

SECTION SIX

SECTION SIX

The history of use or other evidence or safety establishing that the dietary ingredient zeaxanthin purified concentrate from marigold when used under the conditions recommended or suggested in the labeling of dietary supplement products will reasonably be expected to be safe and which is the basis on which the distributor of zeaxanthin purified concentrate from marigold has determined that the use of zeaxanthin purified concentrate from marigold is reasonably expected to be safe. See 21 CFR § 190.6(b)(4).

Abstract

Zeaxanthin and lutein are antioxidant carotenoids that occur naturally in the diet. A new source of these carotenoids, a purified zeaxanthin extract from the marigold flower (*Tagetes erecta*) that contains approximately 30% by weight of the carotenoids zeaxanthin and lutein in a ratio of 85% and 9% respectively, is evaluated for safety for use as a dietary supplement.

The safety of consumption of a zeaxanthin purified concentrate product used as a dietary supplement ingredient has been determined by evaluating the safety of ingestion of the whole product, including the safety of ingestion of the major constituent, zeaxanthin, and of the minor constituents, lutein and xanthophyll epoxides. The approach to evaluating the safety of increased zeaxanthin intake from consumption of zeaxanthin purified concentrate product is based on an evaluation of the incremental increase this ingestion will produce in lutein and zeaxanthin and in total carotenoids, compared to background exposure. In addition, bioavailability of zeaxanthin from zeaxanthin purified concentrate, when used as a dietary supplement, is evaluated in context of average daily consumption of lutein and zeaxanthin consumption for foods. The proposed level of ingestion of zeaxanthin and lutein from the zeaxanthin purified concentrate product would increase intake of zeaxanthin and lutein in the generally vegetable-poor American diet to a level comparable to the mean intake of individuals consuming the recommended number of servings of vegetables per day and is therefore determined to be both prudent and safe. Safety of consumption of the whole product is determined by evaluating the source of the product, production process, nature and quantity of impurities, and product specifications. Corroboration of safety is provided by animal toxicology studies of the zeaxanthin purified concentrate product, as well as human and epidemiologic studies of zeaxanthin and lutein intake. (Kruger et al, 2002)(*See attachment # 5*)

Abbreviations: AMD, age-related macular degeneration; EDI, estimated daily intake; GRAS, generally recognized as safe; IOM, Institute of Medicine; ZPC, zeaxanthin purified concentrate

Article Outline

1. Characterization as a dietary supplement
2. Introduction
3. Approach for the determination of safety of zeaxanthin purified concentrate product
4. Analytical characterization of zeaxanthin purified concentrate product
5. Estimated daily intake (EDI) of Zeaxanthin purified concentrate product from its proposed uses.
6. Ames Test Report
7. 28-Day Subchronic toxicity study in rats
8. Acute Oral Toxicity Test in rats (Up and Down Procedure)
9. Human studies/epidemiologic evidence
10. Conclusions: safety determination
11. References
12. Authors of report

1. Characterization as a dietary supplement: According the definition at 201(ff)(1)(C)(F) of the Food, Drug & Cosmetic Act, zeaxanthin purified extract meets the legal definition of a dietary supplement.

2. Introduction

Studies have suggested a possible association between lutein and zeaxanthin intake and lowered risk of age-related macular degeneration (AMD), the leading cause of visual loss in adults age 65 and older in the United States and Europe. (Seddon et al; 1994)(*see attachment # 6*) As a consequence, there is interest in increasing the levels of ingestion of these carotenoids in the diet. Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet. (Semba, Dagnelie; 2003)(*see attachment # 7*) Zeaxanthin and lutein do not exist independent of one another in the human diet. These carotenoids are commonly found in green leafy vegetables, corn, yellow-orange fruits and vegetables and various bread products. (Humphries, Khachik; 2002)(*see attachment # 8*) Currently, there are no defined dietary reference intakes for the major dietary carotenoids, including lutein and zeaxanthin, but sufficient data exist to support existing recommendations for increased consumption of fruits and vegetables. (Food and Nutrition Board, 2000)(*see bibliography*) Studies of the American diet indicate that vegetable intake is generally lower than is recommended in the Dietary Guidelines for Americans (USDA (*see bibliography*); USDHHS and USDHHS) It is possible to supplement the diet with lutein and zeaxanthin in order to increase serum levels of these carotenoids and presence in ocular tissue; one potential source of these carotenoids is the marigold flower (*Tagetes erecta*).

3. Approach for the determination of safety of zeaxanthin purified concentrate product

The safety assessment of zeaxanthin purified concentrate (ZPC) follows an adaptation of the guidelines of the document entitled “An innovative approach to the determination of safety from a dietary ingredient derived from a new source: case study using a crystalline lutein product”. (Kruger et al., 2002)(*see attachment # 5*). A safety assessment of a new source of such a dietary ingredient requires a two-pronged approach: (1) assess the safety of the intended dietary ingredient(s), in this case, zeaxanthin, and to a lesser extent lutein and xanthophyll epoxides, and (2) assess the safety of the whole product, which includes potential impurities introduced from the source and production. Since the intended dietary ingredients are biologically active, they may produce a range of consequences in the body from physiologic deficiency to therapeutic effect to frank toxicity. The intended use and potential exposure to the intended dietary ingredient(s) must be compared to its determined safe level of ingestion; depending on the ingredient, historical exposure and/or scientific studies (in the case of ZPC, animal toxicology, absorption, metabolism, and clinical trials) may be used to determine that safe level. The safety of the whole product must include appropriate toxicology testing relative to the findings of the analytical characterization.

The constituents of the zeaxanthin purified concentrate product, zeaxanthin and lutein, occur naturally in fruits and vegetables and are part of the normal diet. Unlike the situation for assessing risk of an environmental chemical, however, some dietary components, similar to nutrients, are beneficial or essential for human well-being within a certain range of intakes. A principal feature of the safety assessment for zeaxanthin and lutein utilizes the assumption set forth for nutrients: no risk of adverse effects is expected unless a threshold dose (or intake) is exceeded (IOM, 2000)(*see bibliography*).

Therefore, the approach to evaluating the safety of increased zeaxanthin and lutein intake from consumption of zeaxanthin purified concentrate product is based on an evaluation of the incremental increase this ingestion will produce compared to background exposure. The estimated daily intake (EDI) of zeaxanthin and lutein from zeaxanthin purified concentrate product is compared to background levels of intake of zeaxanthin and lutein. A reasonable assurance of safety is provided when the ingestion of zeaxanthin and lutein from the proposed dietary supplement uses of zeaxanthin purified concentrate product results in only a small increase in the intake of lutein and zeaxanthin and total carotenoids.

Corroboration of safety for the whole product is provided by human studies of zeaxanthin and lutein exposure and animal toxicology studies of the whole zeaxanthin purified concentrate product.

4. Analytical characterization of zeaxanthin purified concentrate product

This safety assessment evaluated a specific zeaxanthin purified concentrate product, zeaxanthin purified concentrate, an extract from the marigold flower (*Tagetes erecta*). A compositional analysis of the product identifies $116 \pm 3\%$ of the components. Approximately 30% of the product consists of the carotenoids zeaxanthin, lutein and xanthophyll epoxides at 85%, 9%, and 4% concentrations, respectively. Fiber, moisture, ash, protein and fat and waxes comprise $\geq 70\%$ of the remainder. Appropriate analytical characterization of the product confirmed the absence of any impurities of toxicologic concern.

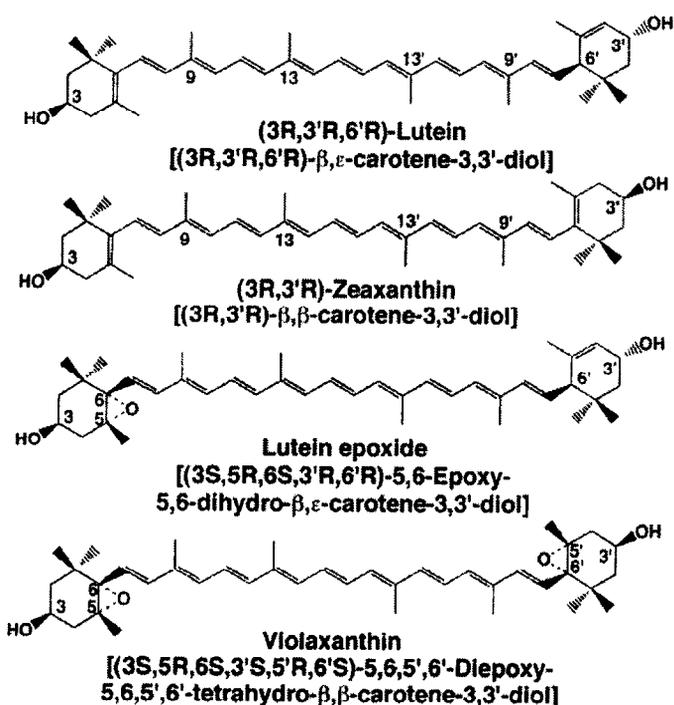


Figure 1: Chemical structure of the major hydroxycarotenoids and carotenoid epoxides in fruits and vegetables. (Humphries, Khachik; 2003)(*see attachment # 8*)

lettuce, romaine	Raw	170	8	21:1
parsley	Raw	10820	502	22:1
peas	canned	719	51	14:1
spinach	Raw	9157	525.1	17:1
YELLOW-ORANGE	Raw			
corn	canned	198	332.7	1:1.7
Cornmeal	Degermed	898	457	2:1
mango	Raw	10	10	1:1
nectarine	Raw	20	170	1:8.5
oranges	Raw	350	250	1.4:1
oranges, mandarin	Raw	70.5	60	1.2:1
Orange Juice*	Frozen Conc.	58	80	1:1.4
Peaches*	canned	14	19	1:1.4
Persimmons*	Raw	346	488	1:1.4
papaya	Raw	22.1	22.1	1:1
peaches	Raw	20	20	1:1
Peach	Canned, syrup	14	19	1:1.4
plum, red	Raw	40	N/A	
spinach	Raw	11607	331	39:1
spinach	cooked, boiled	6864	179	36:1
squash, acorn	Raw	50	N/A	
squash, butternut	Raw	2400	280	8.6:1
Turnip greens	Raw			
Wolfberry**	Dried, fresh		82.4	
Wheat				
Catocin	Raw	32.4	3.1	11:1
Pioneer	Raw	224.1	29.3	7.6:1
Freekeh	Raw	791.9	315.3	2.5:1
Pasta				
egg noodles	cooked, boiled	1391.6	544.9	2.6:1
lasagna	cooked, boiled	288.7	23.8	12.1:1
*Obtained from USDA Carotenoid database, 1998				
**Weller and Breithaupt; 2003 (see attachment # 9)				
Bolded items indicate foods which contain higher amounts of zeaxanthin relative to lutein				

As can be seen in table # 1, the concentration of lutein in green vegetables is predominantly higher than that of zeaxanthin. The abundance of lutein over zeaxanthin in nature has been attributed to the dominant role of lutein in photosynthesis.

The specifications for zeaxanthin purified concentrate are summarized in Table 1.

Table 1. Specifications for Chrysanthis zeaxanthin purified concentrate

Recommended Daily Dosage: 12 mg Zeaxanthin Purified Concentrate		Milligrams
<i>12 mg ZPC contains:</i>		
Fiber (plant cellulose)	48.10%	9.62
Moisture	22.58%	4.52
Ashes (Minerals)	5.04%	1.01
Protein	2.37%	0.47
Fat	7.45%	1.49
Waxes (plant waxes)	0.43%	0.09
Carotenoids	30.0%	6.00
3R 3'R Zeaxanthin 85%		3.06
Lutein 9%		0.324
Xanthophyll Epoxides 4%		0.144

5. Estimated daily intake (EDI) of zeaxanthin purified concentrate product from its proposed uses

5a. Presence in the food supply

The following table includes the ratio of lutein and zeaxanthin that exist in foods available for human consumption:

Table # 1: Quantitative Distribution of Lutein, Zeaxanthin in Selected Fruits, Vegetables, Selected Wheat and Pasta Product (Source: Adapted from Humphries, Khachik, 2003 (*see attachment # 8*), unless otherwise noted; USDA Carotenoid Database, 1998)

Concentration of lutein and zeaxanthin (ug/100g)				
FOODS	Form	Lutein	3R 3'R Zeaxanthin	Lutein/ Zeaxanthin Ratio
GREENS				
beans, green	Raw*	418.1	35	12:1
beans, lima (canned)	canned	356.1	16	22:1
broccoli	Raw	1510.6	42.8	35:1
collards	Raw	5120	140	37:1
kale	Raw	15000	240	63:1

5b. Consumption by target population

Mean daily intake of lutein and zeaxanthin, combined, varies from 0.8 to 4 mg per day, depending on the population studied and the method of dietary assessment employed (Rock et al; 2002 (*see attachment # 10*), Bone et al; 2003 (*see attachment # 11*), Landrum, Bone; 2001)(*see attachment # 12*). However, daily intake of carotenoids such as lutein varies widely between individuals, as illustrated by a standard deviation of 2.45 mg/day in a recently published study (Landrum, Bone; 2001)(*see attachment # 12*). Approximately 78% of dietary lutein and zeaxanthin is sourced from vegetables, spinach (30 g contains 3659 mg of lutein and zeaxanthin – a serving size of 5 grams of spinach contains 610 mg of lutein and zeaxanthin), and orange pepper being particularly rich source of these carotenoids, but a high mole percentage is also found in egg yolk (USDA, 1998)(*see bibliography*). A joint FAO/WHO Expert committee on food additives (FAO, 2004)(*see attachment # 13*) established an acceptable daily intake (ADI) of 0-2 mg/kg body weight (group ADI for lutein and zeaxanthin), which is above the range established as the recommended daily intake for ZPC at 12 mg per day, which provides 3 mg of 3R,3'R-zeaxanthin.

The enrichment of the daily human diet with zeaxanthin-containing plants has been known for a long time; for example, wolfberries (*Lycium barbarum*; Gou Qi Zi), small red berries commonly used in home cooking in China, were traditionally used in Chinese herbal medicine for the improvement of visual acuity. In a single-blind cross-over study using native 3R,3'R-zeaxanthin dipalmitate from wolfberries or non-esterified 3R,3'R-zeaxanthin at a dosage of 5 mg a day for 23 days, subjects (n = 12) experienced no adverse effects. The main phytochemical found in this plant source, which is regarded as the active component, is zeaxanthin dipalmitate, a diester formed from zeaxanthin and palmitic acid. (Breithaupt et al; 2004)(*see attachment # 14*). Wolfberries have been evaluated to yield approximately 82.4 grams of zeaxanthin per 100 grams of dried berries. (Breithaupt, Bamedi; 2001)(*see attachment # 15*)

The largest observational study to date studying the dietary intake of lutein and zeaxanthin involved 2786 subjects were followed for one year. (Rock, CL; 2002)(*see attachment # 10*). The primary aims of the study were to identify the correlates of dietary lutein + zeaxanthin intake and the determinants of serum lutein and zeaxanthin concentrations in a heterogeneous community-based sample of adults aged 18–92 y, recruited and examined at three U.S. sites (n = 2786). Table # 2 summarizes the lutein + zeaxanthin intakes for the U.S. population included in the study:

TABLE # 2

Mean and distributions of intakes of dietary lutein + zeaxanthin, fiber and percent energy from fat, and serum lutein and zeaxanthin concentrations (n = 2,786)				
Dietary or serum factor	Percentile Distributions			Mean (SD)
	10%	50%	90%	
Dietary Lutein + zeaxanthin intake, µg/d	546	1122	2315	1347 (891)
Dietary Fiber, g/d	7	14	25	15 (8)
Energy from fat, %	23	35	45	34 (8)
Serum lutein concentration, µmol/L	0.105	0.201	0.371	0.226 (0.120)
Serum zeaxanthin concentration, µmol/L	0.034	0.065	0.117	.071 (0.039)

*Table adapted from CL Rock et al. 2002(see attachment # 10)

Mean dietary intake of lutein + zeaxanthin in this population was 1.35 mg per day, while the 90% consumed 2.3 mg per day. This data is consistent with absorption rates reported by other research (Beatty et al.; 2004)(see attachment # 16) and also reflects that the intake of lutein and zeaxanthin in the United States is generally lower, but levels of about 3 mg/day can be easily achieved with a high fruit and vegetable diet. (Yeum K-J et al; 1996)(see attachment # 17) Although lutein and zeaxanthin are considered to be major carotenoids in the U.S. diet, data from the 1987 and 1992 National Health Interview Surveys suggest that there was a decline in lutein intake, particularly from dark-green leafy vegetables. (Nebeling et al; 1997)(see attachment # 18)

Serum levels in blood: The abundance of both lutein and zeaxanthin in commonly consumed fruits and vegetables is also reflected in the levels of these carotenoids in human serum and ocular tissues (Rock et al; 2002)(see attachment # 10). Supplement studies in humans have demonstrated varying levels of serum increases of lutein and zeaxanthin after supplementation. This may be because the levels of lutein and zeaxanthin in human serum are largely dependent on dietary habits and carotenoid intake of individuals. It is interesting to note that in the macular region of the human eye, the ratio of lutein to zeaxanthin does not consistently reflect the ratio of these carotenoids found in foods and human serum. (Humphries, Khachik; 2003)(see attachment # 8) For example, it has been show that the concentration of lutein is greater than that of zeaxanthin in the peripheral region of the macula, while zeaxanthin is more abundant in the central region. (Rock et al; 2002)(see attachment # 10). The lutein to zeaxanthin ratio is serum generally reflects the ratio found in the average diet (4:1). (Bone et al; 2003)(see attachment # 11) The results from the observational study listed in Table # 2 demonstrate a consistent ratio of 3:1 for all percentile distributions. It was concluded from this study that every 10% increase in dietary lutein + zeaxanthin intake was associated with a 2.4% increase in serum lutein concentration. In the study by Breithaupt et al.; 2004 (see attachment # 14) in which 5 mg of zeaxanthin (as native 3R,3'R-zeaxanthin dipalmitate from wolfberries or non-esterified 3R,3'R-zeaxanthin) was taken by subjects for 23 days,

only 3.3% of the administered dose was detected in plasma at the highest level of concentration. Based on the assumption that 4% of the body weight is plasma (2.4 liters plasma/60 kg body weight; Barua, 1999)(*see attachment # 19*), this corresponds to an absolute amount of 166 µg 3R,3'R-zeaxanthin, or 122nmol/l (69µg/l). This data, and other data presented indicate that supplementation with zeaxanthin up to 5 mg per day would increase blood serum zeaxanthin concentrations to a level comparable to that achieved by the 90% percentile distribution for consumption of carotenoid-containing vegetables, particularly those with higher amounts of both lutein and zeaxanthin.

5c. Safety of Lutein at 0.324 mg per daily dose

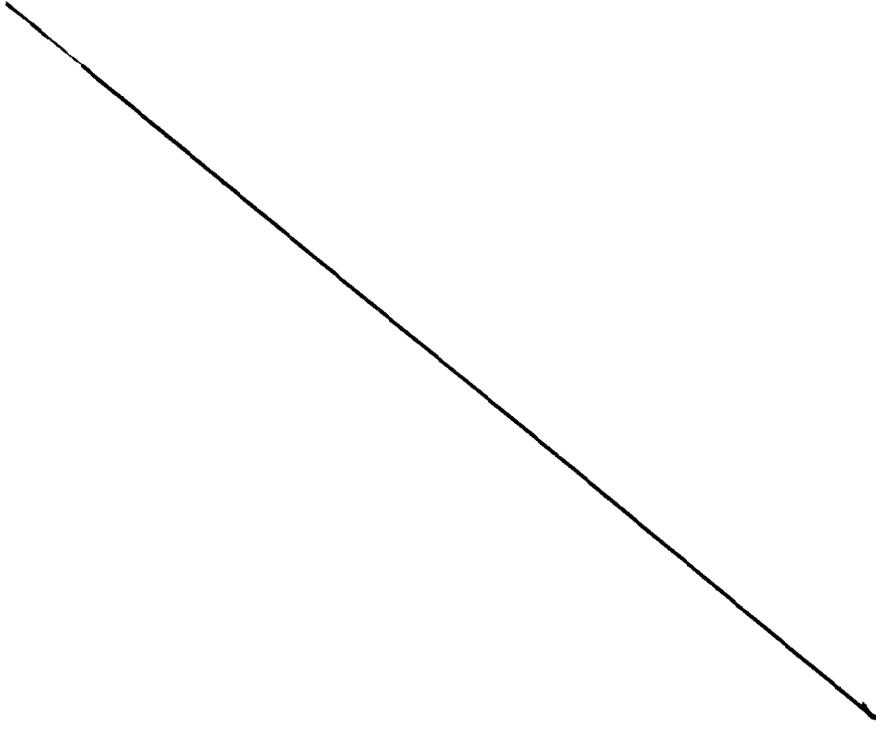
Lutein occurs alongside zeaxanthin in vegetables and fruits. There are no known foods that occur in nature in which one xanthophyll is present in the absence of the other. Lutein has been consumed at dosage of 2 – 30 mg (Bone et al; 2003 [*see attachment # 11*], Semba, Dagnelie; 2003)(*see attachment # 20*) without any report of adverse events. At a level of 0.324 mg per day, lutein is presented in ZPC in a daily dosage below what would be consumed in an ordinary serving of fruits or vegetables (containing lutein and zeaxanthin).

5d. Safety of xanthophyll epoxides at 0.144 mg daily dose

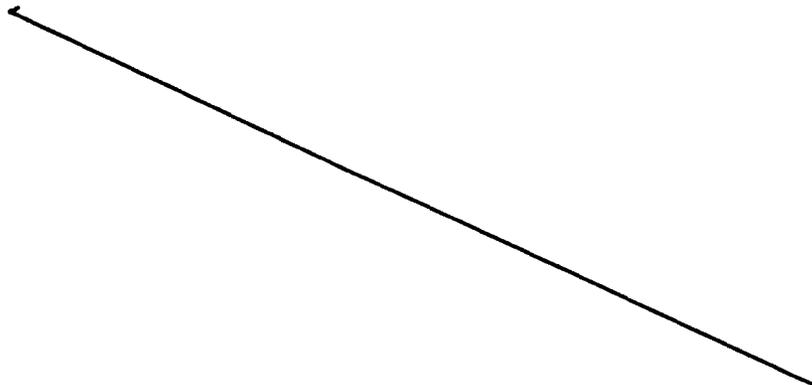
Epoxides of xanthophylls and carotenes are widely distributed in nature, and constitute major dietary carotenoids in a number of fruits and vegetables. (Goodwin; 1980)(*see bibliography*) Information is lacking whether epoxy-carotenoids are absorbed and possess any biological activity. Epoxy-xanthophylls are more abundant in vegetables and fruits and it is not known if these are also absorbed by humans. A study was conducted to determine whether 5, 6-epoxylutein (ELUT) and 5, 6, 5', 6'-diepoxyzeaxanthin (violaxanthin (VIOL)), two of the widely occurring epoxyxanthophylls found in spinach, broccoli, lettuce, cabbage and green peas are absorbed. The two epoxyxanthophylls were chemically synthesized from lutein and zeaxanthin, respectively, and characterized by physio-chemical methods. Analysis of plasma extracts did not show appearance of the administered epoxy-xanthophyll or any metabolite. One possible reason for the observed difference in the absorption of mono-epoxycarotenes and epoxy-xanthophylls is that whereas mono-epoxycarotenes can serve as precursors of vitamin A, the epoxyxanthophylls cannot. (Barua and Olson, 2001)(*see attachment # 22*).

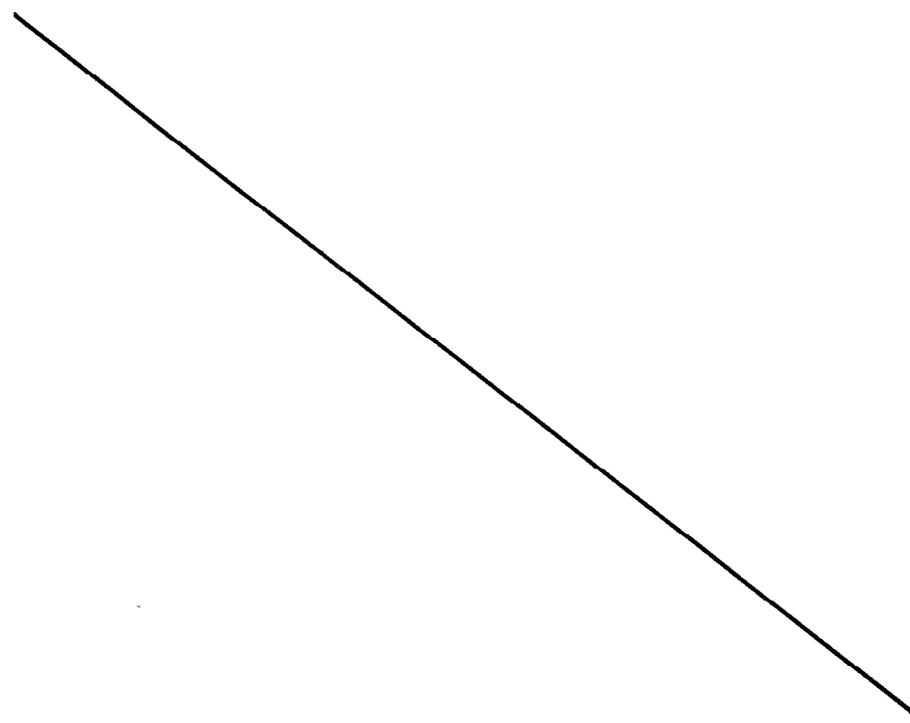
As they appear in foods, epoxy-xanthophylls (xanthophyll epoxides) are present in a variety of commonly consumed foods, as shown by Khachik et al; 1991 (*see attachment # 21*). For a 25 gram serving of green beans (raw), one would consume 0.14 mg of the epoxides neoxanthin, violaxanthin, and lutein-5,6-epoxide, the exact amount provided in the ZPC product. Based on the small dosage of epoxides in ZPC, the ubiquitous presence in fruits and vegetables, the absence of any reported or known adverse effects, and the dubious nature of absorption, xanthophyll epoxides do not present any safety concerns when consumed in the ZPC product.

6. Ames tests

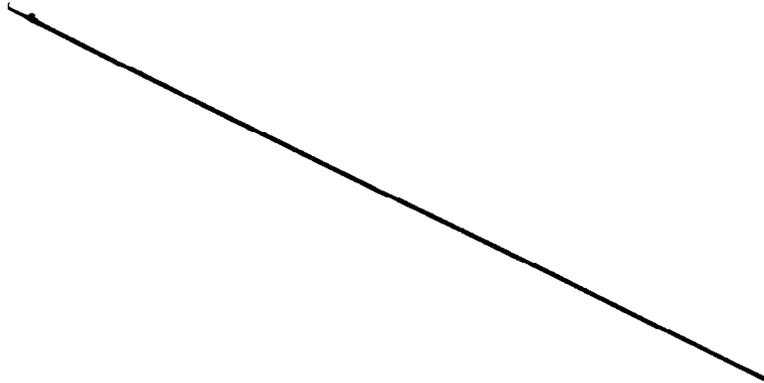


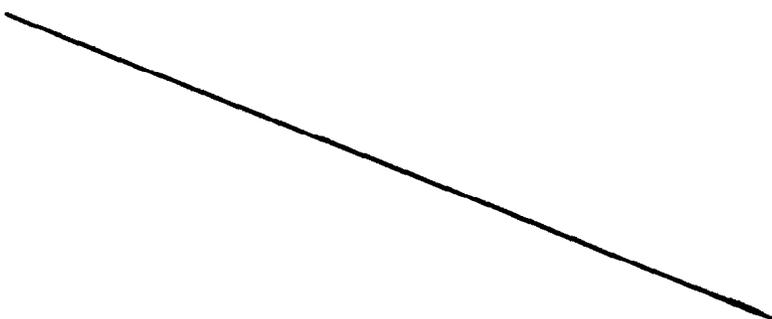
7. Subchronic Toxicity Study – 28 Day Study in Rats





8. Acute Oral Toxicity Test





9. Human studies/epidemiologic evidence

There have been numerous studies looking at the effects of dietary intervention with foods high in zeaxanthin + lutein or zeaxanthin supplements. (Bone et al, 2002 (*see attachment # 11*); Rock et al; 2002(*see attachment # 10*) No adverse health effects were reported in these studies with respect to increased carotenoid intake; these studies provide supporting evidence of safety with increased zeaxanthin and lutein consumption. In many studies, effects on plasma levels were evaluated after zeaxanthin ingestion which ranged from 2 to 30 mg/day for durations as long as 12 months (Bone et al, 2002 (*see attachment # 11*); Rock et al; 2002(*see attachment # 10*))

In one study, 38 subjects were given zeaxanthin and lutein supplements ranging in dosages from 2.4 mg to 30 mg from 60 day to six months. The subjects who consumed 30 mg of zeaxanthin per day did so for a period of 120 and 60 d, respectively. All subjects experienced a general trend in increased concentration of serum lutein and zeaxanthin followed by a exponential decline once supplementation ceased. No adverse events were reported in this study. (Bone et al; 2002)(*see attachment # 11*)

In an independent assessment of the safety of zeaxanthin to establish a theoretical 100-fold NOAEL for zeaxanthin intake, it was determined that a supplement intake of 10 mg/day would provide a 100-fold safety factor. This is based on the ratio between the NOAEL derived from safety studies and the anticipated exposure of the material, the lowest NOAEL documented, the average dietary intake of zeaxanthin in the US using conservatively low ranges, and the highest quintile of dietary intake of zeaxanthin in the US.(*attachment # 26*)

Absorption: Various animal studies have confirmed the ability of primates to absorb and metabolize Lutein or Zeaxanthin supplements, to increase xanthophyll levels rapidly in serum, and to increase macular pigment (MP) optical density.(Neuringer et al, 2004 (*see attachment # 27*); Leung et al, 2001 (*see attachment # 28*)) In one study, Monkeys fed xanthophyll-free diets from birth until 7-16 years were then supplemented with lutein and zeaxanthin over 56 weeks. Both serum lutein or zeaxanthin increased rapidly, reaching steady serum concentration at 24 to 32 weeks, demonstrating that Rhesus monkeys

respond to either dietary L or Z supplementation with increases in serum xanthophylls and MP, even after life-long xanthophyll deficiency.(Neuringer et al, 2004)(*see attachment # 27*) Data on xanthophylls absorption in human varies. In a randomized, single-blind cross-over study in which 12 subjects were administered non-esterified or esterified 3R,3'R-zeaxanthin (5mg) over 23 days, only 3.3% of the administered dose was absorbed (166 mcg of 5 mg).(Breithaupt et al. 2004)(*see attachment # 13*). This is in accordance with data given in the literature, stating a low efficiency of carotenoid absorption, reported to range from 5 to 50% (Olson, 1994)(*see attachment # 29*). Interestingly, the amount absorbed corresponded to that found in an earlier study with β -cryptoxanthin esters (Breithaupt et al. 2003)(*see attachment # 30*), although the carotenoid concentration administered in the present study was nearly four times higher. This underlines the tendency in man to absorb xanthophylls relatively poorly. The majority might have been metabolized to apocarotenals, which were not studied, or excreted through the gastrointestinal tract. The only stereoisomer found in the plasma after the ingestion of 3R,3'R-zeaxanthin palmitate was 3R,3'R-zeaxanthin.

10. Conclusions: safety determination

The safety of zeaxanthin purified concentrate as a source for zeaxanthin and lutein as a dietary supplement is evaluated by determining the safety of ingestion of the whole product, as well as safety of ingestion of the major constituents, zeaxanthin, lutein and xanthophyll epoxides. Zeaxanthin Purified Concentrate provides 3.06 mg of zeaxanthin per recommended daily dose, 0.324 mg of lutein and 0.144 mg of xanthophyll epoxides. Zeaxanthin, along with lutein and xanthophyll epoxides, are present in many foods consumed in the United States. No adverse events have been witnessed or reported from the consumption of zeaxanthin, lutein or epoxides from foods. The daily consumption of zeaxanthin for the United States population is estimated at 1.35 mg. However, it is also significant that Americans do not consume the recommended five to seven servings per day of vegetables (Nebeling et al; 1997)(*see attachment # 18*) indicating consumption of plant-contained xanthophylls is low. Increasing consumption of vegetables to the recommended daily consumption would provide approximately 3 mg of zeaxanthin per day, the equivalent of the recommended daily dosage provided by ZPC. Based on the totality of observational, epidemiological, and clinical studies examined, zeaxanthin (and the minor carotenoids lutein and xanthophyll epoxides) at the level recommended in this product are safe for human consumption. In addition, all independent safety tests, including the AMES mutagenicity test, the 28-day sub-chronic test, and acute oral toxicity test have confirmed the safety of zeaxanthin purified concentrate for use as a dietary supplement.

11. References

Beatty S, Nolan J, Kavanagh H, O'Donovan O. Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin. Archives of Biochemistry and Biophysics, 2004. (received 14 December 2003)

Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and Zeaxanthin Dietary Supplements Raise Macular Pigment Density and Serum Concentrations of these Carotenoids in Humans. *J. Nutr.* 2003; 133: 992-998. .

Breithaupt DE, Weller P, Wolters M, Hahn A. Comparison of plasma responses in human subjects after the ingestion of 3R,3R'-zeaxanthin dipalmitate from wolfberry (*Lycium barbarum*) and non-esterified 3R,3R'-zeaxanthin using chiral high-performance liquid chromatography. *British Journal of Nutrition* 2004; 91: 707-713.

Breithaupt DE, Bamedi. Carotenoid Esters in Vegetables and Fruits: A Screening with Emphasis on *â*-Cryptoxanthin Esters. *J. Agric. Food Chem.* 2001; 49: 2064-2070

Barua AB, Olson JA. Xanthophyll Epoxides, Unlike B-Carotene Monoperoxides, Are Not Detectibly Absorbed by Human. *J. Nutr.* 2001; 131: 3212-3215.

Food and Agriculture Organization of the United Nations: Joint FAO/WHO Expert Committee on Food Additives, Sixty-third meeting, Geneva, 8-17; June 2004. Available at www.fao.org/esn/jecfa/index_en.stm.

Goodwin, TW. In *The Biochemistry of the Carotenoids*, 2nd edn., vol. 1, pp. 96-203, Chapman and Hall, London; 1980.

Humphries JM, Khachik F. Distribution of Lutein, Zeaxanthin, and Related Geometrical Isomers in Fruit, Vegetables, Wheat, and Pasta Products. *J Agric Food Chem.* 2003 Feb 26;51(5):1322-7

Institute of Medicine, 2000. Institute of Medicine (IOM), Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC (2000).

Kruger CL, Murphy M, DeFreitas Z, Pfannkuch F, Heimbach J. An innovative approach to the determination of safety for a dietary ingredient derived from a new source; case study using a crystalline lutein product. *Food and Chemical Toxicology* 2002; 40: 1535-1549; 2002.

Khachik F, Beecher GR, Mudlagiri BG. Separation, Identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure & Appl. Chem*, 1991; 63: 71-80.

Landrum JT, Bone RA. *Arch. Biochem. Biophys.* 2001; 385: 28-40.

Michaud S, Giovannucci EL, Ascherio A, Rimm EB, Forman MRSL, Willett WC, *Cancer Epidemiol. Biomark. Prev.* 1998; 7: 283-290.

Rock CL, Thornquist MD, Neuhouser ML, Kristal AR, Neumark-Sztainer D, Cooper DA, Patterson RE, Cheskin LF. Diet and Lifestyle Correlates of Lutein in the Blood and Diet. *J. Nutr.* 2002; 132: 525S-530S.

Seddon J. M., Ajani U. A., Sperduto R. D., Hiller R., Blair N., Burton T. C. et al. Dietary carotenoids, vitamins A, C, and E and advanced age-related macular degeneration. *JAMA* 1994; 272: 1413–1420.

Semba, RD, Dagnelie, G. Are lutein and zeaxanthin conditionally essential nutrients for eye health? *Medical Hypotheses* 2003; 61(4): 465-472.

Weller, P. and Breithaupt DE. Identification and Quantification of Zeaxanthin Esters in Plants Using Liquid Chromatography-Mass Spectrometry. *Journal of Agricultural and Food Chemistry*. *Journal of Agricultural and Food Chemistry*, Band 51, Heft 24, 2003, S. 7044 – 7049.

USDA, Agricultural Research Service, 1998. Nutrient Data Laboratory Homepage. Available from <http://www.nal.usda.gov/fnic/foodcomp>.

USDA, 2000. US Department of Agriculture (US DA), Agricultural Research Service, 2000. 1994–1996, 1998 Continuing Survey of Food Intakes by Individuals [CD-ROM], data and documentation. National Technical Information Service, Accession No. PB2000–500027.

USDA and USDHHS, 2000. US Department of Agriculture (US DA) and US Department of Health and Human Services (US DHHS), 2000. Nutrition and Your Health: Dietary Guidelines for Americans, fifth ed. Home and Garden Bulletin No. 232, Washington, DC.

USDHHS, 2000. US Department of Health and Human Services (US DHHS), National Center for Health Statistics, 2000. Third National Health and Nutrition Examination Survey, 1988–1994, Healthy Eating Index, NCHS Series 11 No. 1A. National Technical Information Service.

USDHHS, 2000. US Department of Health and Human Services (US DHHS), National Center for Health Statistics, 2000. Third National Health and Nutrition Examination Survey, 1988–1994, Healthy Eating Index, NCHS Series 11 No. 6A. Accessed at: <http://www.cdc.gov/nchs/about/major/nhanes/nh3data.htm#DataFiles1a>.

USDHHS–NCC, 2000. US Department of Health and Human Services (US DHHS), National Center for Health Statistics and University of Minnesota Nutrition Coordinating Center (NCC) Carotenoid Data for NHANES III (US DHHS–NCC), 2000. NCHS CD-Rom, Hyattsville, MD.

12. AUTHORS

This report was prepared by Chris Noonan, M.P.H., associate at IMAGINutrition, Inc., and Anthony Almada, BSc, MSc, President and CSO of IMAGINutrition, Inc.