between low intakes of β-carotene and the risk of CVD, although the results of the studies are inconsistent. In the Rotterdam Study (*n* =4802), there was a significantly reduced risk of myocardial infarction (MI) after a 4-y follow-up in subjects in the highest third of β-carotene intake compared

with the subjects in the lowest third (Klipstein-Grobusch et al. 1999). In an Italian case-control study with 433 cases and 869 paired controls, the risk of nonfatal acute MI in women was inversely related to the intake of β-carotene-containing foods (Tavani et al. 1997).

Knekt et al. (1994) found an inverse nonsignificant association between dietary intake of carotenoids with provitamin A activity and the risk of coronary mortality in women in their longitudinal cohort study of 5133 Finnish men and women aged 30–69 y. Similar findings were observed for β-carotenoid intake and the risk of stroke in the Chicago Western Electric Study, which evaluated 1843 middle-aged men (Daviglus et al. 1997).

In the prospective Health Professionals Study (Ascherio et al. 1999), the dietary intake of lycopene and α- or β-carotene measured by food-frequency questionnaire had no association with stroke risk. From a cross sectional study with 12773 participants of the Atherosclerosis Risk in Communities cohort, Kritchevsky et al. (1998) reported that both women and men in the highest fifth of carotenoid consumption had a lower prevalence of plaques than did those in the lowest fifth, especially in women. The inverse association was somewhat stronger in men aged 55–64 y than in men aged 45–54 y, whereas age made little difference in the women.

From a cohort study pooling 9 prospective studies (10 y follow-up; 4647 major incident CVD events from the 293172 subjects who were free of CVD at baseline), Knekt et al. (2004) found a lower risk of major CVD events at higher total intakes of β-carotene and at higher dietary intakes of several carotenoids after adjustment for only age and energy intake. The relations were considerably reduced after additional adjustment for potential non-dietary confounding factors. The investigators concluded that the CVD risk reductions at high carotenoid intakes appeared to be small.

Interventional studies

Table 21 summarizes clinical trials of β-carotene in CVD. No studies reported adverse effects of β-carotene in a general population, although the risk of CVD was increased in heavy smokers consuming supplemental β-carotene at 20-30 mg/d (corresponding to dietary β-carotene of 120-180 mg/d).

Table 21. Clinical trials of β-carotene in CVD

Dose, mg/d	Subjects	Duration	Results	Reference
Primary prevention study				
20	29133 male smokers aged 50-69 y, ATBC study, Finnish	6.5 y	β-Carotene supplementation increased the post-trial risk of a first-ever nonfatal MI; Nonsignificant increase in IHD and stroke mortality in the β-carotene supplemented group	ATBC group 1994; Tornwall et al. 2004
20	29246 male smokers aged 50-69 y, ATBC study, Finnish	6.5 y	High serum β-carotene doubled the risk of subarachnoid hemorrhage and decreased that of cerebral infarction by one fifth.	Leppälä et al. 1999
50	22071 males; The PHS, American	12 y	No evidence for CVD mortality in the β-carotene supplemented group	Hennekens et al.1996
30	18314 male smokers and asbestos workers; CARET, American	4 y	β-Carotene supplementation had an adverse effect on the incidence of CVD mortality	Omenn et al.1996
50	532 females and 1188 males; SCP Study; American	8.2 y	No evidence for increased CVD mortality in the β-carotene group	Greenberg et al.1996
Secondary prevention study				
20	20536 subjects with CHD, occlusive arterial disease, or diabetes; HPS, British	5.0 y	No evidence for an association between β-carotene intake and the 5 y mortality from, or incidence of, any type of CVD	HPS Collabora-tive Group 2002
20	1862 male smokers, previous MI; ATBC study, Finnish	5.3 y	Significantly increased risk of fatal coronary event in the β-carotene-supplemented group	Rapola et al. 1997
20	Prospective, 6.1 y 26593 M, ATBC Study, Finnish	>5.0 y	Dietary intake of β-carotene was inversely associated with the risk of cerebral infarction. Lycopene intake had no association.	Hirvonen et al. 2000

From Voutilainen et al. 2006. IHD=ischemic heart disease; CHD= coronary heart disease; HPS= Heart Protection Study;

Although a higher plasma β-carotene concentration has been associated with a reduced risk of heart disease in several observational studies, 5 large randomized trials reported inconsistent effects of β-carotene use on cardiovascular events. Two interventional studies showed adverse effects of supplemental β-carotene in heavy smokers (ATBC group 1994, Hennekens et al. 1996). Higher RR of CVD in smokers can be interpreted in a way similar to the cases of lung cancer. From a subset analysis of the ATBC study conducted in Finland, Leppälä et al. (2000) reported that the effect of β-carotene supplementation was significantly modified only by alcohol consumption; the other factors did not have any consistent effect on β-carotene. β-Carotene supplementation increased the risk of intracerebral hemorrhage without statistical

significance and modestly decreased that of cerebral infarction among men with greater alcohol consumption (Leppälä et al. 2000). High serum β-carotene doubled the risk of subarachnoid hemorrhage and decreased that of cerebral infarction by one fifth (Leppälä et al. 1999). In the ATBC Study, the supplementation of β-carotene (20 mg/d for over 6 yr) was inversely associated with the risk of cerebral infarction (RR of highest compared with the lowest quartile: 0.74; 95% CI: 0.60, 0.91; Hirvonen et al. 2000). However, the risk of fatal CHD increased in the groups that received either β-carotene (20 mg/d) or the combination of α-tocopherol and β-carotene compared with the group that received placebo in 1862 male smokers aged between 50 and 69 who had had a previous MI (Rapola et al. 1997). Also, there was an increase the post-trial risk of a first-ever non-fatal MI in the 6-y post-trial effects of α-tocopherol and β-carotene (20 mg/d) supplementation in 29133 male smokers, although there is no plausible mechanism to explain this finding (Tornwall et al. 2004). However, from the ATBC study, Hirnoven et al. (2000) found that dietary intake of β-carotene was inversely associated with the risk for cerebral infarction and that dietary lycopene had no association.

The effects of a combination of β-carotene (30 mg/d) and vitamin A on lung cancer and heart disease were studied for 4 y in the CARET, with a total of 18314 smokers, former smokers, and workers who were exposed to asbestos (Omenn et al.1996). In the supplementation group, the risk of death from any cause and the risk of death from CVD were increased without statistical significance.

However, 12 y of supplementation with β-carotene produced neither benefit nor harm in the incidence of malignant neoplasms, CVD, or death from all causes in the PHS. In this study, β-carotene (50 mg/d on alternate days) was administered to 22071 male physicians aged 40 to 84 y in the United States; 11% of participants were current smokers and 39% were former smokers at the beginning of the study (Hennekens et al. 1996).

In the Heart Protection Study (HPS), a secondary prevention trial, 20536 British adults aged 40–80 y with CHD, other occlusive arterial disease, or diabetes were randomly assigned to receive antioxidant vitamin supplementation (600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene daily) or placebo (HPS Collaborative Group 2002). Antioxidant vitamin supplementation had no effects on 5 y mortality or the incidence of any type of vascular disease, cancer, or any other major outcome.

Also, the Skin Cancer Prevention Study (SCPS) reported that the supplementation with 50 mg β-carotene/d for a median of 4.3 y had no effect on mortality during the median follow-up period of 8.2 y (285 all-cause deaths; Greenberg et al. 1996). It is possible that carotenoids could prevent cellular damage at physiologic concentrations in normal subjects, but that their ability to protect against cellular damage disappears at the higher doses used in the supplementation studies. It appears that the supplementation of β-carotene at pharmacological doses (20-30 mg/d for over 4 y) has adverse effects on CHD in male smokers, heavy alcohol consumers, and asbestos workers.

c. Cervical cancer

No studies linked β-carotene with increased risk of cervical cancer. García-Closas et al. (2005) conducted a systemic review on the role of diet and nutrition on the risk of human papillomavirus (HPV) persistence and cervical neoplasia. The review included all observational studies controlling for HPV infection published between March 1995 and November 2003 and all randomized clinical trials published between January 1991 and November 2003. This review included 10 clinical trials, 8 observational prospective studies, and 15 case-control studies. The few studies on HPV persistence showed a possible protective effect of fruits, vegetables, vitamins C and E, α- and β-carotene, lycopene, and other carotenoids. Evidence for a protective effect of cervical neoplasia was probable for folate, retinol, and vitamin E and possible for vegetables, α- and β-carotene, lycopene, and other carotenoids. Although the available evidence for an association between diet and nutritional status and cervical carcinogenesis taking HPV infection into account is not yet convincing, it appears that α- and β-carotene, and lycopene have a protective or neutral role in cervical carcinogenesis.

d. Prostate cancer

No studies linked β-carotene with increased risk of prostate cancer. Studies on carotenoids and prospate cancer risk produced inconsistent results. Peters et al. (2007) investigated the association between prediagnostic serum carotenoids (lycopene, α- and β-carotene, and other carotenoids) and risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. The study included 692 prostate cancer cases, diagnosed 1 to 8 y after study entry, including 270 aggressive cases and 844 matched controls. There was no association between serum α-carotene or lycopene and total prostate cancer. β-Carotene was associated with an increased risk of aggressive prostate cancer (OR 1.67; 95% CI: 1.03,2.72 for highest versus lowest quintile; P for trend, 0.13); in particular, regional or distant stage disease (OR 3.16; 95% CI: 1.37, 7.31 for highest versus lowest quintile; P for trend, 0.02); other carotenoids were not associated with risk.

However, from a case-control study nested within the prospective Health Professionals Follow-up Study (450 cases and 450 controls), Wu et al.(2004) reported no statistically significant inverse correlations among plasma α- and β-carotene, and lycopene concentrations and overall risk of prostate cancer diagnosis: odds ratio (highest versus lowest quintile; OR), α-carotene: OR 0.67 (CI: 0.40, 1.09); β-carotene: OR 0.78 (95% CI: 0.48,1.25); and lycopene: OR 0.66 (95% CI: 0.38,1.13).

e. Gastric and esophageal cancer

No studies linked β-carotene with increased risk of gastric and esophageal cancer. Most (De Stefani et al. 2000; Ekström et al. 2000; Graham et al. 1990; Ji et al. 1998; Larsson et al. 2007; La Vecchia et al. 1994; Lissowska et al. 2004; Mayne et al. 2001; Nomura et al. 2003; Persson et al. 2008; You et al. 1988), but not all (Garcia-Closas et al. 1999; Hansson et al. 1994; Harrison et al. 1997; Lopez-Carrillo et al. 1999; Palli et al. 2001) case-control studies have

reported a significant inverse association between dietary intake of carotenoids, in particular β-carotene, and gastric cancer risk.

Kubo and Corley (2007) conducted a meta analysis of 10 studies (1 cohort, 9 case-control; 1,057 esophageal and 644 cardia cases). Summary estimates stratified by cancer site suggested that higher intakes of beta-carotene/vitamin A was inversely associated with the risk of esophageal adenocarcinoma (β-carotene, OR 0.46; 95% CI: 0.36, 0.59). β-Carotene intake was also inversely associated with the risk of cardia adenocarcinoma (OR 0.57; 95% CI: 0.46, 0.72). Dose effects were observed for most associations.

Persson et al. (2008) investigated the impact of carotenoids, retinol, and tocopherol on gastric cancer development in a large nested case-control study among Japanese with known *Helicobacter pylori* infection status. A total of 36745 subjects aged 40-69 y in the Japan Public Health Center-based Prospective Study who responded to the baseline questionnaire and provided blood samples in 1990-1995 were followed until 2004. Plasma level of β-carotene was inversely associated with the risk of gastric cancer (compared with the lowest quartile: OR 0.63, OR 0.48, and OR 0.46, for quartiles 2, 3 and 4, respectively, P for trend < 0.01). Inverse associations were evident in men for α-carotene (compared with the lowest quartile: OR 0.78, OR 0.66, and OR 0.60, for quartile 2, 3 and 4, respectively, P for trend = 0.04) and β-carotene (P for trend < 0.01), but not in women, who had relatively higher plasma levels compared with men.

Larsson et al. (2007) conducted a prospective cohort study with 82002 Swedish adults aged 45-83 y who had completed a food-frequency questionnaire in 1997 (7.2 y FU). The multivariate relative risks for the highest versus lowest quartiles of intake were 0.50 (95% CI: 0.30, 0.83; P for trend = 0.03) for α-carotene, and 0.55 (95% CI: 0.32, 0.94; P for trend = 0.07) for β-carotene. No significant associations were found for lycopene intake.

Overall, carotenoid intakes are associated with the reduced risk of gastric or esophageal cancer.

f. Ovarian cancer

No study reported adverse effects of carotenoids on ovarian cancer. From a pooled analysis of 10 prospective cohort studies in North America and Europe, Koushik et al. (2006) reported that the consumption of the major carotenoids during adulthood does not play a major role in the incidence of ovarian cancer. Among 521911 women, 2012 cases of ovarian cancer occurred during a follow-up of 7-22 y across studies. The major carotenoids were not significantly associated with the risk of ovarian cancer. The pooled multivariate RRs (95% CI) were 1.00 (0.95-1.05) for a 600 ug/d increase in α-carotene intake, 0.96 (0.93-1.03) for a 2500 ug/d increase in β-carotene intake, and 1.01 (0.97-1.05) for a 4000 ug/day increase in lycopene intake. These associations did not appreciably differ by study. These results suggest that consumption of the major carotenoids during adulthood does not adversely impact the incidence of ovarian cancer.

Zhang et al. (2007) conducted a case-control study in China during 1999-2000. The cases were 254 patients with histologically confirmed epithelial ovarian cancer and 652 age-matched

controls. Compared with the highest vs. the lowest quartile of intake, the adjusted ORs were 0.39 (95% CI: 0.23, 0.66) for α-carotene, 0.51 (95 % CI: 0.31, 0.84) for β-carotene, and 0.33 (95% CI: 0.20, 0.56) for total carotenoids. The authors concluded that a higher intake of carotenoids can reduce the risk of epithelial ovarian cancer.

g. Breast cancer risk

No studies linked β-carotene with increased risk of breast cancer. In a 8 y follow-up of Women's Health Initiative clinical trials with 5040 postmenopausal women, Kabat et al. (2009) reported that the risk of invasive breast cancer was inversely associated with baseline serum α-carotene concentrations (RR for highest compared with the lowest tertile: 0.55; 95% CI: 0.34, 0.90; P = 0.02) and positively associated with baseline lycopene (RR 1.47; 95% CI: 0.98, 2.22; P = 0.06). Analysis of repeated measurements indicated that α-carotene and β-carotene were inversely associated with breast cancer.

Tamimi et al. (2005) prospectively assessed the relationship between plasma α-carotene, β-carotene, lycopene, other carotenoids, retinol, α-tocopherol, and γ-tocopherol and breast cancer risk by conducting a nested case-control study (969 cases and matched controls) using plasma collected from women enrolled in the Nurses' Health Study. The multivariate risk of breast cancer was 25-35% less for women with the highest quintile compared with that for women with the lowest quintile of α-carotene (OR 0.64; 95% CI: 0.47, 0.88; P for trend = 0.01), β-carotene (OR 0.73; 95% CI: 0.53, 1.02; P for trend = 0.01), and total carotenoids (OR 0.76; 95% CI: 0.55, 1.05; P for trend = 0.05). The association was strongest for α-carotene.

In a case-control study (270 cases, 270 controls) nested within a cohort in New York during 1985-1994, the carotenoids lycopene, α-carotene, and β-carotene were measured (Toniolo et al. 2001). There was an increase in the risk of breast cancer for decreasing β-carotene and α-carotene. The risk of breast cancer approximately doubled among subjects with blood concentrations of β-carotene at the lowest quartile, as compared with those at the highest quartile (OR 2.21; 95% CI: 1.29, 3.79). The OR for the lower quartile of total carotenoids was 2.31 (95% CI: 1.35, 3.96).

However, several observational studies (Wang et al. 1999; Terry et al 2002a; Verhoven 1997) have reported no association between carotenoid intakes and breast cancer risk.

h. Colorectal cancers

It appears that carotenoid intakes showed either protective or no effects on the incidence of colorectal cancer. Männistö et al. (2007) analyzed the associations between intakes of specific carotenoids (α-, and β-carotene, lycopene, and other carotenoids) and risk of colorectal cancer using the primary data from 11 cohort studies carried out in North America and Europe. Carotenoid intakes were estimated from food frequency questionnaires administered at baseline in each study. During 6-20 y of follow-up between 1980 and 2003, 7885 cases of colorectal cancer were diagnosed among 702647 participants. These pooled data did not suggest that carotenoids play an important role in the etiology of colorectal cancer.

From a population-based case-control study of 952 rectal cancer cases and 1205 controls from northern California and Utah, Murtaugh et al. (2004) reported that dietary β-carotene was not associated with rectal cancer risk in men. For women, relative to the highest level of intake, low intake of dietary lycopene (OR 1.7; 95% CI: 1.0, 2.8) was associated with an increased risk of rectal cancer. In the multiple logistic regression, only smoking (OR 3.02; 95% CI: 1.46, 6.25; P = 0.003) and a plasma lycopene concentration < 70 ug/L (OR 2.31; 95% CI: 1.12, 4.77; P = 0.023) were risk factors for adenomatous polyps. Erhardt et al. (2003) reported that the adenoma group had a numerically lower but not statistically significant median plasma β-carotene concentration (-25.5%). Terry et al. (2002b) also reported no association between carotenoid intake and colorectal cancer risk.

However, researchers at the University of North Carolina monitored polyp recurrence and serum concentration of carotenoids and vitamin A concentrations (Steck-Scott et al. 2004). Carotenoids, especially α-carotene, were inversely related to ademonatous polyp recurrence in a sub-cohort of 834 subjects in a low fat, high fiber, high fruit and vegetable dietary intervention, the Polyp Prevention Trial. Compared to the lowest quartile of baseline α-carotene concentrations, the OR of multiple polyp recurrence for the highest quartile was 0.55 (95% CI: 0.30, 0.99). Compared to the lowest quartile of averaged β-carotene concentrations, the OR of multiple adenomas for the highest quartile was 0.40 (95% CI: 0.22, 0.75) with an inverse trend (P = 0.02). This study suggests that both α- and β-carotene may protect against recurrence in nonsmokers and nondrinkers or be indicative of compliance or another healthy lifestyle factor that reduces risk.

i. Other Adverse Effects.

Allergic reactions, increased incidence of prostate cancer, retinopathy, leucopenia, and reproductive disorders have been associated anecdotally with high carotene consumption (IOM 2001). None of these effects has been confirmed by clinical trials. There is no evidence of hypervitaminosis A in individuals consuming high levels of β-carotene or other carotenoids (up to 180 mg/d) (IOM 2001; Mathews-Roth et al. 1974a, 1974b; Mathews-Roth 1986a, 1986b).

j. β-Carotene and treatment of erythropoietic protoporphyria

For treatment of individuals having erythropoietic protoporphyria, carotene can be used at the level of 180 mg/d (IOM 2001). No toxic effects have been reported at extremely high doses (IOM 2001).

k. Interpretations of IOM of the overall studies on β-carotene

The IOM report describes the following interpretations of the overall studies on β-carotene: "The data on the potential for β-carotene to produce increased lung cancer rates in smokers are conflicting and not sufficient for a dose-response assessment and derivation of a Tolerable Upper Intake Level (UL) for this endpoint. Supplements of 30 mg/d or more of β-carotene for long periods of time may be associated with carotenodermia, but this effect is more cosmetic than adverse and can be considered harmless and readily reversible. Because of the inconsistent data on adverse effects of β-carotene, a UL cannot be established at this time. ULs are not established for other carotenoids due to a lack of suitable data."

"Thus, while 20 mg/d of β-carotene in the form of a supplement is sufficient to raise blood concentrations to a range reported to be associated with an increase in lung cancer risk, the same amount of β-carotene in foods is not. Micozzi et al. (1992) demonstrated that 30 mg/d of supplemental β-carotene produced more than a five-fold increase in plasma β-carotene compared to 29 mg/d of β-carotene from carrots."

β-Carotene intake (6.6 mg in the diet which corresponds to 1.1 mg/d in a dietary supplement) under the intended use is approximately 20 fold lower than the pharmacological levels employed for smokers, heavy alcohol consumers, or asbestos workers in CARET and ATBC studies, assuming all the products in each food category will be used at the recommended levles. Even if palm carotenoids are used at the maximum intended levels, β-carotene intake levels would be 7 times lower than the levels used for special at risk population. Also, it is not possible that all the products in each food category can be used at the maximum levels.

2) Human clinical and epidemiology studies of lycopene (Mostly adopted from BASF and HPM)

No adverse effects were noted in human trials in which intake of 5 to 150 mg lycopene daily was observed for up to 24 wk (Bohm and Bitsch 1999, Bowen et al. 1993, Cohn et al. 2001, Micozzi et al. 1992, Paetau et al. 1998, Porrini et al. 1998, Porrini and Riso 2000, Rao and Agarwal 1998, Riso et al. 1999, Steenge et al. 2001). Epidemiology studies of lycopene intake suggest that intake of lycopene, serum concentrations of lycopene, and adipose concentrations of lycopene may be associated with reduced risk for some chronic diseases (Clinton 1998).

Daily doses ranging from 12.0 to 75.0 mg lycopene (*i.e.* 3 to 20 times normal dietary intake) were generally well tolerated, with no reports of any illnesses or adverse biological effects (Agarwal and Rao, 1998; Chen et al. 2001; Hininger et al. 2001; Kucuk et al. 2001; Micozzi et al. 1992). Gastrointestinal intolerances (not specified) were reported in prostate cancer patients supplemented with approximately 30 mg lycopene/d for 3 wk (Chen et al. 2001). However, the effects were considered minor and were not reported in a separate study conducted with a similar protocol (*i.e.*, same population, duration, and treatment; Chen et al. 2001; Kucuk et al. 2001).

Carotenodermia and lycopenodermia

Carotenodermia is characterized by yellowish discoloration of the skin that results from an elevation of plasma carotene concentrations. This condition has been reported in adults taking supplements containing 30 mg/d or more of β-carotene for long periods of time or consuming high concentrations of carotenoid-rich foods such as carrots and is the primary effect of excess carotenoid intake noted in infants, toddlers, and young children (IOM 2001). Carotenodermia is distinguished from jaundice in that the ocular sclera are yellowed in jaundiced subjects but not in those with carotenodermia.

Lycopenodermia results from high intakes of lycopene-rich foods such as tomatoes and is characterized by a deep orange discoloration of the skin (IOM 2001). Lycopene is a more intensely colored pigment than carotene and may cause discoloration at lower concentrations (15 mg lycopene/d for 16 wk) than other carotenoids (Olmedilla et al. 2002). However, no other

clinical studies have been reported symptoms of lycopenodermia even when 75.0 mg lycopene/d was introduced in a clinical study (Reich et al. 1960; La Placa et al. 2000). Both carotenodermia and lycopenodermia are considered harmless and are readily reversible when carotenoid ingestion is discontinued (IOM 2000).

Summary of science

The available clinical data indicate that estimated exposures of 4.8 mg β-carotene, 1.1 mg α-carotene, and 14.8 mg lycopene/person/d (90th percentile), provided through the intended use of palm carotenoids, would not have an adverse effect on body weights, blood counts, serum lipid profiles, immune function, or liver function.

The results of the toxicity studies performed with carotenoids indicate that carotenoid components, β- and α-carotene, and lycopene, are not mutagenic, genotoxic, reprotoxic, teratogenic, or carcinogenic. No signs of organ toxicity were found in subacute, subchronic, or chronic oral toxicity studies in experimental animals receiving doses up to 1000 mg β-carotene / kg BW/d or 1000 mg lycopene/ kg BW/d via the diet. Observational studies have consistently demonstrated an association between high dietary intakes or high plasma concentrations of β-carotene and a reduced cancer risk, in particular for lung cancer. However, the four major primary prevention studies on β-carotene supplementation provided inconsistent results. The supplement dosages of β-carotene used in the ATBC and CARET studies were 20 to 30 mg/d for 2-8 y. Considering bioconversion factors, intake of 20-30 mg β-carotene in a supplement form corresponds to 120 -180 mg of dietary β-carotene. β-Carotene intake under the intended use is 25-35 fold lower than the pharmacological levels employed in CARET and ATBC studies. It is also noteworthy that the IOM (2000; 2001) has not set ULs for β-carotene and other carotenoids.

IV. **Discussion of Information Inconsistent with GRAS Determination**

We are not aware of information that would be inconsistent with a finding that the proposed use of carotenoids preparations in foods and beverages, meeting appropriate specifications and used according to Good Manufacturing Practice, is GRAS.

V. **Conclusion of the expert panel:**
Generally recognized as safe (GRAS) determination for the addition of palm carotnoids to foods

December 2009

CONCLUSION

We, the undersigned expert panel members, Susan Cho, Ph.D., Michael Falk, Ph.D., and George Fahey, Ph.D., have individually and collectively critically evaluated the materials summarized in the palm carotenoids GRAS report, and conclude that palm carotenoids are safe and GRAS for its intended use in foods.

There is broad-based and widely disseminated knowledge concerning the chemistry of carotenoids, including α-, β-, and γ- carotenes, and lycopene. They cannot be synthesized in the body: thus, vitamin A and carotenoids must be obtained from the diet. Vitamin A deficiency results in serious health hazards. Symptoms caused by vitamin A deficiency can be alleviated by provitamin A carotenoids. Although carotenoids are not essential for human health, they have biological actions that may be important in maintaining health. Palm oil represents a major nutritional source of natural carotenoids. The palm carotenoids described in this self-affirmation are prepared from palm oil. Taken orally, palm carotenoids are bioavailable to all vital organs.

Palm carotenoids (a mixture of α -, β-, and γ- carotenes and lycopene with an average α- and β-carotene content of 33.1% and 64.2% of total carotenoids, respectively) proposed for use in this food product is well characterized. Palm carotenoids will be used as an ingredient in foods and beverages as a source of natural pro-vitamin A and colorants. Intended food applications include fats or oils, such as salad dressing, mayonnaise, margarine, and spread, bakery products, ready-to-eat cereals, desserts, potato chips, meal replacement beverages, cereal bars, beverages, and medical foods. The 90th percentile intakes including Malaysian Palm Oil Board (MPOB) β-carotene from all GRAS proposed use categories by users of one or more foods were estimated to be 5.9, 1.7, and 14.8 mg/d for β-carotene, α-carotene, and lycopene, respectively. These intake levels correspond to 0.10, 0.03, and 0.24 mg/kg BW/d for β-carotene, α-carotene, and lycopene, respectively. The proposed food use results in levels of exposure at concentrations significantly below those associated with any adverse effects of β-carotene and lycopene, i.e. 1000x below the No Observable Adverse Effect Level (NOAEL). Although there is limited data on α- and γ-carotene, based on their structural similarity the panel has no safety concerns. Also, the testing of the mixed palm carotenoid suggests the lack of safety concerns at the intended use levels.

Carotenoids have been evaluated and found safe for intended use by various scientific bodies. In particular β-carotene, is listed as a GRAS nutrient under the 21 CFR Part 184. Also, the FDA and the FAO/WHO have approved β-carotene as a coloring agent. Also the FDA had no question on GRAS notification of BASF synthetic lycopene (GRN 000119) and lycopene from *Blakeslea trispora* (GRN 000173). Currently, dietary supplements containing carotenoids, in

particular β-carotene and lycopene, have been on the market in the U.S. as dietary supplements since 1999 under the *Dietary Supplements Health and Education Act of 1994* (DSHEA).

Palm carotenoids have a long history of safe use as natural pro-vitamin A and colorants in foods. There are no indications of significant adverse effects related to palm carotenoids in the publicly available literature on animal toxicology studies and human clinical studies reviewed by the panel. Therefore, not only is the proposed use of palm carotenoids safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also *Generally Recognized as Safe* (GRAS) according to the Title 21 Code of Federal Regulations (21 CFR).

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Our Reference	Doc / GRAS / 2
COA No	CC_105169_A1B
Sampling Date	12th February 2008
Released Date	20th February 2008
Manufacturing Date	12th February 2008
Best Before Date	11th February 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0076/04	10 0 Min	10 2
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 11
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 7
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
8	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10

Certified By

Ms Chung Yoke Tying
Senior Quality Control Chemist
Issued Date 2nd July 2009

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Carotech

CERTIFICATE OF ANALYSIS

CAROMIN® 10% (H) (NON-GMO)

KOSHER MS 1500:2004

NATURAL MIXED CAROTENOIDS COMPLEX 10% OIL SUSPENSION

Our Reference	Doc / GRAS / 3
COA No	CC_103214_A1
Sampling Date	19th February 2008
Released Date	25th February 2008
Manufacturing Date	*19th February 2008*
Best Before Date	18th February 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0076/04	10 0 Min	10 1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 29
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 7
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
8	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10

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KOSHER MS 1500:2004

NATURAL MIXED CAROTENOIDS COMPLEX 10% OIL SUSPENSION

Our Reference	Doc / GRAS / 1
COA No	CC_106118_A1/9
Sampling Date	10th December 2008
Released Date	17th December 2008
Manufacturing Date	10th December 2008
Best Before Date	9th December 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0076/04	10 0 Min	10 6
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 06
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 9
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CERTIFICATE OF ANALYSIS

CAROMIN® 20% (NON-GMO)

KOSHER

NATURAL MIXED CAROTENOIDS COMPLEX 20% OIL SUSPENSION

Our Reference	Doc / GRAS / 3
COA No	CC_205789_B1C/5
Sampling Date	14th May 2009
Released Date	20th May 2009
Manufacturing Date	14th May 2009
Best Before Date	13th May 2012
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0070/04	20 0 Min	20 4
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 08
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 6
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER

NATURAL MIXED CAROTENOIDS COMPLEX 20% OIL SUSPENSION

Our Reference	Doc / GRAS / 2
COA No	CC_206299_B1/6
Sampling Date	9th March 2009
Released Date	13th March 2009
Manufacturing Date	9th March 2009
Best Before Date	8th March 2012
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0070/04	20 0 Min	21 1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 05
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 4
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER

NATURAL MIXED CAROTENOIDS COMPLEX 20% OIL SUSPENSION

Our Reference	Doc / GRAS / 1
COA No	CC_206372_B1/6
Sampling Date	23rd March 2009
Released Date	27th March 2009
Manufacturing Date	23rd March 2009
Best Before Date	22nd March 2012
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0070/04	20 0 Min	20 7
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 06
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 9
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER

NATURAL MIXED CAROTENOIDS COMPLEX 25% OIL SUSPENSION

Our Reference	Doc / GRAS / 3
COA No	CC_256038_C1/3
Sampling Date	17th June 2009
Released Date	19th June 2009
Manufacturing Date	17th June 2009
Best Before Date	16th June 2012
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0064/04	25 0 Min	25 4
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 07
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 9
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER

NATURAL MIXED CAROTENOIDS COMPLEX 25% OIL SUSPENSION

Our Reference	Doc / GRAS / 2
COA No	CC_256057_C1A/6
Sampling Date	20th March 2009
Released Date	25th March 2009
Manufacturing Date	20th March 2009
Best Before Date	19th March 2012
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0064/04	25 0 Min	25 6
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 11
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 3
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CAROMIN® 25% (NON-GMO)

KOSHER MS 1500:2004

NATURAL MIXED CAROTENOIDS COMPLEX 25% OIL SUSPENSION

Our Reference	Doc / GRAS / 1
COA No	CC_256132_C1/6
Sampling Date	17th December 2008
Released Date	22nd December 2008
Manufacturing Date	17th December 2008
Best Before Date	16th December 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0064/04	25 0 Min	25 5
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 08
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 5
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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CAROMIN® 30% (NON-GMO)

KOSHER MS 500:2004

NATURAL MIXED CAROTENOIDS COMPLEX 30% OIL SUSPENSION

Our Reference	Doc / GRAS / 1
COA No	CC_30P8299Z_C1/7
Sampling Date	22nd October 2008
Released Date	29th October 2008
Manufacturing Date	22nd October 2008
Best Before Date	21st October 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0058/05	30 0 Min	31 0
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 02
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 2
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
8	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10

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KOSHER MS 500:2004

NATURAL MIXED CAROTENOIDS COMPLEX 30% OIL SUSPENSION

Our Reference	Doc / GRAS / 2
COA No	CC_30P8173Z_C1/7
Sampling Date	9th April 2008
Released Date	12th April 2008
Manufacturing Date	9th April 2008
Best Before Date	8th April 2011
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0058/05	30 0 Min	30 9
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 02
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	1 6
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
8	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10

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KOSHER MS 1500 2004

NATURAL MIXED CAROTENOIDS COMPLEX 30% OIL SUSPENSION

Our Reference	Doc / GRAS / 3
COA No	CC_30P7039Z_C1
Sampling Date	7th October 2007
Released Date	12th October 2007
Manufacturing Date	7th October 2007
Best Before Date	6th October 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Opaque Reddish Oil Suspension	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0058/05	30 0 Min	31 6
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	1 00 Max	0 02
4	*Peroxide Value, meq/kg	AOCS 5th Edition Cd 8-53	10 0 Max	2 5
5	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	< 3 0
6	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	< 10 0
7	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
8	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10

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CAROSOL™ 3% (GMO-FREE)

KOSHER MS 1500:2004

COLD WATER DISPERSIBLE NATURAL MIXED CAROTENOIDS POWDER 3%

Our Reference	Doc / GRAS / 1
COA No	344/28/A8_1/11
Sampling Date	4th March 2009
Released Date	4th March 2009
Manufacturing Date	3rd March 2009
Best Before Date	2nd December 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Powder	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0082/06	3 0 Min	3 1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	6 00 Max	Passes Analysis
4	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	Passes Analysis
5	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	Passes Analysis
6	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	Passes Analysis
7	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	Passes Analysis

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CAROSOL™ 3% (GMO-FREE)

KOSHER

COLD WATER DISPERSIBLE NATURAL MIXED CAROTENOIDS POWDER 3%

Our Reference	Doc / GRAS / 2
COA No	CC_03_151208_1/6
Sampling Date	17th December 2008
Released Date	17th December 2008
Manufacturing Date	15th December 2008
Best Before Date	14th September 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Powder	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0082/06	3 0 Min	3 1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	6 00 Max	Passes Analysis
4	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	Passes Analysis
5	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	Passes Analysis
6	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	Passes Analysis
7	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	Passes Analysis

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STANDARDS MALAYSIA ACCREDITED LABORATORY
MS ISO/IEC 17025
TESTING
SAMM NO 364

Carotech

CERTIFICATE OF ANALYSIS

CAROSOL™ 3% (GMO-FREE)

KOSHER MS 1500:2004

COLD WATER DISPERSIBLE NATURAL MIXED CAROTENOIDS POWDER 3%

Our Reference	Doc / GRAS / 3
COA No	CC_03_304/40/A8 _1/14
Sampling Date	3rd November 2008
Released Date	3rd November 2008
Manufacturing Date	3rd November 2008
Best Before Date	2nd August 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Powder	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0082/06	3 0 Min	3 1
3	Moisture (WT, %) (Karl Fisher)	AOCS 5th Edition Ca 2e-84	6 00 Max	Passes Analysis
4	*Lead (Pb), ppm	AOCS 5th Edition Ca 15-75	3 0 Max	Passes Analysis
5	*Heavy Metals, ppm	AOCS 5th Edition Ca 15-75	10 0 Max	Passes Analysis
6	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	Passes Analysis
7	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	Passes Analysis

Certified By

Ms Chung Yoke Tying
Senior Quality Control Chemist
Issued Date 2nd July 2009

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STANDARDS MALAYSIA ACCREDITED LABORATORY
MS ISO/IEC 17025
TESTING
SAMM NO 364

Carotech

CERTIFICATE OF ANALYSIS

ALPHABEADS® 7.5% (NON-GMO)

KOSHER MS 500 2004

NATURAL MIXED CAROTENOIDS COMPLEX 7.5% BEADLETS

Our Reference	Doc / GRAS / 3
COA No	BB-75-05881WN/2
Sampling Date	13th December 2008
Released Date	19th December 2008
Manufacturing Date	13th December 2008
Best Before Date	12th December 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dark Reddish Beadlets	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0254/03	7 5 Min	7 7
3	Moisture Content, %	Karl Fischer Method	8 0 Max	6 1
4	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
5	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005/Volume IV/Appendix XVI B	1 cfu/g Max	< 1
7	***Eschenchia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
8	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

Certified By

Ms Chung Yoke Lying
Senior Quality Control Chemist
Issued Date 3rd July 2009

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STANDARDS MALAYSIA ACCREDITED LABORATORY

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TESTING
SAMM NO 364

Carotech

CERTIFICATE OF ANALYSIS

ALPHABEADS® 7.5% (NON-GMO)

KOSHER MS 1500 2004

NATURAL MIXED CAROTENOIDS COMPLEX 7.5% BEADLETS

Our Reference	Doc / GRAS / 2
COA No	BB-75-08146WN/2
Sampling Date	30th July 2008
Released Date	5th August 2008
Manufacturing Date	30th July 2008
Best Before Date	29th July 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dark Reddish Beadlets	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0254/03	7 5 Min	8 0
3	Moisture Content, %	Karl Fischer Method	8 0 Max	5 4
4	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 10
5	** Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005/Volume IV/Appendix XVI B	1 cfu/g Max	< 1
7	***Eschenchia coli*	BP 2005/Volume IV/Appendix XVI B	Absent/1g	Absent
8	***Salmonella*	BP 2005/Volume IV/Appendix XVI B	Absent/10g	Absent

Certified By

Ms Chung Yoke Tying
Senior Quality Control Chemist
Issued Date 3rd July 2009

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MS ISO/IEC 17025
TESTING
SAMM NO 364

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ALPHABEADS® 7.5% (NON-GMO)

KOSHER MS 1500 2004

NATURAL MIXED CAROTENOIDS COMPLEX 7.5% BEADLETS

Our Reference	Doc / GRAS / 1
COA No	BB-75-09605WN/2
Sampling Date	19th February 2008
Released Date	24th February 2008
Manufacturing Date	19th February 2008
Best Before Date	18th February 2009
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Dark Reddish Beadlets	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0254/03	7 5 Min	8 1
3	Moisture Content, %	Karl Fischer Method	8 0 Max	6 2
4	**Total Aerobic Microbial Count	BP 2005, Plate Count Method	1000 cfu/g Max	< 10
5	**Total Combined Molds And Yeasts	BP 2005, Plate Count Method	100 cfu/g Max	< 10
6	**Coliform Count	BP 2005, Plate Count Method	1 cfu/g Max	< 1
7	***Eschenchia coli*	BP 2005, Plate Count Method	Absent/1g	Absent
8	***Salmonella*	BP 2005, Plate Count Method	Absent/10g	Absent

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Senior Quality Control Chemist
Issued Date 3rd July 2009

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SAMM NO 364

Carotech

CERTIFICATE OF ANALYSIS

CAROSEM™ 1%

KOSHER

MS 1500 2004

NATURAL MIXED CAROTENOIDS EMULSION 1%

Our Reference	Doc / GRAS
COA No	CCsem1 / A / OF0996_060809/3
Sampling Date	6th August 2009
Released Date	12th August 2009
Manufacturing Date	6th August 2009
Best Before Date	5th August 2010
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Liquid	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0202/00	1 0 ± 0 2 Min	0 96
3	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 1000
4	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 100

Certified By

Ms Chung Yoke Tying
Senior Quality Control Chemist
Issued Date 23rd December 2009

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STANDARDS MALAYSIA ACCREDITED LABORATORY
MS ISO/IEC 17025
TESTING
SAMM NO 364

Carotech

CERTIFICATE OF ANALYSIS

CAROSEM™ 5%

KOSHER MS 1500:2004

NATURAL MIXED CAROTENOIDS EMULSION 5%

Our Reference	Doc / GRAS
COA No	CCsem5 / A / OF3221_150909/3
Sampling Date	6th August 2009
Released Date	12th August 2009
Manufacturing Date	6th August 2009
Best Before Date	5th August 2010
Batch No	(b)(4)
Country Of Origin	Malaysia

	Analysis	Test Method	Specification	Test Results
1	*Physical Appearance		Orange Liquid	
2	Mixed Carotene (UV-VIS), %	In House Method – CTM_0202/00	5 0 ± 0 3 Min	5 1
3	**Total Viable Aerobic Count	BP 2005/Volume IV/Appendix XVI B	1000 cfu/g Max	< 1000
4	**Total Combined Molds & Yeast Count	BP 2005/Volume IV/Appendix XVI B	100 cfu/g Max	< 100

Certified By

Ms Chung Yoke Tying
Senior Quality Control Chemist
Issued Date 23rd December 2009

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CAROMIN® 20%

Natural Mixed Carotenoids Complex 20% Concentrate

Description

CAROMIN® 20% - Natural Mixed Carotenoids Complex 20% Concentrate is a reddish vegetable oil suspension of naturally occurring mixture of carotenoids, extracted from palm fruits (***Elaeis guineensis)***

CAROMIN® 20% contains natural ***alpha-carotene, beta-carotene, gamma-carotene, lycopene*** and other carotenoids, a unique mixture in natural proportion as found in carrots. Palm mixed carotene complex is the only true full spectrum carotenoids complex with the highest ratio of ***alpha-carotene***. It also contains a mixture of other carotenoids such as ***beta-carotene, gamma-carotene*** and ***lycopene*** that are commonly found in fruits and vegetables

The unique combination of carotenoids in natural proportion and their synergistic effects may ultimately confer the many beneficial health effects associated with carotene.

Typical Chemical Properties

TOTAL MIXED CAROTENOIDS COMPLEX	**200 mg/g (20%) Minimum**
Isomers	**Typical Value ***
Alpha-Carotene	65 mg/g
Beta-Carotene	135 mg/g
Gamma-Carotene	1 0 mg/g
Lycopene	1.0 mg/g
TOTAL MIXED CAROTENOIDS COMPLEX	**202 mg/g**
VITAMIN A EQUIVALENCE	**240,000 I.U. /g Minimum**

**** Typical Value refers to the average analytical results accumulated over the past years***

ISOMERIC FORMS	Approximately 34% typical cis-isomer and 66% trans-isomer
VITAMIN A ACTIVITY	Each gram of this product contains not less than 240,000 I.U. of Vitamin A Activity Biological Activity 1mg of *trans beta-carotene* = 1,667 I U. of Vitamin A Activity. [Martindale The Extra Pharmacopoeia, 30th ed., page 1051]

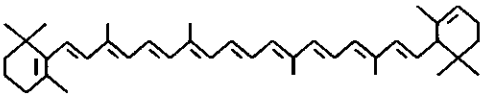
APPLICATION	***Dietary Supplement***	Soft Gelatin Capsules
	Natural Colorant	Oil-Based Food Systems Margarine / Butter / Oil System (As a natural colorant and source of Vitamin A)
	Water Dispersible Carotene	Emulsions & Powder
	Cosmetics & Personal Care Products	

CAROMIN® 20%

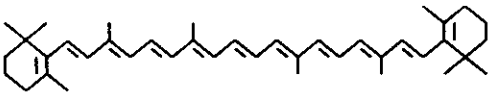
PEROXIDE VALUE	Maximum 10 meq/kg
MOISTURE	Maximum 1.0%
SOLUBILITY	Soluble in oils and fats. Insoluble in water. Partially soluble in eth
STABILITY	**CAROMIN® 20%** is stabilized with 0.3% tocopherol. It has a shelf l 24 months when stored in cool and dry place in unopened on containers. Once opened, the product should be used immediately.
MICROBIOLOGY	Total Aerobic Microbial Count Max 1000/g Total Combined Molds & Yeasts Max 100/g
PACKAGING	**CAROMIN® 20%** is available in 1-kg, 3-kg and 20-kg containers i nitrogen. Other types of packages are available upon request. Smal samples are available for evaluation purposes.
STORAGE	**CAROMIN® 20%** is sensitive to air, light and heat. Store in airtight con at or below room temperature.
STANDARDS	In full compliance with European Communities Code· E160a (i) - N Carotenes.
KOSHER CERTIFICATION	**CAROMIN® 20%** is certified Kosher by the Court of the Chief Rabb Din, London.
LABELING	Products formulated with **CAROMIN® 20%** can be labeled as cont all natural carotenoids.

CHEMICAL STRUCTURE

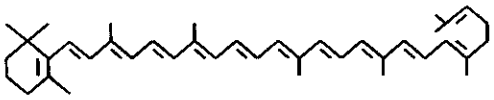
α-carotene



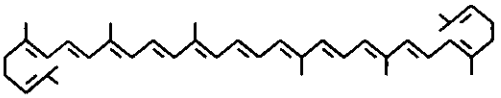
β-carotene



γ-carotene



Lycopene



TECHNICAL SUPPORT

Carotech's technical support team is ready to assist you in the use of our range of phytonutrients and other ingred your formulations at all stages of your product development. Emphasis is given to supplying the most cost effect efficient solution to your particular requirement. Contact us for further information

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Fax +60 (05)-2014213
E-mail info@carotech net
Website www carotech net

CAROTECH INC
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08817 USA

Tel +1 (732)-90
Fax +1 (732)-90
E-mail carot3@aol
Website www tocotri

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ALPHABEADS® 7.5%

Natural Mixed Carotenoids Complex 7.5% Beadlets

Description

ALPHABEADS® 7.5% - Natural Mixed Carotenoids Complex 7.5% Beadlets is a source of dry-stabilized mixed carotenoids (**Caromin®**), extracted and purified from palm fruits (***Elaeis guineensis***)

ALPHABEADS® 7.5% contains natural ***alpha-carotene, beta-carotene, gamma-carotene, lycopene*** and other carotenoids, a unique mixture in natural proportion as found in carrots. These beadlets are highly stable, free-flowing and directly compressible. It is suitable for the production of tablets, two-piece hard shell capsules and vitamin / food premixes.

Palm mixed carotene complex is the only true full spectrum carotenoids complex with the highest ratio of ***alpha-carotene***. The unique combination of carotenoids in natural proportion and their synergistic effects may ultimately confer the many beneficial health effects associated with carotene.

Typical Chemical Properties

TOTAL MIXED CAROTENOIDS COMPLEX	**75 mg/g (7.5%) Minimum**
Isomers	**Typical Value ***
Alpha-Carotene	25 mg/g
Beta-Carotene	51 mg/g
Gamma-Carotene	300 mcg/g
Lycopene	300 mcg/g
TOTAL MIXED CAROTENOIDS COMPLEX	**76.6 mg/g**
VITAMIN A EQUIVALENCE	**88,000 I.U. /g Minimum**

**** Typical Value refers to the average analytical results accumulated over the past years***

ISOMERIC FORMS	Approximately 34% typical cis-isomer and 66% trans-isomer
APPEARANCE	Dark Reddish Beadlets
VITAMIN A ACTIVITY	Each gram of this product contains typically 88,000 I U. of Vitamin A Activity. Biological Activity 1mg of *trans beta-carotene* = 1,667 I.U. of Vitamin A Activity [Martindale The Extra Pharmacopoeia, 30th ed., page 1051]

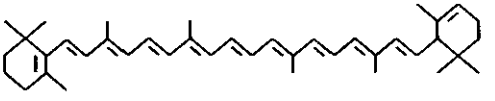
APPLICATION	***Dietary Supplement***	Two-Piece Hard Shell Capsules / Tablets
	Vitamin Premixes	
	Functional Ingredient	Food Premixes

ALPHABEADS® 7.5%

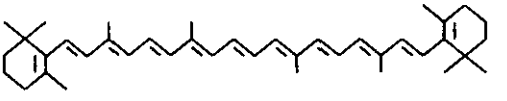
MESH SIZE	100% through No 20 U.S.STD Sieve
MOISTURE	Maximum 8 0%
SOLUBILITY	Soluble in water . Ca 1g/100ml in cold water : Ca 10g/100ml in hot water Partially soluble in organic solvent
STABILITY	**ALPHABEADS® 7.5%** is physically and chemically stable. It has a shelf life of 24 months and is recommended that the bulk containers be stored at 4°C for optimum stability.
ODOR	Characteristic
PACKAGING	**ALPHABEADS® 7.5%** is packaged in resealable double polyethylene-lined fiber drums, available in 1-kg, 5-kg, 10-kg and 20-kg. Other types of packages are available upon request for evaluation purposes
STORAGE	**ALPHABEADS® 7.5%** is sensitive to air, light and heat. Store in airtight containers at or below room temperature
STANDARDS	In full compliance with European Communities Code: E160a (i) - Mixed Carotenes
KOSHER CERTIFICATION	**ALPHABEADS® 7.5%** is certified Kosher
LABELING	Products formulated with **ALPHABEADS® 7.5%** can be labeled as containing all natural carotenoids.

CHEMICAL STRUCTURE

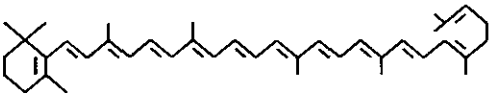
α-carotene



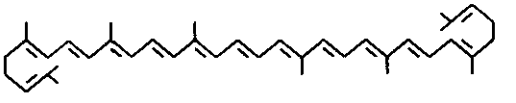
β-carotene



γ-carotene



Lycopene



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Website www tocotrienol org

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SUBMISSION END

Reference List for Industry Submission, GRN 000320

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