ORIGINAL SUBMISSION

**Via Express Courier**

Paulette Gaynor, PhD
GRAS Notification Program
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

**GRAS NOTIFICATION FOR KEMIN ZEAONE™**

Dear Dr. Gaynor:

March 10, 2016

On behalf of Kemin Food, L.C. ("the Notifier"), Ramboll Environ US Corporation is pleased to submit this Notification of the Generally Recognized as Safe (GRAS) Determination for the ingredient, ZeaONE™ manufactured using an extract of zeaxanthin from marigold flowers. This Notification contains 1) the Notifier's GRAS Exemption Claim; 2) the Expert Panel Report on the GRAS Status of the ingredient; and 3) the Safety Evaluation report including reference literature.

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Yours sincerely

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**Gavin Thompson**
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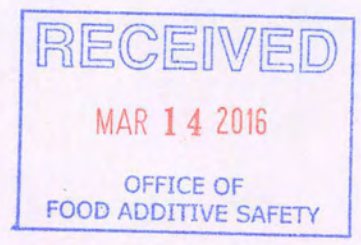
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cc:         Joanne Lasrado Hollis, PhD

Enclosures:   One original GRAS Dossier Submission with Appendices 1-6
              One CD-ROM with electronic versions of the GRAS Dossier and references

**RAMBOLL** ENVIRON

# 639

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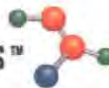
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Enclosures:    One original GRAS Dossier Submission with Appendices 1-6
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# GRAS Exemption Claim for Use of Zeaxanthin

Kemin Foods, L.C. (Kemin) has determined that the use of zeaxanthin purified extract concentrate derived from the flowers of a cultivar of *Tagetes erecta* (marigold) with enhanced levels of zeaxanthin relative to lutein and other carotenoids is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (FFDCA) because Kemin has determined such use to be Generally Recognized As Safe (GRAS). This determination is in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR § 170.36), as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

## A. Name and Address of Notifier

Anita Norian
President
Kemin Foods, L.C.
2100 Maury St.
Des Moines, IA 50317
Telephone: 515-559-5432

## B. Chemical and Common or Usual Name of GRAS Substance

The substance that is the subject of this GRAS determination commonly known as zeaxanthin or ß,ß-carotene-3,3'-diol or (3R, 3'R)-zeaxanthin. The full chemical name of zeaxanthin is 4-[18-(4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-3-en-1-ol.

Kemin zeaxanthin that is the subject of this GRAS determination is produced by Kemin via a cultivar of *T. erecta* with enhanced levels of zeaxanthin relative to lutein and other carotenoids. Kemin will make their zeaxanthin commercially available under the trade name ZeaONE™ which is formulated into safflower oil suspensions (14% or 20%) or put into beadlets (5% or 10%).

## C. Intended Use

Kemin intends to provide Kemin ZeaONE™ zeaxanthin as an ingredient in baby and toddler foods, candies, cereals, dairy products, soups and beverages at up to 300 micrograms per serving. The specific food categories and use levels for Kemin, along with the maximum amounts of zeaxanthin and combined lutein and zeaxanthin provided per serving of food in each category, are shown in Table 1. The estimated daily intakes from the proposed uses are shown in Table 2.

## D. Basis for GRAS Determination

This GRAS determination is based upon scientific procedures as described under 21 CFR § 170.30(b). The use of Kemin zeaxanthin as an ingredient in the food categories and the levels described above has been shown to be safe and GRAS, using scientific procedures, under the FFDCA, Section 201(s). To demonstrate that the Kemin zeaxanthin product is safe, and GRAS, under the intended conditions of use, the safety of the proposed intake of Kemin zeaxanthin product has been reviewed by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

**Table 1. Proposed Maximum Use Levels of Kemin ZeaONE™ with the Corresponding Amounts of Zeaxanthin and Combined Lutein and Zeaxanthin Per Serving (µg/serving[a])**

| Food Category | Fortification Levels | Zeaxanthin[b] | Lutein + Zeaxanthin[b] |
|---|---|---|---|
| Baby and toddler foods | 100 | 50 | 53 |
| Candy, hard and soft | 300 | 150 | 159 |
| Cereal, instant and regular | 600 | 300 | 318 |
| Cereal, ready-to-eat, high fiber | 600 | 300 | 318 |
| Cereal, ready-to-eat, other | 300 | 150 | 159 |
| Crackers | 600 | 300 | 318 |
| Drinks, carbonated, fruit-flavored, or energy | 300 | 150 | 159 |
| Egg substitutes | 600 | 300 | 318 |
| Frozen yogurt | 600 | 300 | 318 |
| Fruit juices and nectars | 300 | 150 | 159 |
| Gum | 300 | 150 | 159 |
| Margarine-like spreads | 600 | 300 | 318 |
| Meal replacement beverages and mixes | 600 | 300 | 318 |
| Milk, dry | 300 | 150 | 159 |
| Milk, fermented | 600 | 300 | 318 |
| Milk, flavored | 300 | 150 | 159 |
| Milk, soy and imitation | 600 | 300 | 318 |
| Salad dressing | 300 | 150 | 159 |
| Soup | 600 | 300 | 318 |
| Tea, ready-to-drink | 300 | 150 | 159 |
| Tomato-based sauces | 300 | 150 | 159 |
| Vegetable juices | 600 | 300 | 318 |
| Water, bottled | 600 | 300 | 318 |
| Yogurt | 600 | 300 | 318 |

[a] Serving sizes correspond to Reference Amounts Customarily Consumed (21 CFR § 101.12).
[b] Kemin ZeaONE™ contains a minimum of 50% zeaxanthin and 3% lutein by weight.

**Table 2. Estimated Daily Intakes from Proposed Uses for ZeaONE™ Zeaxanthin (mg/day)**

| Population | Purified Zeaxanthin Extract Concentrate | | Zeaxanthin | | Combined Lutein + Zeaxanthin | |
|---|---|---|---|---|---|---|
| | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile |
| Infants < 12 mo | 0.6 | 1.5 | 0.3 | 0.8 | 0.3 | 0.8 |
| Infants 12-23 mo | 2.2 | 3.7 | 1.1 | 1.9 | 1.2 | 2.0 |
| Children 2-5 y | 2.9 | 5.2 | 1.5 | 2.6 | 1.5 | 2.7 |
| Males, 6-11 y | 3.3 | 6.0 | 1.7 | 3.0 | 1.8 | 3.2 |
| Females, 6-11 y | 3.3 | 5.7 | 1.6 | 2.8 | 1.7 | 3.0 |
| Males, 12-18 y | 4.1 | 7.8 | 2.1 | 3.9 | 2.2 | 4.1 |
| Females, 12-18 y | 3.7 | 7.1 | 1.9 | 3.5 | 2.0 | 3.8 |
| Males, 19-49 y | 4.9 | 9.4 | 2.4 | 4.7 | 2.6 | 5.0 |
| Females, 19-49 y | 3.9 | 7.3 | 2.0 | 3.6 | 2.1 | 3.9 |
| Males, 50+ y | 3.4 | 6.3 | 1.7 | 3.1 | 1.8 | 3.3 |
| Females, 50+ y | 3.1 | 5.9 | 1.6 | 2.9 | 1.6 | 3.1 |
| Total population, 1+ y | 3.8 | 7.2 | 1.9 | 3.6 | 2.0 | 3.8 |
| Pregnant and Lactating Females 20-59 y | 4.7 | 7.8 | 2.3 | 3.9 | 2.5 | 4.1 |
| Females 14-45 y | 4.0 | 7.4 | 2.0 | 3.7 | 2.1 | 3.9 |

*Based on NHANES 2009-2010 data.

## E. Basis for GRAS Determination

This GRAS determination is based upon scientific procedures as described under 21 CFR § 170.30(b). The use of Kemin zeaxanthin as an ingredient in the food categories and the levels described above has been shown to be safe and GRAS, using scientific procedures, under the FFDCA, Section 201(s). To demonstrate that the Kemin zeaxanthin product is safe, and GRAS, under the intended conditions of use, the safety of the proposed intake of Kemin zeaxanthin product has been reviewed by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

Determination of the GRAS status of Kemin's zeaxanthin, under the intended conditions of use, has been made through the deliberations of Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine); John Thomas, Ph.D. (Adjunct Professor, Department of Pharmacology & Toxicology, Indiana University School of Medicine); and Elizabeth J. Johnson, Ph.D. (Research Scientist, Jean Mayer USDA Human Nutrition Research Center on Aging and Associate Professor, Tufts University). These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts, independently and collectively, carefully and critically reviewed and evaluated the safety dossier assembled by Ramboll Environ U.S. Corporation. The safety dossier (attached) incorporates publicly available information regarding the safety of zeaxanthin, safety evaluations carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) on lutein and zeaxanthin obtained from *T. erecta* and synthetic zeaxanthin and unpublished toxicological studies on the ZeaONE™ product.

The experts concluded that the proposed uses of zeaxanthin are GRAS based on scientific procedures (the Expert Panel Report). Therefore, zeaxanthin is GRAS at the proposed levels of inclusion, and thus, is excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

## F. Availability of Information

The data and information that serve as the basis for this GRAS determination will be sent to the U.S. FDA upon request, or will be made available to FDA for reviewing and copying at reasonable times at the office of Gavin P. Thompson, Ph.D., Principal, Ramboll Environ U.S. Corporation, 2111 E. Highland Ave., Suite 402, Phoenix, Arizona, 85016 or 4350 North Fairfax Drive, Suite 300, Arlington, Virginia 22203; telephone: 602-734-7704; facsimile: 602-734-7701; e-mail: gthompson@ramboll.com.

## G. Signature

Kemin Foods, L.C. hereby makes and submits this notice of a GRAS Exemption Claim for Kemin ZeaONE™ (zeaxanthin) under the intended conditions of use.

(b) (6)

3-7-16

Anita Norian
President
Kemin Foods, L.C.

Date

# Expert Panel Report
# on the Generally Recognized as Safe Status
# of the Proposed Uses of ZeaONE™,
# an Extract of Zeaxanthin from Marigold

Ramboll Environ US Corporation (Ramboll Environ; formerly ENVIRON International Corporation), on behalf of Kemin Foods, L.C. (Kemin), convened a panel of experts (Expert Panel), qualified by their scientific training and experience to evaluate the safety of food ingredients, to determine the safety, suitability and the Generally Recognized As Safe (GRAS) status of the proposed uses of a purified zeaxanthin extract derived from a marigold cultivar (*Tagetes erecta* 'Scarletade'), referred to by Kemin as ZeaONE™ (also herein "the Ingredient"). The Expert Panel Members were Joseph F. Borzelleca, Ph.D. (Professor Emeritus, Virginia Commonwealth University School of Medicine); John Thomas, Ph.D. (Adjunct Professor, Department of Pharmacology & Toxicology, Indiana University School of Medicine); and Elizabeth J. Johnson, Ph.D. (Research Scientist, Jean Mayer USDA Human Nutrition Research Center on Aging and Associate Professor, Tufts University).

The Expert Panel, independently and collectively, critically evaluated the available information presented in documents prepared by Ramboll Environ (the GRAS dossier). This information consisted of the description of the substance (including the identity and physical and chemical properties), analyses demonstrating and confirming the purity and manufacturing consistency of the product, the chemical identity of ZeaONE™, and product specifications. A critical overview of the history of use (consumer exposure), intended conditions of use, levels of use and estimated daily intakes (EDIs) from the intended conditions of use, its regulatory status, product stability and safety assessment of the Kemin ZeaONE™ ingredient was provided by Ramboll Environ to the Expert Panel. The Expert Panel reviewed and evaluated this information and also evaluated other materials deemed appropriate and necessary for this review.

As part of its independent and collective critical evaluation, the Expert Panel convened by teleconference on 8 September 2015 with representatives of Ramboll Environ. At the conclusion of its deliberations, the Expert Panel unanimously agreed to the conclusions described herein. A summary of the basis for these conclusions follows.

## Background Information

On September 25, 2007 members of this Expert Panel determined the proposed uses of purified zeaxanthin extract concentrate (ZEC) to be GRAS based on a safety assessment dossier prepared by ENVIRON International Corporation (now Ramboll Environ US Corporation) that consisted of a safety evaluation of the ZEC and supporting documentation (documents collectively known as "the 2007 GRAS dossier"). Kemin has since obtained a license from Ball Horticultural Company (BHC) and Chrysantis, Inc. to sell zeaxanthin ingredients derived from altered marigold plants covered by certain BHC patent rights under U.S. Patent numbers 6,784,351, 7,033,622 and 7,575,766, acquired Chrysantis' purified ZEC product business and now Kemin produces ZeaONE™, a zeaxanthin with a higher purity than ZEC. Kemin's ZeaONE™ uses the same marigold source and manufacturing process for the production of the marigold oleoresin for ZEC; however, Kemin's subsequent processing uses fewer solvents and results in a zeaxanthin product, ZeaONE™, that is of higher purity than ZEC.

Since the 2007 GRAS determination, Kemin has modified the substance identity, the manufacturing process, and the specifications and also conducted three key additional toxicological studies. Ramboll Environ also identified additional studies regarding zeaxanthin, lutein and related carotenoids not available for review at the time of the 2007 GRAS determination.

To determine that ZeaONE™ is safe and GRAS at the proposed use levels and uses, the Expert Panel reviewed a revised safety dossier prepared for the ZeaONE™ product and proposed uses. The safety dossier presents a safety assessment for the consumption of Kemin ZeaONE™ for the original uses and a new use as a dietary ingredient in prenatal dietary supplements for pregnant women.

## Description of ZeaONE™ the Manufacturing Process and Product Specifications

The substance in this GRAS determination is zeaxanthin purified extract concentrate (ZeaONE™) derived from extracts of a hybrid cultivar of *Tagetes erecta* with enhanced levels of zeaxanthin relative to lutein and other carotenoids. Zeaxanthin is a naturally occurring carotenoid with a chemical formula of $C_{40}H_{56}O_2$ and the Chemical Abstracts Services (CAS) Registry Number 144-68-3. The structure of zeaxanthin is:



Kemin manufactures ZeaONE™ from *T. erecta* hybrids obtained by chemically inducing genetic mutations on a commercial open pollinated variety known as Scarletade. The chemical used for the mutation is ethyl methanesulfonate (EMS). This improvement results in a higher flower tonnage yield per acre and also a higher effective concentration due to a higher ratio of petal to calyx. The breeding techniques used to create the hybrid are not considered genetic engineering under U.S. regulations (7 CFR §340.1).

Flowers from the mature plant are processed into meal and then compressed into small pellets and extracted with hexane. The thick paste that remains after solvent removal, "oleoresin," is an extract containing the fatty acid esters of the desired carotenoids. The oleoresin is purified by saponification and filtration with ethanol resulting in a cake that is screened crystalline zeaxanthin, which is also known as the zeaxanthin cake. The zeaxanthin cake is formulated into safflower oil suspensions (14% or 20%) or put into beadlets (5 or 10%). These products are then sold in the marketplace.

Kemin has established specifications for their ZeaONE™ ingredient to ensure that a consistent food-grade ingredient is produced. The chemical, physical and microbiological specifications of ZeaONE™ are presented in Table 1. Batches were analyzed with regard to the chemical and microbiological parameters listed in the specifications including the following parameters:

- carotenoid content: zeaxanthin > 50%, lutein > 3%, and meso-zeaxanthin ≤ 1.5%;
- heavy metals: arsenic ≤ 1.0 mg/kg, cadmium ≤ 0.5 mg/kg, lead ≤ 1.0 mg/kg, and mercury ≤ 0.1 mg/kg;
- microbial contamination: yeasts and molds meet specifications, and coliform, *Listeria*, *Salmonella*, and *Staphylococcus* all absent; and
- residual solvents: ethanol ≤ 125 ppm and hexanes ≤ 25 ppm.

These tested batches met the established specifications demonstrating that the Kemin ZeaONE™ ingredient complies with appropriate specifications for the food-grade ingredient and that a consistent product can be and is produced. This compliance and consistency in specifications is confirmed in the certificates of analysis of the ingredient.

Stability tests confirmed that ZeaONE™ zeaxanthin in a 14% oil suspension and ZeaONE™ zeaxanthin in a 5% beadlet remained above label claims for all lots tested at both 25 °C and 60% relative humidity and at 40 °C and 75% relative humidity for 12 months and 6 months, respectively.

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Expert Panel Report:
GRAS Determination for Kemin Foods, L.C. ZeaONE™, a Zeaxanthin Extract from Marigold

**Table 1. Specifications and Batch Analysis Results for Kemin ZeaONE™**

| Characteristics | Test Method | Specifications Min. | Specifications Max. | Frequency | 1410109212 | 1501103870 | 1411112625 |
|---|---|---|---|---|---|---|---|
| Zeaxanthin | KHM-005-082 | 50% of Cake | | Every lot | 55.1 | 50.52 | 53.9 |
| Lutein | KHM-005-082 | 3% of Cake | | Every lot | 7.5 | 6.90 | 8.4 |
| Meso-zeaxanthin | KHM-005-082 | | 1.5% | Every lot | 0.36 | 0.37 | 0.20 |
| Appearance | KHM-005-916 | Orange to red, free flowing powder | | Every lot | Pass | Pass | Pass |
| Color | KHM-005-916 | Orange to red | | Every lot | Pass | Pass | Pass |
| Odor | KHM-005-916 | Bland | | Every lot | Pass | Pass | Pass |
| Ash | KHM-005-940 | -- | 5% | VTP[1] | 0.14 | 0.09 | 0.1 |
| Moisture | KHM-005-035 | -- | 5% | Every lot | 1.2 | 1.2 | 0.8 |
| **Heavy Metals** | | | | | | | |
| Lead | ICP-MS AOAC 993.14 | -- | 1 ppm | VTP[1] | 0.0418 | 0.02 | 0.03 |
| Cadmium | ICP-MS AOAC 993.14 | -- | 0.5 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| Mercury | ICP-MS AOAC 993.14 | -- | 0.1 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| Arsenic | ICP-MS AOAC 993.14 | -- | 1.0 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| **Solvents** | | | | | | | |
| Ethanol | KHM-005-957 | -- | 125 ppm | VTP[1] | 3 | 9 | ND |
| Hexanes | KHM-005-957 | -- | 25 ppm | VTP[1] | 3 | 4 | 1 |
| Methanol[2] | KHM-005-957 | Record | | VTP[1] | 3 | 38 | 2 |
| **Microbiological tests** | | | | | | | |
| Aerobic Plate count | AOAC 966.23 | -- | 1000 cfu/g | | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| Escherichia coli | USP | Negative/10 g | | Every lot | Neg/10 g | Neg/10 g | Neg/10 g |
| Listeria monocytogenes | AOAC 999.06 | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| Salmonella | USP | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| Staphylococcus (enrichment) | USP | Negative/10 g | | Every lot | Neg/10 g | Neg/10 g | Neg/10 g |
| Total Coliforms | CMMEF 4th Ed. | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| Yeast and Mold Count | FDA-BAM | -- | 100 cfu/g | Every lot | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| Propylene Glycol[3] | KHM-005-033 | -- | 1000 ppm | VTP[1] | 311 | 489 | 441 |
| Thiophenes | KHM-005-072 | -- | 300 ppm | VTP[1] | N/A | 5 | N/A |
| Dioxins | EPA 1613 | -- | 86 pg/g | VTP[1] | 22.34 | 24.16 | 23.38 |
| Pesticides | USP Pesticides | Conforms to USP 561 | | VTP[1] | Conforms | Conforms | Conforms |

ND = non-detect; N/A = not applicable.

[1] VTP (Validated Testing Program) evaluates the existing data for analytes of interest from the first 10 lots of each product. The mean and standard deviation of these data are then utilized to determine an appropriate skip lot testing frequency.

[2] Methanol is not used as a solvent but is present in USP ethanol (current USP specification is not more than 200 µL methanol/L ethanol).

[3] Propylene glycol is a processing aid.

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## History of Exposure and Use

Humans do not synthesize zeaxanthin and lutein *de novo* and depend on a dietary supply. Zeaxanthin occurs naturally in corn and corn products, peppers, artichoke hearts and egg yolks and at lower concentrations in grapes, nectarines and peaches among other foods.[1] The FDA permits the use of *Tagetes* (marigold) extract as a natural flavoring substance in accordance with Good Manufacturing Practice (21 CFR §172.510) and in chicken feed as a color additive (21 CFR §73.295).

The current Dietary Guidelines for Americans advises consumption of fruit and vegetables including rich sources of lutein and zeaxanthin such as dark green vegetables, orange vegetables, starchy vegetables, legumes and other vegetables several times each week.[2] Approximately 25% of individuals two years and older consume the recommended number of vegetable servings with mean and 90th percentile intakes of lutein+zeaxanthin approximately 3.8 and 7.3 mg/day, respectively. These intakes were regarded as "prudent" intakes of carotenoids by the Institute of Medicine.[3] The estimated mean and 90th percentile intakes of lutein+zeaxanthin by the total population one year and older from naturally occurring dietary sources and food consumption data reported in the United States Department of Health and Human Service's 2009-2012 National Health and Nutrition Examination Surveys (NHANES) are 1.4 and 13.4 mg/day, respectively (Table 4). Based on these intake data and dietary recommendations, the general population of Americans falls short of "prudent" levels of carotenoid intakes by approximately 2.0 mg per day.

## Proposed Uses and Estimated Daily Intakes

The proposed food categories and use levels for Kemin ZeaONE™, along with the maximum amounts of zeaxanthin and combined lutein and zeaxanthin provided per serving of food in each category, are shown in Table 2. The fortification levels are lower than the original 2007 levels due to the higher purity of the product from the revised manufacturing process. The final Kemin ZeaONE™ products are mixed with either food-grade oils or other food-grade excipients (sucrose, modified food starch) to create standardized products that meet the zeaxanthin specification (for example, 14% or 20% oil formulations and 5% or 10% beadlet formulations) and allows users to maintain a consistent formulation.

Estimates of potential intakes of ZeaONE™ resulting from these intended uses were calculated using food consumption data reported in the 2009-2010 and 2011-2012 NHANES. Estimates of potential intake of Kemin ZeaONE™, zeaxanthin, and of combined lutein and zeaxanthin from Kemin ZeaONE™ are shown in Table 3. The estimated mean and 90th percentile 2-day average intakes of Kemin ZeaONE™ are 3.8 and 7.2 mg per day, respectively, by the population ages 1 year and older. The estimated mean and 90th percentile 2-day average intakes of zeaxanthin by the population ages 1 year and older are 1.9 and 3.6 mg, respectively. The estimated mean and 90th percentile 2-day average intakes of combined lutein and zeaxanthin by this population are 2.0 and 3.8 mg, respectively. Across all of the subpopulations in this analysis, the maximum estimated 2-day average 90th percentile combined lutein and zeaxanthin intake is 5.0 mg; this intake was estimated for males ages 19-49 years.

Estimates of potential combined intake of lutein and zeaxanthin from dietary sources and Kemin ZeaONE™ are shown in Table 4. For an individual with average intakes of lutein+zeaxanthin from current dietary sources, combined lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 3.4 to 5.2 mg per day (1.4 + [2.0- or 3.8] mg/day). For an individual with lutein+zeaxanthin intakes from current dietary sources at the 90th percentile of intake, total lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 15.4 to 17.2 mg per day (13.4 + [2.0 or 3.8] mg/day) and potentially an additional 2 mg zeaxanthin from a 10 mg Kemin ZeaONE™ dietary supplement.[4]

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[1] Perry A, Rasmussen H, Johnson EJ. 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. J Food Compost Anal. 22:9-15; doi:10.1016/j.jfca.2008.07.006.

[2] U.S. Department of Health and Human Services, U.S. Department of Agriculture (USDHHS/USDA). 2015. Scientific Report of the 2015 Dietary Guidelines Advisory Committee.

[3] Institute of Medicine. 2000. β-carotene and other carotenoids. In: National Academy Press, ed. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. 325-382. Washington, D.C.

[4] Due to 10 mg Kemin ZeaONE supplementation at 20% zeaxanthin in safflower oil (10 mg x 0.02 = 2 mg).

| Table 2. Proposed Maximum Use Levels of Zeaxanthin Extract Concentrate and Kemin ZeaONE™ and Corresponding Amounts of Zeaxanthin and Combined Lutein and Zeaxanthin Per Serving (µg/serving[a]) | | | | | | |
|---|---|---|---|---|---|---|
| | 2007 | Zeaxanthin Extract Concentrate[b] | | 2015 | Kemin ZeaONE™[c] | |
| Food Category | Fortification Levels | Zeaxanthin | Lutein + Zeaxanthin | Fortification Levels | Zeaxanthin | Lutein + Zeaxanthin |
| Baby and toddler foods | 188 | 50 | 54 | 100 | 50 | 53 |
| Candy, hard and soft | 565 | 150 | 162 | 300 | 150 | 159 |
| Cereal, instant and regular | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, high fiber | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, other | 565 | 150 | 162 | 300 | 150 | 159 |
| Crackers | 1130 | 300 | 324 | 600 | 300 | 318 |
| Drinks, carbonated, fruit-flavored, or energy | 565 | 150 | 162 | 300 | 150 | 159 |
| Egg substitutes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Frozen yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |
| Fruit juices and nectars | 565 | 150 | 162 | 300 | 150 | 159 |
| Gum | 565 | 150 | 162 | 300 | 150 | 159 |
| Margarine-like spreads | 1130 | 300 | 324 | 600 | 300 | 318 |
| Meal replacement beverages and mixes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, dry | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, fermented | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, flavored | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, soy and imitation | 1130 | 300 | 324 | 600 | 300 | 318 |
| Salad dressing | 565 | 150 | 162 | 300 | 150 | 159 |
| Soup | 1130 | 300 | 324 | 600 | 300 | 318 |
| Tea, ready-to-drink | 565 | 150 | 162 | 300 | 150 | 159 |
| Tomato-based sauces | 565 | 150 | 162 | 300 | 150 | 159 |
| Vegetable juices | 1130 | 300 | 324 | 600 | 300 | 318 |
| Water, bottled | 1130 | 300 | 324 | 600 | 300 | 318 |
| Yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |

Notes:

a Serving sizes correspond to Reference Amounts Customarily Consumed (21 CFR §101.12).

b Purified Zeaxanthin Extract Concentrate contains approximately 27% zeaxanthin and 2% lutein by weight.

c Kemin ZeaONE™ contains a minimum of 50% zeaxanthin and 3% lutein by weight.

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**Table 3. Intakes from Proposed Uses:**
**Zeaxanthin, and Combined Lutein + Zeaxanthin**

| Population | Zeaxanthin Concentrate* (mg/day) | | | | Zeaxanthin (mg/day) | | | | Lutein + Zeaxanthin (mg/day) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ZEC 2007 | | ZeaONE™ 2015 | | ZEC 2007 | | ZeaONE™ 2015 | | ZEC 2007 | | ZeaONE™ 2015 | |
| | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile |
| Infants < 12 mo | 1.0 | 2.1 | 0.6 | 1.5 | 0.3 | 0.6 | 0.3 | 0.8 | 0.3 | 0.6 | 0.3 | 0.8 |
| Infants 12-23 mo | 2.1 | 3.6 | 2.2 | 3.7 | 0.6 | 1.0 | 1.1 | 1.9 | 0.6 | 1.0 | 1.2 | 2.0 |
| Children 2-5 y | 2.7 | 4.2 | 2.9 | 5.2 | 0.7 | 1.1 | 1.5 | 2.6 | 0.8 | 1.2 | 1.5 | 2.7 |
| Males, 6-11 y | 3.3 | 5.5 | 3.3 | 6.0 | 0.9 | 1.5 | 1.7 | 3.0 | 0.9 | 1.6 | 1.8 | 3.2 |
| Females, 6-11 y | 2.8 | 4.8 | 3.3 | 5.7 | 0.8 | 1.3 | 1.6 | 2.8 | 0.8 | 1.4 | 1.7 | 3.0 |
| Males, 12-18 y | 3.8 | 6.1 | 4.1 | 7.8 | 1.0 | 1.7 | 2.1 | 3.9 | 1.1 | 1.8 | 2.2 | 4.1 |
| Females, 12-18 y | 3.1 | 5.1 | 3.7 | 7.1 | 0.8 | 1.4 | 1.9 | 3.5 | 0.9 | 1.5 | 2.0 | 3.8 |
| Males, 19-49 y | 3.9 | 6.7 | 4.9 | 9.4 | 1.0 | 1.8 | 2.4 | 4.7 | 1.1 | 2.0 | 2.6 | 5.0 |
| Females, 19-49 y | 3.1 | 5.6 | 3.9 | 7.3 | 0.8 | 1.5 | 2.0 | 3.6 | 0.9 | 1.6 | 2.1 | 3.9 |
| Males, 50+ y | 3.0 | 5.2 | 3.4 | 6.3 | 0.8 | 1.4 | 1.7 | 3.1 | 0.9 | 1.5 | 1.8 | 3.3 |
| Females, 50+ y | 2.6 | 4.8 | 3.1 | 5.9 | 0.7 | 1.3 | 1.6 | 2.9 | 0.7 | 1.4 | 1.6 | 3.1 |
| Total population, 1+ y | 3.2 | 5.6 | 3.8 | 7.2 | 0.9 | 1.5 | 1.9 | 3.6 | 0.9 | 1.6 | 2.0 | 3.8 |
| Pregnant and Lactating Females 20-59 y | NA | NA | 4.7 | 7.8 | NA | NA | 2.3 | 3.9 | NA | NA | 2.5 | 4.1 |
| Females 14-45 y | NA | NA | 4.0 | 7.4 | NA | NA | 2.0 | 3.7 | NA | NA | 2.1 | 3.9 |

Notes:
*Purified Zeaxanthin Extract Concentrate (ZEC) 2007 data are based on NHANES 2003-2004 data; ZeaONE™ 2015 data are based on NHANES 2009-2010 data.

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**Table 4. Potential Combined Intake of Lutein+Zeaxanthin from Dietary Sources and Kemin ZeaONE™ (mg/day)**

| | Source of Lutein+Zeaxanthin (L+Z) | | | | | |
| | Current Diet | | Kemin ZeaONE™ | | Total | |
| Population | Intake level | L+Z | Intake level | L+Z | L+Z | |
|---|---|---|---|---|---|---|
| Total population, 1+ y | Mean | 1.4 | Mean | 2.0 | 3.4 | |
| | | | 90th percentile | 3.8 | 5.2 | |
| | 90th percentile | 13.4 | Mean | 2.0 | 15.4 | |
| | | | 90th percentile | 3.8 | 17.2 | |

Data source: NHANES 2009-2012 (Calculations made by Ramboll Environ)

The estimates of intake presented in Table 4 are likely large overestimates of actual combined lutein and zeaxanthin intakes resulting from the proposed uses in the food supply. In the calculations of estimated intakes, any reported intake of a food corresponding to one of the proposed use categories (Table 2) was assumed to contain added Kemin ZeaONE™. Additionally, all foods were assumed to contain the maximum proposed concentration of Kemin ZeaONE™ per serving. It is likely that consumers may consume only a subset of these foods containing added Kemin ZeaONE™, and not all products may contain the maximum proposed use levels; therefore, these estimated intakes are likely overestimates of typical intakes.

## Intended Effect

Kemin ZeaONE™ is intended to provide an additional dietary source of zeaxanthin in a variety of foods and beverages as a nutrient supplement which is necessary for the body's nutritional and metabolic processes in accordance with 21 CFR 170.3(o)(20).

## Safety Assessment

Numerous GRAS notifications (GRNs) for carotenoids have been submitted to FDA. These publicly-available carotenoid GRNs, including GRNs 110, 140, 221, 291, 385, 390, 542 and 543 for lutein or lutein esters, and 481 and 550 for meso-zeaxanthin. These GRNs received FDA response letters with "no questions at this time".

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) established an ADI of 1 mg/kg bw/day, for lutein and zeaxanthin derived from *T. erecta* with high concentrations of total carotenoids present at levels ≥ 80%.[5] The EFSA ADI is equivalent to 60 mg of lutein derived from marigolds for a 60 kg person.[6] In addition, an ADI of 2 mg/kg bw/day for lutein and zeaxanthin combined from marigold and for zeaxanthin (synthetic) has been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for lutein and zeaxanthin.[7,8]

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[5] EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the reevaluation of lutein (E 161b) as a food additive on request of the European Commission. EFSA Journal 2010; 8(7):1678 [57 pp.]. doi:10.2903/j.efsa.2010.1678.

[6] The NOAEL for the Kemin ZeaONE 90-d rat study is 550 mg/kg bw/day which is 14% zeaxanthin and therefore 77 mg zeaxanthin/kg bw/day. The NOAELs for two additional zeaxanthin/lutein 90-d oral toxicity studies in the rat were 400 mg/kg bw/d.

[7] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. WHO/FAO Joint Expert Committee on Food Additives. 63rd meeting Geneva, 8-17 June 2004; http://www.who.int/ipcs/publications/jecfa/en/Summary63final.pdf.

[8] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. WHO/FAO Joint Expert Committee on Food Additives. 63rd meeting Geneva, 8-17 June 2004; http://www.inchem.org/documents/jecfa/jecmono/v54je01.pdf.

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## Absorption, Distribution, Metabolism and Excretion in Humans

Carotenoids are fat-soluble compounds and consequently are absorbed like dietary fat. Carotenoids appear to be absorbed by the mucosa of the small intestine, mainly in the duodenum, via passive diffusion and rapidly taken up by the liver. Ingestion of zeaxanthin or lutein typically results in increases in blood concentrations of the ingested carotenoid and increased concentrations of their respective metabolites. In metabolic reactions of zeaxanthin and lutein, the general skeleton of the polyene chain of the carotenoids remains intact; however, chemical transformations of the carotenoid end-group can result in the formation of polar conjugates that are likely excreted in urine. Intake of zeaxanthin does not appear to affect plasma concentrations of other dietary carotenoids.

Zeaxanthin is one of the carotenoids in the retina, particularly in the central region, the macula lutea. Macular pigment, which is composed solely of lutein and zeaxanthin, absorbs blue-light prior to its reaching the underlying photoreceptor cell layer, protecting it from light and presumably oxidative damage. Increased ingestion of zeaxanthin and lutein has been reported to increase macular pigment density.

## Toxicological Studies

In 2007 the Expert Panel found that ZEC is safe and GRAS at the originally proposed levels of addition to foods and based on the safety data (Table 2).

Since the 2007 GRAS determination, Kemin has revised the manufacturing process to create ZeaONE™, a zeaxanthin product with higher purity. The resulting product consists of ≥ 50% zeaxanthin and ≥ 3% lutein with a corresponding decrease in residual components.

Recent toxicological studies with Kemin ZeaONE™ support the safety of zeaxanthin generally but also demonstrate the safety of the ZeaONE™ product specifically. The studies with Kemin ZeaONE™ reported a NOAEL of 550 mg ZeaONE™/kg-bw/day (77 mg zeaxanthin/kg bw/day) in rats; this was the highest dose tested. Kemin ZeaONE™ is neither mutagenic nor genotoxic. Kemin ZeaONE™ does not contain residual components from the manufacturing process with allergenic potential and there is no evidence to suggest that it may cause adverse effects in sensitive populations because the revised manufacturing process reduces residual components from the 2007 ZEC product and does not introduce allergenic substances.

The estimated mean (4.9 mg/day) and 90th percentile (9.4 mg/day) intakes of Kemin ZeaONE™ correspond to intakes of 82 and 157 µg Kemin ZeaONE™/kg bw/day for a 60 kg adult, respectively, or 67 µg Kemin ZeaONE™/kg bw/day for a 60 kg pregnant woman taking the prenatal supplement.

For an individual with average intakes of lutein+zeaxanthin from current dietary sources, combined lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 3.4 to 5.2 mg/d. For an individual with lutein+zeaxanthin intakes from current dietary sources at the 90th percentile of intake, total lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 15.4 to 17.2 mg/d. These estimates are well below the ADI of 60 mg/d established by EFSA for marigold-derived lutein.

A critical evaluation of the available evidence indicates that foods containing up to 600 µg Kemin ZeaONE™/serving are safe and suitable for the general population, daily supplementation of up to 10 mg Kemin ZeaONE™ is safe and suitable for the adult general population, and that prenatal supplements containing up to 4.0 mg Kemin ZeaONE™ are safe and suitable for pregnant women.

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## Conclusions

We, the members of the Expert Panel, have independently and collectively, critically evaluated the available information on ZeaONE™, a purified zeaxanthin extracted from *Tagetes erecta* flowers, manufactured by Kemin Foods, L.C., presented in the dossier prepared by Ramboll Environ and summarized herein, and other information deemed appropriate. We unanimously conclude that the proposed uses of ZeaONE™, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting the food grade specifications presented in the dossier, in baby and toddler foods, candies, specific cereals, dairy products, soups and sauces, drinks including teas, fruit and vegetable juices, and water, crackers and gum and as a dietary ingredient in prenatal supplements, are safe and suitable.

We further unanimously conclude that the proposed uses of ZeaONE™, manufactured consistent with cGMP and meeting the food grade specifications presented in the dossier, in baby and toddler foods, candies, specific cereals, dairy products, soups and sauces, drinks including teas, fruit and vegetable juices, and water, crackers and gum and as a dietary ingredient in prenatal supplements, are Generally Recognized As Safe (GRAS) based on scientific procedures.

It is our opinion that other experts, qualified by scientific training and experience, and evaluating the same data and information, would concur with these conclusions.

**Joseph F. Borzelleca, Ph.D.**       Signature:    (b) (6)
*Chair of the Expert Panel*
Professor Emeritus
Pharmacology & Toxicology      Date:    20 January 2016
School of Medicine,
Virginia Commonwealth University
Richmond, Virginia

**John Thomas, Ph.D., A.T.S.**      Signature:    (b) (6)
Adjunct Professor
Department of Pharmacology & Toxicology
Indiana University School of Medicine      Date:    2 Feb. 2016
Indianapolis, Indiana

**Elizabeth J. Johnson, Ph.D.**      Signature:    (b) (6)
Research Scientist
Jean Mayer USDA Human Nutrition
Research Center on Aging      Date:    29 January 2016
Associate Professor, Tufts University
Boston, Massachusetts

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Safety Evaluation of
ZeaONE™
For Use in Select Foods

Prepared for:
**Kemin Foods, L.C.**
**Des Moines, Iowa**

Prepared by:
**Ramboll Environ US Corporation**
**Phoenix, Arizona**

Date:
**August 28, 2015**

Project Number:
**2826967C**

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List of Appendices

# 1 Executive Summary

## 1.1 Introduction and Background

On September 25, 2007, an Expert Panel determined the proposed uses of ZEC to be generally recognized as safe (GRAS) based on scientific procedures. The Expert Panel critically evaluated available information on the safety of ZEC and zeaxanthin including a safety assessment dossier prepared by ENVIRON International Corporation (now Ramboll Environ US Corporation) that consists of a safety evaluation of the ZEC and supporting documentation (documents collectively known as "the 2007 GRAS dossier") and the opinion of an expert panel (Appendix 1). Kemin Foods, L.C. ("Kemin") obtained a license from Chrysantis, Inc. to sell zeaxanthin food ingredients and also acquired Chrysantis® Purified Zeaxanthin Extract Concentrate (ZEC). ZEC is a purified extract derived from a marigold cultivar (*Tagetes erecta* 'Scarletade') and was marketed under the trademark EZ Eyes®. Since acquiring ZEC, Kemin produces ZeaONE™, a zeaxanthin with a higher purity than ZEC. Kemin's ZeaONE™ uses the same marigold source and manufacturing process for the production of a marigold oleoresin (MOR) as ZEC; however, Kemin's subsequent MOR processing uses fewer solvents and results in a zeaxanthin product that is of higher purity than ZEC. Kemin states that ZeaONE™ is substantially chemically equivalent to ZEC and proposes to amend the 2007 GRAS dossier to include Kemin's crystalline ZeaONE™.

To determine that foods containing ZeaONE™ are safe and GRAS at the proposed use levels in these additional uses, Kemin engaged Ramboll Environ US Corporation (Ramboll Environ) to revise the safety assessment and safety dossier prepared for ZEC for the proposed uses of the ZeaONE™ product. This document presents a safety assessment for the consumption of Kemin ZeaONE™ from the original uses and a new use as an ingredient in prenatal dietary supplements for pregnant women.

Since the 2007 GRAS determination by the Expert Panel, Kemin has not modified the history of exposure (Chapter 3), the intended technical effect of ZeaONE™ (Chapter 4) since the previous GRAS determination. Hence, those chapters are unmodified herein. Kemin has modified the substance identity, the manufacturing process, and the specifications (Chapter 2) and also conducted three key additional toxicological studies. Ramboll Environ also identified additional studies regarding zeaxanthin, lutein and related carotenoids not available for review in the previous GRAS dossier. The Kemin ZeaONE™ toxicity studies and the additional studies were reviewed by Ramboll Environ and are abstracted herein (Chapter 5).[1]

Kemin ZeaONE™ is intended to be added to a variety of foods to increase dietary intakes of zeaxanthin. The food categories and the respective maximum concentrations of the concentrate to be added are shown in Table 1-1. Table 1-1 also identifies the amount of zeaxanthin and lutein+zeaxanthin per food category that will be provided by the proposed uses of Kemin ZeaONE™. Kemin also intends to use ZeaONE™ as a dietary ingredient in dietary supplements marketed to adults over 18 years of age in an amount up to 10 mg daily and to

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[1] Note that much of this document is retained from the 2007 GRAS dossier. Minor edits to the original material have been made for clarity and consistency; however, "Chrysantis ZEC" is retained to refer to the 2007 material and "ENVIRON" is retained to demonstrate that the work was performed in 2007.

pregnant and lactating women, and women intending to become pregnant in an amount up to 4 mg daily. The discussion of intended uses (Chapter 3) has been revised significantly due to the additional proposed uses, in particular, the products, fortification levels and estimated dietary intakes (EDIs) for consumers. The fortification levels are lower because Kemin has increased the purity of the product. Therefore, the changes in the EDIs are predominantly a result of the updated food consumption data.

For an individual with average intakes of lutein+zeaxanthin from current dietary sources, combined lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 3.4 to 5.2 mg/d. For an individual with lutein+zeaxanthin intakes from current dietary sources at the 90th percentile of intake, total lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 15.4 to 17.2 mg/d. These estimates are well below the ADI of 60 mg/d established by the European Food Safety Authority for marigold-derived lutein which is structurally analogous to zeaxanthin.

A critical evaluation of the available evidence indicates that foods containing up to 600 µg ZeaONE™/serving are safe and suitable for the general population and that daily supplementation of up to 10 mg Kemin ZeaONE™ (0.2 mg zeaxanthin/day) is safe and suitable for the adult general population. This evaluation also indicates that ZeaONE™ as an ingredient in prenatal dietary supplements at up to 4 mg per day (0.8 mg zeaxanthin/day) is safe and suitable for pregnant and lactating women.

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| Table 1-1. Proposed Maximum Use Levels of Zeaxanthin Product (Kemin ZeaONE™[d]) and Corresponding Amounts of Zeaxanthin and Combined Lutein and Zeaxanthin Per Serving (µg/serving[a]) | | | | | | |
|---|---|---|---|---|---|---|
| | **2007** | **Chrysantis Purified Zeaxanthin Extract Concentrate[b]** | | **2015[c]** | **Kemin ZeaONE™[d]** | |
| **Food Category** | **Fortification Levels** | **Zeaxanthin** | **Lutein + Zeaxanthin** | **Fortification Levels** | **Zeaxanthin** | **Lutein + Zeaxanthin** |
| Baby and toddler foods | 188 | 50 | 54 | 100 | 50 | 53 |
| Candy, hard and soft | 565 | 150 | 162 | 300 | 150 | 159 |
| Cereal, instant and regular | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, high fiber | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, other | 565 | 150 | 162 | 300 | 150 | 159 |
| Crackers | 1130 | 300 | 324 | 600 | 300 | 318 |
| Drinks, carbonated, fruit-flavored, or energy | 565 | 150 | 162 | 300 | 150 | 159 |
| Egg substitutes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Frozen yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |
| Fruit juices and nectars | 565 | 150 | 162 | 300 | 150 | 159 |
| Gum | 565 | 150 | 162 | 300 | 150 | 159 |
| Margarine-like spreads | 1130 | 300 | 324 | 600 | 300 | 318 |
| Meal replacement beverages and mixes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, dry | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, fermented | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, flavored | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, soy and imitation | 1130 | 300 | 324 | 600 | 300 | 318 |
| Salad dressing | 565 | 150 | 162 | 300 | 150 | 159 |
| Soup | 1130 | 300 | 324 | 600 | 300 | 318 |
| Tea, ready-to-drink | 565 | 150 | 162 | 300 | 150 | 159 |
| Tomato-based sauces | 565 | 150 | 162 | 300 | 150 | 159 |
| Vegetable juices | 1130 | 300 | 324 | 600 | 300 | 318 |
| Water, bottled | 1130 | 300 | 324 | 600 | 300 | 318 |
| Yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |

a Serving sizes correspond to Reference Amounts Customarily Consumed (21 CFR §101.12).

b Chrysantis® Purified Zeaxanthin Extract Concentrate contains approximately 27% zeaxanthin and 2% lutein by weight.

c Fortification levels revised so that the zeaxanthin levels match the 2007 levels.

d Kemin ZeaONE™ contains a minimum of 50% zeaxanthin and 3% lutein by weight.

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# 2  Description of Substance

## 2.1    Identity

The identity of the GRAS substance is zeaxanthin purified extract concentrate (ZeaONE™) derived from extracts of a cultivar of *Tagetes erecta* with enhanced levels of zeaxanthin relative to lutein and other carotenoids.

The variety used by Kemin was obtained by chemically inducing genetic mutations on a commercial open pollinated variety known as Scarletade. The chemical used for the mutation is ethyl methanesulfonate (EMS). This mutation process is detailed in the Ball Horticulture Company patent document presented in Appendix 1.

## 2.2    Common and Trade Names

Common and/or trade names include: zeaxanthin; ß,ß-carotene-3,3'-diol; and (3R, 3'R)-zeaxanthin.

## 2.3    Chemical Identification

Zeaxanthin has been assigned Chemical Abstracts Services (CAS) Registry Number 144-68-3.

## 2.4    Chemical Formula

The chemical formula of zeaxanthin is $C_{40}H_{56}O_2$.

## 2.5    Chemical Structure

The chemical structure of zeaxanthin is 4-[18-(4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethyl-cyclohex-3-en-1-ol. Figure 1 is a schematic representation of the compound.

**Figure 1. Chemical Structure of Zeaxanthin**

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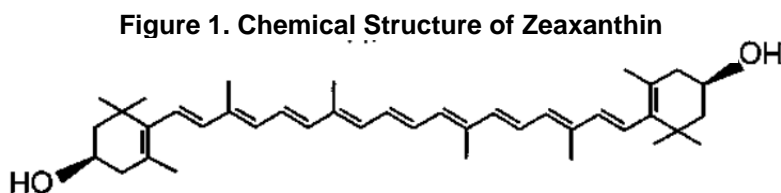
## 2.6    Chemical and Physical Properties

The properties of zeaxanthin are summarized in Table 2-1.

| Table 2-2. Properties of Zeaxanthin | |
| --- | --- |
| **Property** | **Value and Units** |
| Appearance | Orange to red, free flowing powder |
| Molecular weight | 568.88 |
| Bulk density | 0.6-0.68 $g/cm^3$ |
| Solubility in water | Insoluble |
| Flash point | Negative |
| Autoignition temperature | 200 – 220 °C |
| Melting point | 215.5 °C |
| Stability | Needs to be protected from light, oxygen and temperature |
| Source: Kemin Foods, L.C.; Budavari et al. (1996) | |

## 2.7    Production Process

*Tagetes erecta* is the source of the xanthophylls (lutein and zeaxanthin) used in the production of Kemin ZeaONE™. These carotenoids are found in the petals in commercial quantities. A schematic presentation of the production process of Kemin ZeaONE™ from *Tagetes erecta* is presented in Figures 2 and 3.

## 2.8    Planting and Cultivation of *Tagetes erecta* Lut 2 257 Mutant

The *Tagetes erecta* variety, Lut 2 257 Mutant, is crossed with a *Tagetes erecta* male (Figure 2, Step 2) to create a hybrid seed of the Lut 2 257 mutant (Figure 2, Step 3). Hybrid 21238 was the first hybrid plant which had 50% fully double flowers and 50% semi-double flowers, which were the plants used to derive purified ZEC. These hybrids were improved through traditional breeding practices to produce 100% fully double flowers. The current hybrids 71955 and 71952 used to make ZeaONE™ are the result of improved female hybrids crossed with same male parent hybrid as 21238. This improvement resulted in a higher flower tonnage yield per acre and also a higher effective concentration due to a higher ratio of petal to calyx.

It is important to note that the breeding techniques used to create the hybrid *Tagetes erecta* plants used in the production of Chrysantis ZEC and Kemin ZeaONE™ are not considered genetic engineering[2] and the hybrids are not genetically modified organisms (GMO) in the context of EU Directive 2001/18/EC.

These hybrid seeds are planted and developed into hybrid plants (Figure 2, Steps 4-5). The hybrid plants are grown in India where they are cultivated into mature plants (Figure 2, Steps 6-7).

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[2] The genetic modification of organisms by recombinant DNA techniques. 7 C.F.R. 340.1
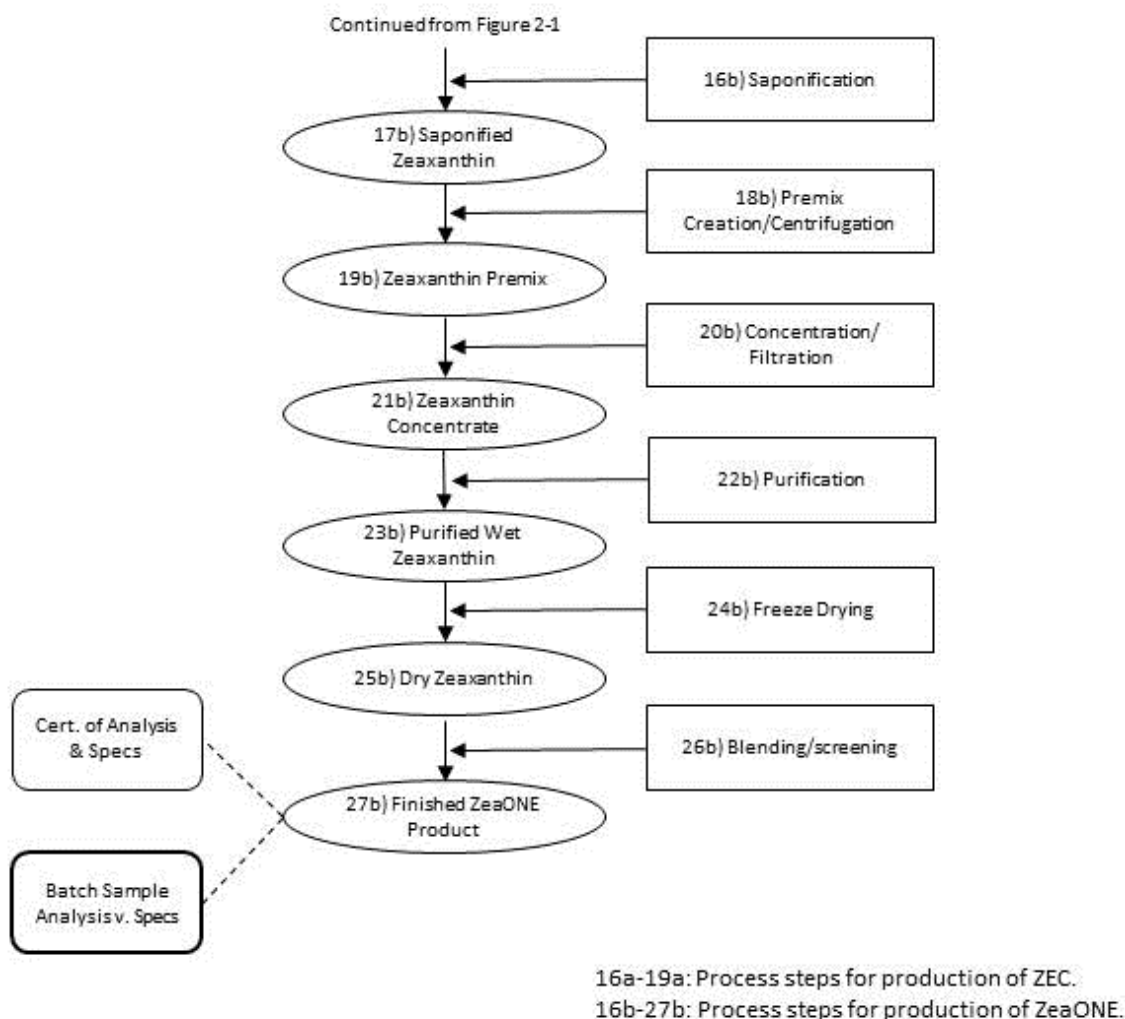
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**Figure 2. Production Process of Kemin ZeaONE™ and ZEC**
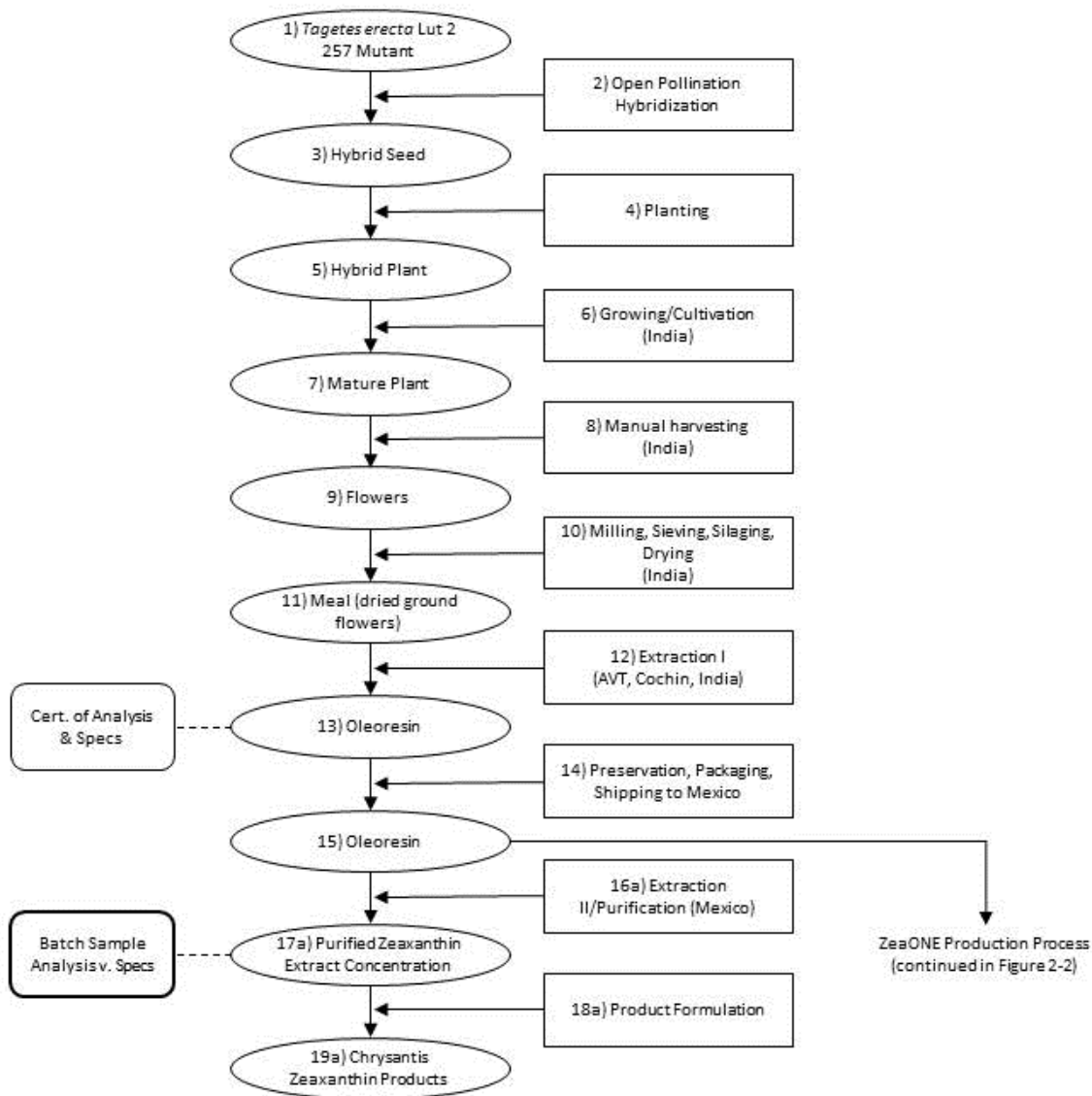


16a-19a: Process steps for production of ZEC.
16b-27b: Process steps for production of ZeaONE.

Source:  Kemin Foods, L.C.

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**Figure 3. Purification of Kemin ZeaONE™**



Source: Kemin Foods, L.C.

## 2.9 Harvesting of the Mature Plant and First Extraction

Flowers from the mature plant are manually harvested (Figure 2, Steps 8-9) and then silaged, dried and ground into a meal through a process of milling, sieving and drying (Figure 2, Steps 10-11). The marigold meal is then compressed into small pellets and extracted with hexane (Figure 2, Step 12). The hexane solvent is removed by evaporation in several stages and recycled back into the extraction process. A certificate of analysis of the hexane used in the production process is presented in Appendix 1. The thick paste that remains after solvent removal, "oleoresin," is an extract containing the fatty acid esters of the desired xanthophylls (Figure 2, Step 13). Specifications are set for the content of the oleoresin extract. Once it is produced, its contents are analyzed. The certificate of analysis for Kemin' oleoresin is also presented in Appendix 1. The oleoresin is then shipped to Des Moines, Iowa for further processing and purification (Figure 2, Step 14).

## 2.10 Purification of Oleoresin

The purification process for Kemin ZeaONE™ has been modified from the Chrysantis method provided in the 2007 GRAS dossier (Figure 3). The crystalline ZeaONE™ is manufactured using the following process:

Saponification

- Heat marigold oleoresin in heat tent set for 48 hours at 150-155 °F.

- Transfer heated oleoresin to saponification vessel and add equal parts propylene glycol.

- Mix/hold oleo and glycol for a minimum of 3 hours between 130-140 °F.

- Add potash over minimum of 60 minutes.

  – Potash amount (kg) = Oleoresin (kg) x 43.9%

- Increase saponification vessel temperature to 180 °F. Mix/hold for a minimum of 16 hours between 174-180 °F.

Premix Creation / Centrifuge

- Charge premix vessel with deionized (DI) water heated to 158 °F and saponified zeaxanthin in a ratio of 20:1 (DI water to saponified zeaxanthin).

- Filter premix through basket centrifuge with 35-40 micron filter cloth at a flow rate of 1.2 gallons per minute (GPM).

- Pull cake and repeat premix creation and centrifuge procedure until all saponified zeaxanthin has been processed.

Concentrate / Filtrate

- Concentrate the first pass filtrate using an Alfa Laval disc stack centrifuge.

- Charge premix vessel with DI water heated to 158 °F and saponified zeaxanthin in a ratio of 60:40 (DI water to saponified zeaxanthin).

- Filter premix through basket centrifuge at a flowrate of 1.2 GPM.

- Pull cake and charge premix vessel with undiluted filtrate from the second centrifuge pass.

- Filter premix through basket centrifuge at a flowrate of 1.20 GPM.

- Pull cake.

Purification

- Mix wet cake and measure %solids, kg zeaxanthin.

- Charge Nutsche Filter with ethanol and zeaxanthin wet cake in a ratio of 10:1 (ethanol to zeaxanthin).

- Mix for 30 minutes.

- Discharge ethanol filtrate to filter press with 1.5 micron filters and rinse cake with water at a ratio of 40:1 (water to zeaxanthin).

- Collect purified wet cake.

Drying
- Dry purified wet cake using freeze dryer.

Blending / Screening
- Purified dry cake lots are blended and screened to form the finished crystalline zeaxanthin product.

## 2.11  Product Formulation

After analysis to determine compliance with specifications, the Kemin ZeaONE™ is formulated into safflower oil suspensions (14% or 20%) or put into beadlets (5 or 10%). These products are then sold in the marketplace.

## 2.12  Product Specifications and Batch Analyses Results

The specifications for the Kemin ZeaONE™ are summarized in Table 2-2.

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| Table 2-3. Specifications and Batch Analysis Results for Kemin ZeaONE™ | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Characteristics** | **Test Method** | **Specifications** Min. | Max. | **Frequency** | **1410109212** | **1501103870** | **1411112625** |
| Zeaxanthin | KHM-005-082 | 50% of Cake | | Every lot | 55.1 | 50.52 | 53.9 |
| Lutein | KHM-005-082 | 3% of Cake | | Every lot | 7.5 | 6.90 | 8.4 |
| Meso-zeaxanthin | KHM-005-082 | | 1.5% | Every lot | 0.36 | 0.37 | 0.20 |
| Appearance | KHM-005-916 | Orange to red, free flowing powder | | Every lot | Pass | Pass | Pass |
| Color | KHM-005-916 | Orange to red | | Every lot | Pass | Pass | Pass |
| Odor | KHM-005-916 | Bland | | Every lot | Pass | Pass | Pass |
| Ash | KHM-005-940 | -- | 5% | VTP[1] | 0.14 | 0.09 | 0.1 |
| Moisture | KHM-005-035 | -- | 5% | Every lot | 1.2 | 1.2 | 0.8 |
| **Heavy Metals** | | | | | | | |
| Lead | ICP-MS AOAC 993.14 | -- | 1.0 ppm | VTP[1] | 0.0418 | 0.02 | 0.03 |
| Cadmium | ICP-MS AOAC 993.14 | -- | 0.5 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| Mercury | ICP-MS AOAC 993.14 | -- | 0.1 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| Arsenic | ICP-MS AOAC 993.14 | -- | 1.0 ppm | VTP[1] | <0.01 | <0.01 | <0.01 |
| **Solvents** | | | | | | | |
| Ethanol | KHM-005-957 | -- | 125 ppm | VTP[1] | 3 | 9 | ND |
| Hexanes | KHM-005-957 | -- | 25 ppm | VTP[1] | 3 | 4 | 1 |
| Methanol[2] | KHM-005-957 | Record (ppm) | | VTP[1] | 3 | 38 | 2 |
| **Microbiological tests** | | | | | | | |
| Aerobic Plate count | AOAC 966.23 | -- | 1000 cfu/g | | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| *Escherichia coli* | USP | Negative/10 g | | Every lot | Neg/10 g | Neg/10 g | Neg/10 g |
| *Listeria monocytogenes* | AOAC 999.06 | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| *Salmonella* | USP | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| *Staphylococcus* (enrichment) | USP | Negative/10 g | | Every lot | Neg/10 g | Neg/10 g | Neg/10 g |
| Total Coliforms | CMMEF 4th Ed. | Negative/25 g | | Every lot | Neg/25 g | Neg/25 g | Neg/25 g |
| Yeast and Mold Count | FDA-BAM | -- | 100 cfu/g | Every lot | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| Propylene Glycol[3] | KHM-005-033 | -- | 1000 ppm | VTP[1] | 311 | 489 | 441 |
| Thiophenes | KHM-005-072 | -- | 300 ppm | VTP[1] | N/A | 5 | N/A |
| Dioxins | EPA 1613 | -- | 86 pg/g | VTP[1] | 22.34 | 24.16 | 23.38 |
| Pesticides | USP Pesticides | Conforms to USP 561 | | VTP[1] | Conforms | Conforms | Conforms |

ND – non-detect; N/A – not applicable
[1]VTP (Validated Testing Program evaluates the existing data for analytes of interest from the first 10 lots of each product. The mean and standard deviation of these data are then utilized to determine an appropriate skip lot testing frequency).
[2]Methanol is not used as a solvent but is present in USP Ethanol.
[3]Propylene glycol is a processing aid.

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## 2.13   Product Characterization

The carotenoid content is predominantly zeaxanthin (up to 60%) with some lutein (approximately 10%) that is then normalized to 50% zeaxanthin upon mixing with safflower oil. The zeaxanthin within the product is 99% 3R, 3'R-zeaxanthin and less than 1% 3S,S'S-zeaxanthin and 3R,3'S-zeaxanthin (Appendix 2). The summarized results of the analysis of three lots of Kemin ZeaONE™ are presented in Tables 2-3 and 2-4. The product characterization table including vitamin and fatty acid quantities along with documentation for the extracts and methods of analysis are presented in Appendix 2.

| Table 2-3. Distribution of Carotenoids in Kemin's ZeaONE™ (% of ingredient) | | | |
|---|---|---|---|
| | **ZeaONE™ lots**[a] | | |
| **Analytical parameters** | **1404101637** | **1404108253** | **1406105374** |
| Carotenoids (%) | 75.23 | 76.41 | 73.74 |
| *Zeaxanthin* | *55.60* | *60.40* | *52.30* |
| Lutein | 10.90 | 7.10 | 11.40 |
| β-carotene[b] | 8.20 | 8.38 | 9.31 |
| β-cryptoxanthin[b] | 0.53 | 0.53 | 0.73 |
| [a]HPLC analysis of ZeaONE™; Appendix 2<br>[b]AOAC 941.15; Quackenbush (1987) modified. (Covance Laboratories)<br>Notes:<br>AOAC – Association of Analytical Communities; HPLC = high-performance liquid chromatography | | | |

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| Table 2-4. Additional Characterization Data of Kemin ZeaONE™ | | | | |
|---|---|---|---|---|
| | ZeaONE™ Batch Number | | | |
| Analytical parameters | 1404101637 | 1404108253 | 1406105374 | Method[a] |
| Protein (%) | 0.49 | 0.61 | 0.50 | AOAC 968.06, 992.15 (modified) |
| Fat (%) | 0.99[2] | 0.78[2] | 2.60[2] | AOAC 922.06 (modified), 933.05 (modified), 925.32 (modified) |
| Ash (%) | <0.1 | <0.1 | 0.29 | AOAC 923.03 |
| Moisture (%) | 0.71 | <0.1 | 0.49 | AOAC 925.08 |
| **Sugars (%)** | | | | |
| Fructose | <0.1 | <0.1 | <0.1 | |
| Glucose | <0.1 | <0.1 | <0.1 | Mason and Slover, 1971(modified); Brobst, 1972 |
| Sucrose | <0.1 | <0.1 | <0.1 | (modified) |
| Lactose | <0.1 | <0.1 | <0.1 | |
| Maltose | <0.1 | <0.1 | <0.1 | |
| Waxes (%) | 10.0 | 11.1 | 10.0 | |
| **Vitamins** | | | | |
| Vitamin A as Retinol | <1.00 | <1.00 | <1.00 | AOAC 992.04, 992.06, 2001.13 |
| Vitamin C (mg/100g) | <1.00 | <1.00 | <1.00 | Fontannaz et al. 2006 (modified); |
| Vitamin D3 (IU/g) | <0.020 | <0.020 | <0.020 | AOAC 2011.11; Huang et al. 2009. |
| Vitamin D2 (IU/g) | <0.080 | <0.080 | <0.080 | |
| Vitamin E (IU/g) | 0.045 | n/a | n/a | Cort et al. 1983 (modified); Speek et al. 1985 (modified); McMurray et al. 1980 (modified) |
| Vitamin K1 (µg/100 g) | <5 | 15.0 | <5.00 | AOAC 992.27, 999.15 |
| Thiamin (mg/100 g) | <0.01 | <0.010 | <0.010 | AOAC 942.23, 953.17, 957.17, 960.46 |
| Riboflavin (mg/100 g) | <0.02 | <0.02 | <0.02 | AOAC 940.33 |
| Niacin (mg/100 g) | <0.03 | <0.03 | <0.03 | AOAC 944.13 |
| Vitamin B6 (mg/100 g) | <0.007 | <0.007 | <0.007 | AOAC 961.15; Atkins et al. 1943 |
| Folic Acid (µg/g) | <0.060 | 0.09 | <0.06 | AOAC 992.05, 960.46 |
| Vitamin B12 (µg/g) | <0.001 | <0.001 | <0.001 | AOAC 952.20, 960.46; USP; |
| Biotin (µg/100 g) | <0.005 | <0.005 | <0.005 | AOAC 49:882; Scheiner, J. and DeRitter, 1975; Wright, L.D. and Skeggs, 1944; |
| Pantothenic Acid (µg/100 g) | <0.4 | 0.9 | <0.4 | AOAC 945.74 |
| **Fatty Acids (%)** | | | | |
| Saturated | | 0.413 | 2.15 | AOAC 996.06; Official Methods and Recommended Practices of the AOCS, Official methods Ce 2b-11 (2011), Ce 1h-05 (2009), Ce 1j-07 (2013), Ce 2-66 (2009),The American Oil Chemists' Society, Champaign, IL (modified). |
| Total Cis Unsaturated | 0.3 | 0.32 | 0.292 | |
| Monounsaturated | | 0.301 | 0.182 | |
| Polyunsaturated | | 0.019 | 0.11 | |
| Trans | <0.01 | 0.014 | 0.04 | |
| Omega 3 | <0.01 | <0.01 | 0.097 | |
| Omega 6 | 0.014 | 0.02 | 0.018 | |

0.287

0.634  0.013

**Table 2-4. Additional Characterization Data of Kemin ZeaONE™**

| Analytical parameters | ZeaONE™ Batch Number 1404101637 | 1404108253 | 1406105374 | Method[a] |
|---|---|---|---|---|
| Omega 9 | <0.01 | <0.01 | <0.010 | |
| Total Fatty Acids | 0.988 | 0.782 | 2.6 | |
| 4:0 Butyric | <0.01 | <0.01 | <0.010 | |
| 6:0 Caproic | <0.01 | <0.01 | <0.010 | |
| 8:0 Caprylic | <0.01 | <0.01 | <0.010 | |
| 10:0 Capric | <0.01 | <0.01 | <0.010 | |
| 12:0 Lauric | <0.01 | 0.026 | <0.010 | |
| 14:0 Myristic | 0.037 | <0.01 | 0.094 | |
| 14:1 Myristoleic | <0.01 | <0.01 | <0.010 | |
| 15:0 Pentadecanoic | 0.01 | <0.01 | <0.010 | |
| 15:1 Pentadecenoic | <0.01 | <0.01 | <0.010 | |
| 16:0 Palmitic | 0.347 | 0.231 | 1.17 | |
| 16:0 Palmitoleic | 0.038 | 0.042 | <0.010 | |
| 17:0 Heptadecanoic | <0.01 | <0.01 | 0.012 | |
| 17:1 Heptadecenoic | <0.01 | <0.01 | <0.010 | |
| 18:0 Stearic | 0.215 | 0.135 | 0.661 | |
| 18:1 Oleic | <0.01 | <0.01 | <0.010 | |
| 18:2 Linoleic | 0.014 | 0.02 | 0.018 | |
| 20:0 Arachidic | 0.018 | 0.012 | 0.049 | |
| 18:3 Gamma Linoleic | <0.01 | <0.01 | <0.010 | |
| 20:1 Eicosenoic | <0.01 | <0.01 | <0.010 | |
| 18:3 Linolenic | <0.01 | <0.01 | <0.010 | |
| 18:4 Octadecatetraenoic | <0.01 | <0.01 | <0.010 | |
| 20:2 Eicosadienoic | <0.01 | <0.01 | <0.010 | |
| 22:0 Behenic | 0.013 | <0.01 | 0.208 | |
| 22:1 Erucic | <0.01 | <0.01 | <0.010 | |
| 20:3 Eicosatrienoic | <0.01 | <0.01 | <0.010 | AOAC 996.06; Official Methods and Recommended |
| 20:4 Arachidonic | <0.01 | <0.01 | <0.010 | Practices of the AOCS, Official methods Ce 2b-11 (2011), |
| 20:5 Eicosapentaenoic | <0.01 | <0.01 | 0.025 | Ce 1h-05 (2009), Ce 1j-07 (2013), Ce 2-66 (2009),The |
| 24:0 Linoceric | 0.034 | 0.028 | 0.057 | American Oil Chemists' Society, Champaign, IL |
| 22:5 Docosapentaenoic | <0.01 | <0.01 | 0.072 | (modified). |
| 22:6 Docosahexaenoic | <0.01 | <0.01 | <0.010 | |
| Total 18:1 Trans | <0.01 | <0.01 | 0.042 | |
| Total 18:1 Cis | 0.262 | 0.273 | 0.19 | |
| Total 18:2 trans | <0.01 | 0.015 | <0.010 | |
| Total 18:3 trans | <0.01 | <0.01 | <0.010 | |

Notes:
[a]All the analyses were carried out by Covance Laboratories Inc., Madison, WI (Appendix 2)

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## 2.14 Product Stability

Figures 4 through 8 provide schematic representations of market products of ZeaONE™ suspended in 14% safflower oil (i.e., a softgel) and ZeaONE™ 5% beadlet (also containing sucrose and modified food starch), respectively. The stability of ZeaONE™ zeaxanthin in a 14% oil suspension and ZeaONE™ zeaxanthin 5% beadlet were monitored at both 25 ⁰C and 60% relative humidity and at  40 ⁰C and 75% relative humidity for 12 months and 6 months, respectively. Zeaxanthin concentrations remained above label claim for all lots tested. The measurements recorded in the stability studies are presented in Appendix 3.

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**Figure 4. Storage Stability of Total Carotenoid as Zeaxanthin in Six Batches of Kemin ZeaONE™ over a 120-day Period at 40 ºC and 75 % Relative Humidity**
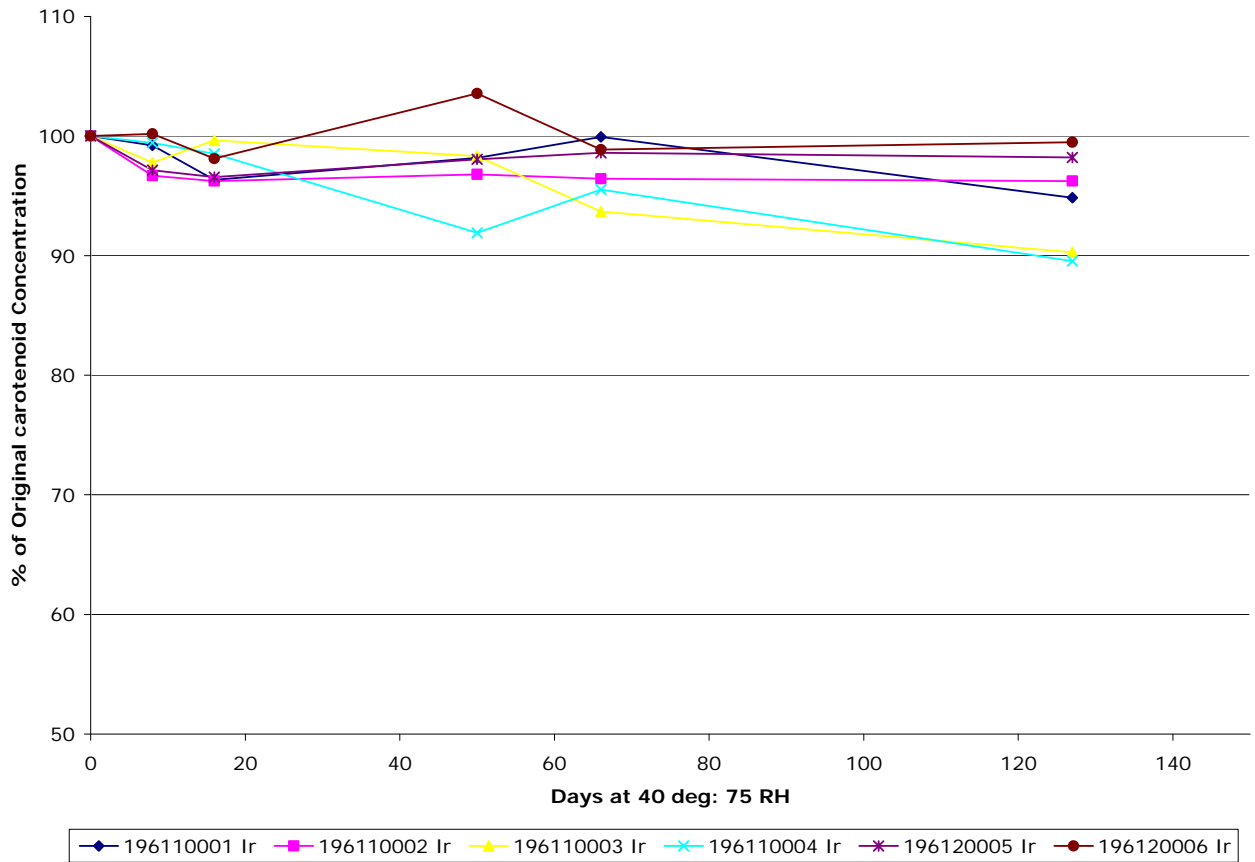


Source: Chrysantis GRAS dossier, 2007
Note: Each data point is a representation of replicate analysis.

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**Figure 5. Storage Stability of ZeaONE™ Zeaxanthin in 14% Safflower Oil Market Product**
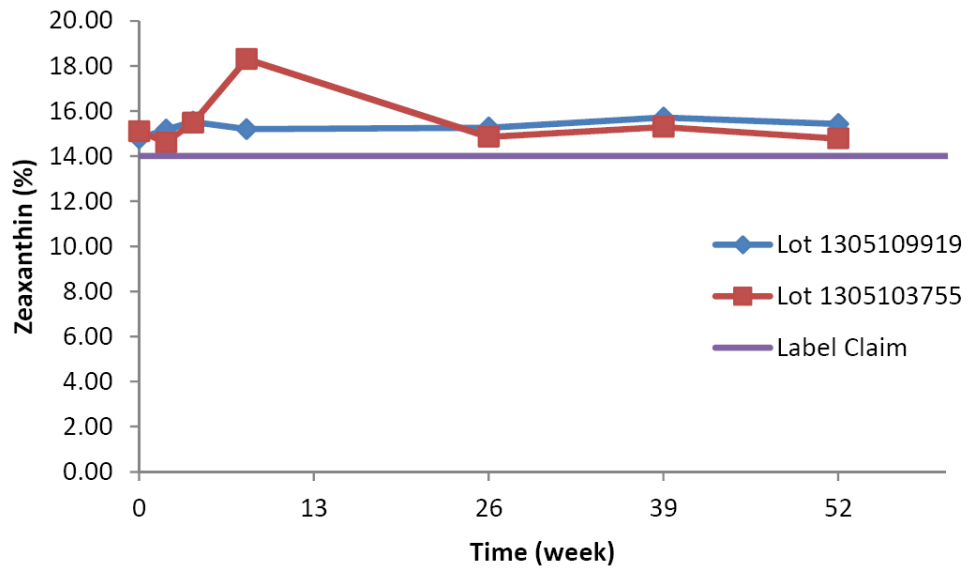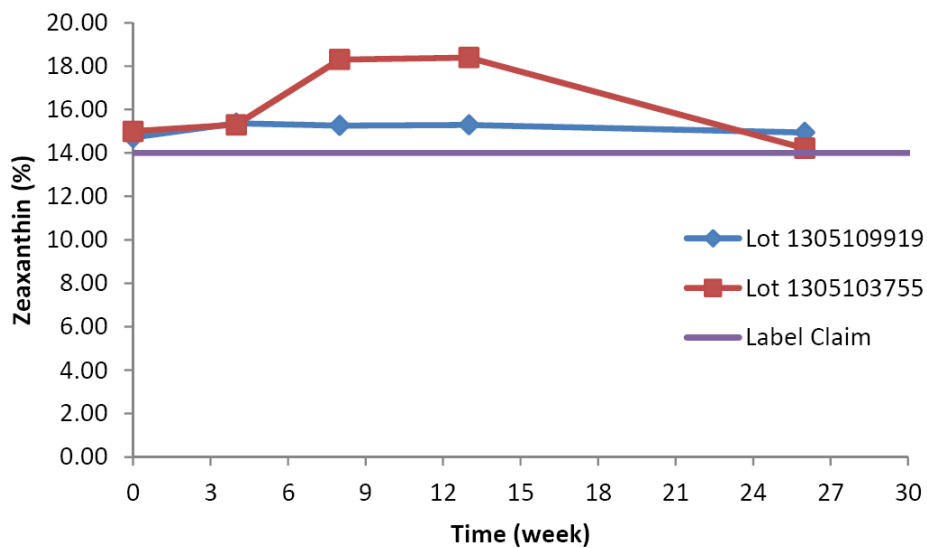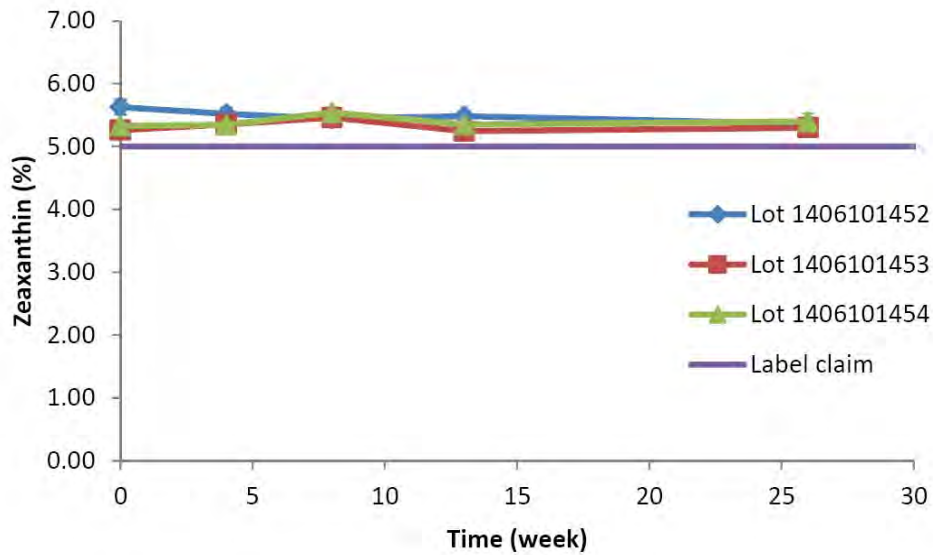Suspension over a 12 month period at 25 °C and 60% relative humidity



**Figure 6. Storage Stability of ZeaONE™ Zeaxanthin in 14% Safflower Oil Market Product**
Suspension over a 6 month period at 40 °C and 75% relative humidity

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ZeaONE™ Safety Evaluation
Prepared for Kemin Foods, L.C.

**Figure 7. Storage Stability of ZeaONE™ Zeaxanthin 5% Beadlet Market Product**
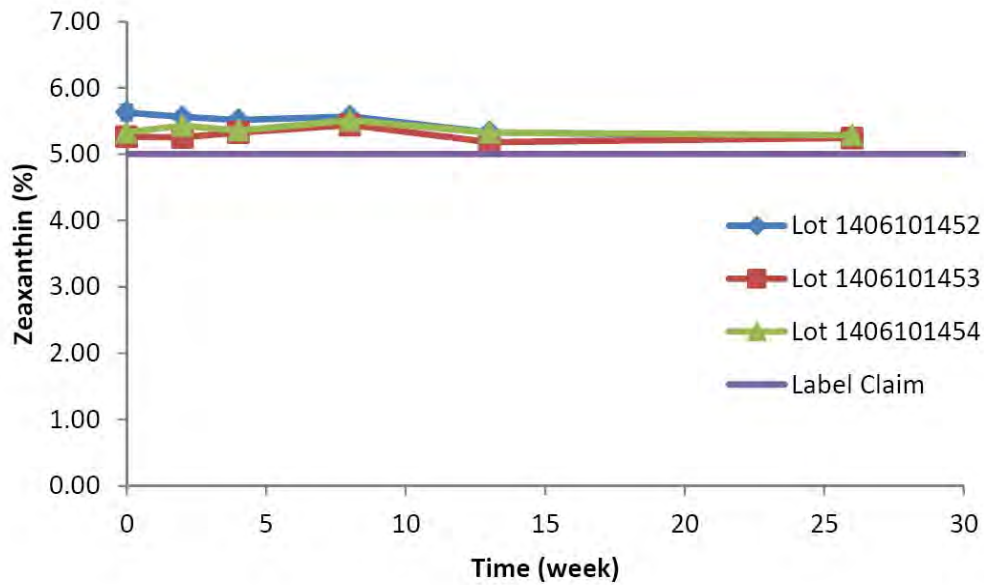Suspension over a 6 month period at 25 °C and 60% relative humidity



**Figure 8. Storage Stability of ZeaONE™ Zeaxanthin 5% Beadlet Market Product**
Suspension over a 6 month period at 40 °C and 75% relative humidity

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# 3 History of Use, Proposed Use and Consumer Exposure

## 3.1 Exposure to Zeaxanthin and Related Carotenoids from Dietary Sources

### 3.1.1 Naturally Occurring Sources

Most carotenoids are composed of a central carbon chain of alternating single and double bonds and carry different cyclic or acyclic end groups. Their major biochemical functions are determined by the extended system of conjugated double bonds, which is also responsible for their color (Stahl and Sies 2005). Carotenoids containing at least one oxygen atom are classified as the xanthophylls, and the hydrocarbon carotenoids are classified as carotenes. Lutein and zeaxanthin, which are xanthophylls, are isomers of each other and differ only in the position of one double bond (Figure 9).

**Figure 9. Structures of Lutein and Zeaxanthin**
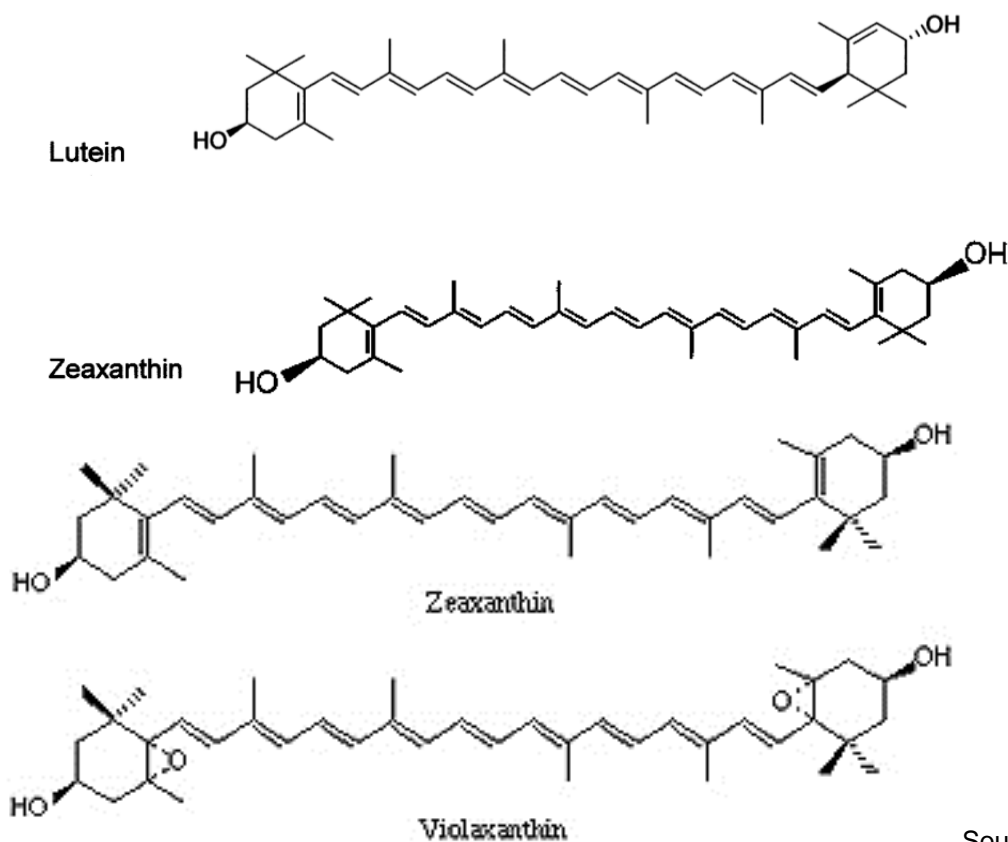


Source: Yonekura and Nagao (2007) & IOM (2000)

Animals and humans cannot synthesize carotenoids de novo and depend on a dietary supply (Stahl and Sies 2005). Approximately 60 carotenoids are consumed in the human diet (Yonekura and Nagao 2007). Dietary sources of carotenoids are primarily vegetables and fruits (Handelman 2001; Khachik et al. 1991; Olson 1994). Some carotenoids are consumed in eggs and fish as a result of carotenoid-rich poultry and fish feeds. Key dietary carotenoids that are monitored in nationwide survey data include α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and lycopene.

The primary carotenoids in Kemin ZeaONE™ are the xanthophylls zeaxanthin and lutein. Other carotenoids identified in Kemin ZeaONE™ include β-cryptoxanthin, β-carotene, antheraxanthin, violaxanthin, canthaxanthin and mutatoxanthin.

In many analyses of carotenoid concentrations in foods, the concentrations of lutein and zeaxanthin are reported as a single value, making it difficult to identify the zeaxanthin content of a food product. Perry et al. (2009) measured the individual concentrations of lutein and zeaxanthin in 66 food samples. The foods included in the study are the major dietary sources of the xanthophylls in the US based on contributions to total xanthophyll intakes as assessed using data from NHANES 2009 - 2012.

The mean concentrations of trans-zeaxanthin, trans-lutein, cis-zeaxanthin, and cis-lutein identified for each food are shown in Table 3-1. Total concentrations of zeaxanthin and lutein were derived from the sum of the trans and cis isomers. Overall, trans isomers of lutein and zeaxanthin were the predominant form of the carotenoids in the foods included in the study. Corn and corn products tended to have the highest concentrations of zeaxanthin (up to 556 µg/100 g food). Cooked egg yolks were found to contain 127 µg zeaxanthin per 100 g. The most concentrated vegetable sources of zeaxanthin included orange peppers (1665 µg zeaxanthin per 100 g) followed by red and yellow peppers and artichoke hearts (18-22 µg zeaxanthin per 100 g). Relatively low concentrations of zeaxanthin (3-6 µg zeaxanthin per 100 g) were found in fruits including grapes, nectarines and peaches. In most of the foods included in the analysis, the concentration of zeaxanthin was considerably lower than the concentration of lutein. Xanthophyll concentration in orange peppers was an exception; the concentration of zeaxanthin in orange peppers was approximately 8 times the concentration of lutein. Lutein was found in the highest concentrations in green leafy vegetables.

The carotenoid pathways that exist in plants may explain the relatively low concentrations of zeaxanthin and higher concentrations of lutein found in most plant sources of these xanthophylls (Rodriguez-Amaya 2001). Zeaxanthin is derived from β-carotene: β-carotene undergoes hydroxylation to form β-cryptoxanthin; then undergoes hydroxylation to form zeaxanthin. Zeaxanthin is then easily transformed to antheraxanthin and violaxanthin by epoxidation reactions. Lutein, however, is derived from α-carotene by hydroxylation to α-cryptoxanthin or zeinoxanthin and subsequent hydroxylation to form lutein. Unlike zeaxanthin, lutein appears to undergo limited epoxidation to form other carotenoids.

| Table 3-1. Mean concentrations of Lutein and Zeaxanthin in Selected Foods | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Lutein | | | Zeaxanthin | | | |
| | trans | cis | trans + cis | trans | cis | trans + cis | Z/La |
| Food | (µg/100 g food) | | | | | | |
| **Corn and corn products** | | | | | | | |
| Apple Jacks®, cereal | 43 | 2 | 45 | 24 | 2 | 26 | 0.6 |
| Cap'n Crunch®, cereal | 42 | 4 | 46 | 20 | 4 | 24 | 0.5 |
| Cheetos® | 66 | 48 | 114 | 73 | 12 | 85 | 0.7 |
| Chex Mix® | 48 | 4 | 52 | 25 | 4 | 29 | 0.6 |
| Corn, cooked from frozen | 202 | 37 | 239 | 202 | 25 | 227 | 0.9 |

| | Lutein | | | Zeaxanthin | | | |
|---|---|---|---|---|---|---|---|
| **Food** | **trans** | **cis** | **trans + cis** | **trans** | **cis** | **trans + cis** | **Z/La** |
| | | | **(µg/100 g food)** | | | | |
| Corn Chex, cereal® | 151 | 17 | 168 | 115 | 20 | 135 | 0.8 |
| Corn Flakes, cereal | 40 | 15 | 55 | 49 | 12 | 61 | 1.1 |
| Corn Muffin | 86 | 10 | 96 | 51 | 44 | 95 | 1.0 |
| Corn Pops®, cereal | 42 | 5 | 47 | 36 | 9 | 45 | 1.0 |
| Cornmeal, yellow | 1001 | 63 | 1064 | 531 | 25 | 556 | 0.5 |
| Cornmeal, white | 13 | 0 | 13 | 13 | 0 | 13 | 1.0 |
| Crispix®, cereal | 25 | 2 | 27 | 21 | 3 | 24 | 0.9 |
| Fritos® | 17 | 11 | 28 | 33 | 5 | 38 | 1.4 |
| Frosted Flakes®, cereal | 33 | 13 | 46 | 81 | 16 | 97 | 2.1 |
| Fruit Loops®, cereal | 41 | 4 | 45 | 24 | 4 | 28 | 0.6 |
| Life®, cereal | 51 | 3 | 54 | 25 | 3 | 28 | 0.5 |
| Popcorn, Smartfood® | 64 | 59 | 123 | 141 | 83 | 224 | 1.8 |
| Reese's Puffs®, cereal | 46 | 7 | 53 | 34 | 9 | 43 | 0.8 |
| Tortilla | 0 | 0 | 0 | 0 | 0 | 0 | NA[b] |
| Tortilla chip, Tostitos® | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| **Eggs and foods containing eggs** | | | | | | | |
| egg noodles, cooked | 16 | 0 | 16 | 0 | 0 | 0 | NA |
| egg yolk, cooked | 159 | 0 | 159 | 127 | 0 | 127 | 0.8 |
| macaroni & cheese, Kraft | 3 | 0 | 3 | 0 | 0 | 0 | 0.0 |
| mayonnaise, Hellman's® | 35 | 6 | 41 | 21 | 6 | 27 | 0.7 |
| mayonnaise, Hellman's®, fat free | 5 | 0 | 5 | 3 | 1 | 4 | 0.8 |
| spinach pasta, cooked | 176 | 0 | 176 | 0 | 0 | 0 | 0.0 |
| **Fruits and vegetables** | | | | | | | |
| apple, red delicious, with skin | 15 | 0 | 15 | 0 | 0 | 0 | 0.0 |
| apricot, dried | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| artichoke heart | 62 | 33 | 95 | 18 | 0 | 18 | 0.2 |
| asparagus, cooked | 991 | 0 | 991 | 0 | 0 | 0 | 0.0 |
| broccoli, cooked | 772 | 0 | 772 | 0 | 0 | 0 | 0.0 |
| Brussels sprouts, cooked | 155 | 0 | 155 | 0 | 0 | 0 | 0.0 |
| cabbage, red | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| cantaloupe, raw | 19 | 0 | 19 | 0 | 0 | 0 | 0.0 |
| cilantro | 7703 | 0 | 7703 | 0 | 0 | 0 | 0.0 |
| cucumber | 361 | 0 | 361 | 0 | 0 | 0 | 0.0 |
| endive | 399 | 0 | 399 | 3 | 0 | 3 | 0.0 |
| grapes, green | 53 | 0 | 53 | 6 | 0 | 6 | 0.1 |
| grapes, red | 24 | 0 | 24 | 4 | 0 | 4 | 0.2 |
| green beans, cooked from frozen | 306 | 0 | 306 | 0 | 0 | 0 | 0.0 |
| honeydew | 25 | 0 | 25 | 0 | 0 | 0 | 0.0 |
| kale, cooked | 8884 | 0 | 8884 | 0 | 0 | 0 | 0.0 |
| kiwi | 171 | 0 | 171 | 0 | 0 | 0 | 0.0 |
| lettuce, romaine | 3824 | 0 | 3824 | 0 | 0 | 0 | 0.0 |

| Table 3-1. Mean concentrations of Lutein and Zeaxanthin in Selected Foods | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Lutein | | | Zeaxanthin | | | |
| | trans | cis | trans + cis | trans | cis | trans + cis | Z/La |
| Food | (µg/100 g food) | | | | | | |
| lima beans, cooked | 155 | 0 | 155 | 0 | 0 | 0 | 0.0 |
| mango | 6 | 0 | 6 | 0 | 0 | 0 | 0.0 |
| nectarine | 8 | 0 | 8 | 4 | 0 | 4 | 0.5 |
| olive, green | 79 | 76 | 155 | 0 | 0 | 0 | 0.0 |
| parsley | 4326 | 0 | 4326 | 0 | 0 | 0 | 0.0 |
| peach | 11 | 0 | 11 | 3 | 0 | 3 | 0.3 |
| pepper, green | 173 | 0 | 173 | 0 | 0 | 0 | 0.0 |
| pepper, orange | 208 | 0 | 208 | 1665 | 0 | 1665 | 8.0 |
| pepper, red | 0 | 0 | 0 | 22 | 0 | 22 | NA |
| pepper, yellow | 139 | 0 | 139 | 18 | 0 | 18 | 0.1 |
| scallions, raw | 782 | 0 | 782 | 0 | 0 | 0 | 0.0 |
| scallions, cooked in oil | 2488 | 0 | 2488 | 0 | 0 | 0 | 0.0 |
| spinach, cooked | 12640 | 864 | 13504 | 0 | 0 | 0 | 0.0 |
| spinach, raw | 6603 | 621 | 7224 | 0 | 0 | 0 | 0.0 |
| squash, acorn, raw, no skin | 47 | 0 | 47 | 0 | 0 | 0 | 0.0 |
| squash, butternut, cooked | 57 | 0 | 57 | 0 | 0 | 0 | 0.0 |
| squash, yellow, cooked | 150 | 0 | 150 | 0 | 0 | 0 | 0.0 |
| watermelon | 4 | 0 | 4 | 0 | 0 | 0 | 0.0 |
| zucchini, cooked, with skin | 1355 | 0 | 1355 | 0 | 0 | 0 | 0.0 |
| **Miscellaneous foods** | | | | | | | |
| hot sauce | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| salsa | 40 | 21 | 61 | 0 | 0 | 0 | 0.0 |
| pistachio, shelled | 1405 | 0 | 1405 | 0 | 0 | 0 | 0.0 |

Data source: Perry et al. 2009
[a] Ratio of total zeaxanthin to total lutein
[b] NA = not applicable

The carotenoids β-cryptoxanthin and β-carotene are widely distributed in fruits and vegetables. β-cryptoxanthin is present in relatively high concentrations (up to 3500 µg per 100 g food) in many red, orange or yellow-colored fruits and vegetables such as winter squash, red peppers, pumpkins, tangerines, oranges, peaches, apricots, corn and carrots (USDA 2006). β-carotene is found in a wide variety of both yellow-orange or green fruits and vegetables. Concentrations of β-carotene in cooked sweet potatoes and cooked carrots are approximately 11500 and 8300 µg per 100 g food, respectively, and concentrations in cooked kale and cooked spinach are approximately 8200 and 6300 µg β-carotene per 100 g food, respectively.

Antheraxanthin, violaxanthin, and mutatoxanthin are found in a variety of fruits and vegetables. Concentrations of these carotenoids are summarized in Table 3-2. Spinach, broccoli, red peppers, mango, oranges and orange juice are generally the most concentrated sources of antheraxanthin (351 to 7400 µg per 100 g food) and violaxanthin (141 to 3868 µg per 100 g).

Mutatoxanthin is found primarily in orange juice (1208 to 4808 µg per 100 g) and ripe mango (333 µg per 100 g).

**Table 3-2. Concentrations of Antheraxanthin, Violaxanthin and Mutatoxanthin in Fruits and Vegetables**

| Food | Antheraxanthin | Violaxanthin | Mutatoxanthin | Reference |
|---|---|---|---|---|
| | µg carotenoid per 100 g food (fresh weight) | | | |
| **Fruits** | | | | |
| Cherry | 12 | 36 | ----[a] | Takagi 1985 |
| Mandarin, Satsuma | | 250 | ---- | Kato et al. 2004 |
| Mango (partially ripe) | 32 | 31 | 96 | Subbarayan and Cama 1970 |
| Mango (fully ripe) | 268 | 632 | 333 | Subbarayan and Cama 1970 |
| Orange, Valencia | | 1200 | ---- | Kato et al. 2004 |
| Orange juice, untreated | 162 | 445[c] | ---- | Cortes et al. 2006 |
| Orange juice, pasteurized | 141 | 351[c] | ---- | Cortes et al. 2006 |
| Orange juice, Valencia concentrated (commercial) | ---- | ---- | 4048 | Philip et al. 1988 |
| Orange juice, navel concentrated (commercial) | ---- | ---- | 1208 | Philip et al. 1988 |
| **Vegetables** | | | | |
| Asparagus, green | 40 | 79 | 8 | Deli et al. 2000 |
| Avocado, dark green flesh | ---- | 100 | ---- | Ashton et al. 2006 |
| Avocado, pale green flesh | ---- | 20 | ---- | Ashton et al. 2006 |
| Avocado, yellow flesh | ---- | 70 | ---- | Ashton et al. 2006 |
| Broccoli, raw | ---- | 1370 | ---- | Khachik et al. 1992 |
| Broccoli, steamed | ---- | 450 | ---- | Khachik et al. 1992 |
| Broccoli, microwaved | ---- | 580 | ---- | Khachik et al. 1992 |
| Cabbage | 26 | 25 | ---- | Takagi 1985 |
| Carrot | 6 | 7 | ---- | Takagi 1985 |
| Green beans, raw | ---- | 230 | ---- | Khachik et al. 1992 |
| Green beans, steamed | ---- | 80 | ---- | Khachik et al. 1992 |
| Green beans, microwaved | ---- | 90 | ---- | Khachik et al. 1992 |
| Lettuce[b] | 9 | 10 | ---- | Takagi 1985 |
| Lettuce[b] | ---- | 18 | ---- | Kimura and Rodriguez-Amaya 2003 |
| Maize | 9 | 6 | ---- | Takagi 1985 |
| Mustard leaf | 41 | 33 | ---- | Takagi 1985 |
| Paprika | ---- | 24 | ---- | Perez-Galvez et al. 2003 |
| Peppers, red | 3863 | 6893 | ---- | Minguez-Mosquera et al. 1993 |
| Peppers, green | ---- | 917 | ---- | Minguez-Mosquera et al. 1993 |
| Potato, white | 2 | 27 | ---- | Nesterenko and Sink 2003 |
| Potato, yellow | 6 | 71 | ---- | Nesterenko and Sink 2003 |
| Potato, orange | 228 | 99 | ---- | Nesterenko and Sink 2003 |
| Potato, white | 16 | 12.4 | ---- | Breithaupt and Bamedi 2002 |
| Potato, yellow | 37 | 33 | ---- | Breithaupt and Bamedi 2002 |
| Radish | 8 | 9 | ---- | Takagi 1985 |
| Soya | 9 | 5 | ---- | Takagi 1985 |
| Spinach, raw | ---- | 7400 | ---- | Khachik et al. 1992 |
| Spinach, steamed | ---- | 490 | ---- | Khachik et al. 1992 |
| Spinach, microwaved | ---- | 4800 | ---- | Khachik et al. 1992 |

a ---- = Not tested in study.
b Represents average calculated from all types included in study.

## 3.2 Dietary Intakes of Lutein and Zeaxanthin from Naturally Occurring Sources

### 3.2.1 Estimates of Intake Based on Dietary Recalls Reported in Nationwide Food Consumption Surveys

Estimates of carotenoid intakes have been generated based on dietary recall data collected in nationwide food consumption surveys and databases of carotenoid concentrations in foods. As previously described, researchers at Tufts University measured lutein and zeaxanthin concentrations in 66 foods (Perry et al. 2009) and combined these concentration data with recall data reported in the most recent nationwide food consumption survey, namely the 2009-2012 National Health and Nutrition Examination Survey (NHANES). Estimates of lutein and zeaxanthin intakes based on these two sources of concentration data are presented below.

Estimated intakes of zeaxanthin and lutein from naturally occurring dietary sources are shown in Table 3-3. These estimates are based on the lutein and zeaxanthin concentration data measured Perry et al. (2009) (Table 3-1) and food consumption data reported in the 2009-2012 NHANES. The specific foods included in the xanthophyll analyses represent key dietary sources of lutein and zeaxanthin, though not all dietary sources of the carotenoids were captured in the analyses.

Results from the analyses indicate that mean intakes of trans-lutein by males ages one year and older range from 120 ± 10 to 829 ± 94 µg/day, and mean intakes by females range from 191 ± 27 to 1226 ± 401 µg/day. Mean intakes of trans-zeaxanthin range from 41 ± 3 to 110 ± 12 µg/day and 41 ± 3 to 95 ± 23 µg/day by males and females ages one year and older, respectively.

Estimates of 2-day average intakes of α-carotene, β-carotene, β-cryptoxanthin, lutein+zeaxanthin, and lycopene by adults based on food consumption reported in NHANES 2009-2012 and the corresponding nutrient composition data files are shown in Table 3-4. Of the five types of carotenoids included in the NHANES data set, mean daily intakes of lycopene were the highest, followed by β-carotene and lutein+zeaxanthin. The estimated mean intakes of lutein+zeaxanthin by males and females 19+ y are 1.6 and 1.65 mg/day, respectively.

## 3.3 Dietary Supplement Sources

In the U.S., zeaxanthin is approved as a dietary supplement ingredient. Chrysantis Zeaxanthin Extract Concentrate is an example of an approved source of zeaxanthin in dietary supplements (FDA 2001). The recommended intake of the Chrysantis's Extract Concentrate in supplements is 12 mg per day, which provides approximately 3000 µg zeaxanthin, 320 µg lutein and 140 µg epoxides. A synthetic zeaxanthin beadlet formulation marketed under the trade name Optisharp™ is another example of zeaxanthin that is available for use as a dietary supplement ingredient (FDA 2001); the recommended daily intake is 1000 µg zeaxanthin. The dietary supplement ingredient is manufactured by DSM Nutritional Products (formerly known as Roche Vitamins, Inc.) and supplements containing this ingredient are available throughout the world.

Based on Internet searches of zeaxanthin dietary supplements, most commercially available supplemental zeaxanthin appears to be provided as part of a multivitamin, but some is provided only in combination with lutein. With the exception of a few supplements, the concentration of lutein tends to be higher than that of zeaxanthin. In the sample of products identified in these

searches, the manufacturers' suggested daily dose of zeaxanthin in supplements ranged from 50-8000 µg though NHANES data suggest most consumers receive lower amounts (Table 3-5).

| Table 3-3. 2-Day Average Dietary Intakes of Lutein and Zeaxanthin in the US | | | | | | |
|---|---|---|---|---|---|---|
| **Sex** | | *trans*-Lutein | | | *trans*-Zeaxanthin | |
| Age | | *mean ± SEM (µg/day)* | | | | |
| **Males** | | | | | | |
| 1-3 y | 120 | ± | 10 | 47 | ± | 7 |
| 4-8 y | 195 | ± | 21 | 41 | ± | 3 |
| 9-13 y | 229 | ± | 33 | 55 | ± | 5 |
| 14-18 y | 355 | ± | 50 | 64 | ± | 5 |
| 19-30 y | 484 | ± | 49 | 90 | ± | 8 |
| 31-50 y | 748 | ± | 71 | 110 | ± | 12 |
| 51-70 y | 829 | ± | 94 | 82 | ± | 5 |
| 71+ y | 541 | ± | 72 | 72 | ± | 5 |
| 19+ y | 696 | ± | 47 | 93 | ± | 5 |
| **Females** | | | | | | |
| 1-3 y | 541 | ± | 72 | 41 | ± | 3 |
| 4-8 y | 191 | ± | 27 | 59 | ± | 16 |
| 9-13 y | 223 | ± | 32 | 44 | ± | 4 |
| 14-18 y | 325 | ± | 67 | 68 | ± | 17 |
| 19-30 y | 296 | ± | 45 | 57 | ± | 3 |
| 31-50 y | 541 | ± | 79 | 63 | ± | 3 |
| 51-70 y | 748 | ± | 70 | 83 | ± | 10 |
| 71+ y | 875 | ± | 69 | 52 | ± | 5 |
| 19+ y | 740 | ± | 47 | 67 | ± | 3 |
| Pregnant/lactating; 20-59 y | 1226 | ± | 401 | 95 | ± | 23 |
| 14-45 y | 577 | ± | 47 | 63 | ± | 4 |

Source: Based on data in NHANES 2009-2012.

**Table 3-4. 2-Day Average Carotenoid Intakes by Adults and the General Population (µg/day)**

| Carotenoid | Males 19+ y (n = 4619) | | Females 19+ y (n = 4991) | | Total Population 1+ y (n=15765) | | Pregnant or Lactating Females 20 to 59 y (n =123) | | Females 14 to 45 y (n = 4991) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean | 90th percentile | mean | 90th percentile | mean | 90th percentile | mean | 90th percentile | mean | 90th percentile |
| α-carotene | 484 | 1345 | 423 | 1174 | 404 | 1147 | 540 | 1204 | 353 | 1027 |
| β-carotene | 2409 | 5614 | | 5773 | 2093 | 5162 | | 5088 | 1859 | 4701 |
| β-cryptoxanthin | 88 | 208 | | 194 | 83 | 194 | | 198 | 72 | 177 |
| lutein+zeaxanthin | 1625 | 3141 | | 3661 | 1428 | 1336 | 4105 | 2708 | 1313 | 2810 |
| lycopene | 6498 | 16462 | | 11945 | 5296 | 2888 | | 14737 | 5026 | 12942 |
| Total | 11104 | 26769 | 9160 | 22747 | 9304 | 22755 | 12582 | 23936 | 8623 | 21657 |

Data source: NHANES 2009-2012.

*(stray marginal numbers visible: 2371, 85, 1649, 4631, 123, 2369, 6444)*

**Table 3-5. 30 Day Average Intakes from Dietary Supplements by Adults and the General Population (µg/day)**

| Carotenoid | Males 19+ y | | | Females 19+ y | | | Total Population 1+ y | | | Pregnant or Lactating Women 20 to 59 y | | | Females 14 to 45 y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | mean | 90th percentile | n | mean | 90th percentile | n | mean | 90th percentile | n | mean | 90th percentile | n | mean | 90th percentile |
| lutein+zeaxanthin | 2 | 0.81 | NA | 3 | 2.00 | NA | 5 | 1.89 | NA | 0 | NA | NA | 0 | NA | NA |
| lutein | 528 | 1.19 | 1.70 | 578 | 1.72 | 1.93 | 1160 | 1.47 | 1.99 | | 1.72 | NA | 108 | 1.44 | 1.99 |
| zeaxanthin | 31 | 1.18 | 1.97 | | 1.84 | 1.99 | 48 | 1.34 | 1.98 | | NA | NA | 8 | 1.81 | 1.99 |

Data source: NHANES 2009-2012.

*(stray marginal numbers visible: 14, 3, 0)*

## 3.4    Summary of Lutein+Zeaxanthin Intakes in the US

Estimates of lutein and zeaxanthin intake based on responses to dietary recalls and food frequency questionnaires indicate that the range of usual lutein+zeaxanthin intakes in the U.S. population is wide. The median intakes of these xanthophylls by males and females 19 years and older is estimated to be approximately 1.6 mg lutein+zeaxanthin/day, which is comparable to the median intake of 1.7 mg lutein+zeaxanthin/day by older adults participating in the Eye Disease Case-Control Study (Seddon et al. 1994), and slightly lower than median intakes reported in other observational studies (Brown et al. 1999; Chasen-Taber et al. 1999; Taylor et al. 2002). Individuals who routinely consume relatively high amounts of lutein+zeaxanthin from dietary sources, as indicated by 90th percentile intakes or the top quintile of intake, ingest in the range of 3.1 to 13.4 mg lutein+zeaxanthin/day or more, or approximately 2 to 6 times more lutein+zeaxanthin than the median intake of these xanthophylls.

The current Dietary Guidelines for Americans (USDHHS/USDA 2015) specifically encourage increased consumption of fruits and vegetables. Individuals consuming a reference diet of 2000 calories per day are advised to consume 1.5 – 2.5 cups of fruit and 2 to 3.5 cups of vegetables per day, and to eat a varied diet of vegetables that includes selections of dark green vegetables, orange vegetables, starchy vegetables, legumes and other vegetables several times each week. As previously discussed, dark green and orange vegetables tend to be particularly rich sources of lutein and zeaxanthin and therefore would contribute to increased intakes of these carotenoids.

Given that lutein and zeaxanthin are found primarily in vegetables, it is reasonable to assume that individuals whose diets include the recommended number of servings of vegetables have relatively high intakes of lutein and zeaxanthin. Using food consumption data reported in NHANES III, Kruger and colleagues (2002) estimated intakes of lutein+zeaxanthin by the total U.S. population and also intakes by the subset of the Americans who met dietary recommendations for vegetable intakes. The mean intake of lutein+zeaxanthin by the American population ages two months and older was estimated to be 1.7 mg/day (IOM 2001; as cited in Kruger et al. 2002). Among the approximately 25% of individuals two years and older consuming the recommended number of vegetable servings, mean intakes of lutein+zeaxanthin were approximately 3.8 mg/day, while 90th percentile intakes of lutein+zeaxanthin were approximately 7.3 mg/day. These intakes were regarded as "prudent" intakes of the carotenoids. Intakes of lutein+zeaxanthin by adults 20 years and older who consumed the recommended number of vegetables were slightly higher than intakes by the general population, with mean and 90th percentile intakes of 4.0 and 7.8 mg lutein+zeaxanthin/day, respectively. Based on these intake data and dietary recommendations for vegetable intakes, the general population of Americans age two years and older falls short of "prudent" levels of lutein+zeaxanthin intakes by approximately 2.0 mg per day.

## 3.5    US FDA Regulatory Status of Marigold Extracts

The FDA permits the use of *Tagetes* (marigold) extract in human food products. Dried marigold petals and marigold petal concentrates obtained from xanthophyll marigolds are used as feed additives in the poultry industry to intensify the yellow color of egg yolks and broiler skin (Piccaglia et al. 1998). The carotenoids desired in poultry tissues are a function of their dietary concentration, because poultry do not have the ability to synthesize carotenoids de novo

(Balnave and Bird 1996). Table 3-6 presents a summary of the U.S. Codes of Federal Regulations (CFR) sections that address the use of marigold extract in food and feed.

| Table 3-6. US FDA Approvals for the Use of Marigold Extract | |
|---|---|
| **CFR Citation** | **Uses and Restrictions** |
| 21 CFR § 172.510<br>*Tagetes patula*<br>*Tagetes erecta*<br>*Tagetes minuta*<br>*Tagetes glandulifera* | Natural flavoring substance; must be used in the minimum quantity required to produce the intended physical effect and in accordance with Good Manufacturing Practices (GMP).<br><br>*T. erecta* may be used as oil only. |
| 21 CFR § 73.295<br>*Tagetes erecta* | Meal and extracts may be safely used in chicken feed as a color additive |

Two ingredients derived from marigold extracts have been determined to be Generally Recognized As Safe (GRAS) for use in selected foods and beverages. Cognis Corporation determined that addition of their lutein ester ingredient, which is derived from marigold flowers, is GRAS at maximum ester levels of 2000 to 6000 µg lutein ester per serving in a variety of foods; FDA reviewed the GRAS determination and had no questions regarding the conclusions made by Cognis Corporation (FDA 2003). The product contains more than 93% lutein esters and no more than 7% zeaxanthin esters. Based on the proposed uses of the product, users of the ingredient at the 90th percentile intake level may consume up to approximately 22 mg lutein esters and 1.7 mg zeaxanthin esters per day.

In 2004, Chrysantis determined that their marigold-derived crystalline lutein product is GRAS for use in selected foods and beverages at levels corresponding to 300 to 3000 µg lutein per serving. The crystalline lutein product is approximately 76% lutein and 7% zeaxanthin by weight. FDA reviewed the GRAS determination and had no questions regarding the conclusions of Chrysantis (FDA 2004).

Synthetic zeaxanthin manufactured by DSM Nutritionals and marketed under the trade name Optisharp™ also has been determined to be GRAS for use as an ingredient in ready-to-eat cereals, cereal bars, spaghetti sauces, egg substitutes, yogurt, energy bars, fruit juices and fruit drinks, energy drinks, meal replacement drinks, and soy-based beverages (NPI Center 2007). Optisharp™ is used as a source of zeaxanthin in an instant powder drink sold in Indonesia, and dry soup mix containing Optisharp™ is available in Chile (Optisharp 2007).

## 3.6   International Regulatory Status of Marigold Extracts

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) established an ADI of 1 mg/kg bw/day, for lutein derived from *Tagetes erecta* with high concentrations of total carotenoids present as esters at levels ≥ 60%. This ADI was based on the NOAEL of 200 mg/kg bw/day (the highest dose level tested) from a 90-day rat study, and an uncertainty factor of 200 due to the absence of a multigeneration reproductive toxicity study and of chronic

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toxicity/carcinogenicity studies." (EFSA 2011). The EFSA ADI is equivalent to 60 mg of lutein derived from marigolds for a 60 kg person.[3]

In addition, an ADI of 2 mg/kg bw/day for lutein and zeaxanthin combined has been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for lutein and zeaxanthin meeting the purity specifications set forth by JECFA (JECFA, 2004). The JECFA evaluation of lutein and zeaxanthin from *Tagetes erecta* stated "In view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin, the Committee decided to include zeaxanthin [and synthetic zeaxanthin] in the ADI (0 - 2mg/kg bw) for lutein, which had a stronger toxicological database, and to make this a group ADI for these two substances" (JECFA 2004, JECFA 2006).

## 3.7   GRAS Notifications for Carotenoids Since 2007

Since the 2007 GRAS determination for the Chrysantis ZEC product, several notifications have been made to FDA for a variety of carotenoids. FDA has indicated that they had no questions regarding the safety determinations (Table 3-7) of these products.

| Table 3-7. GRAS Notifications for Carotenoids Since 2007 | | | |
|---|---|---|---|
| **GRN #** | **Substance** | **Company** | **Date** |
| 550 | Meso-zeaxanthin | INNOBIO Limited | March 2015 |
| 543 | Lutein esters | INNOBIO Limited | March 2015 |
| 543 | Lutein | INNOBIO Limited | March 2015 |
| 481 | Meso-zeaxanthin | Industrial Oganica S.A. de C.V. (IOSA) | April 2014 |
| 432 | Lutein diacetate | Industrial Oganica S.A. de C.V. (IOSA) | November 2012 |
| 390 | Suspended lutein | Abbott Nutrition | January 2012 |
| 385 | Lutein | OmniActive Health Technologies Ltd. | December 2011 |
| 294 | Astaxanthin esters | Fuji Chemical Industry Co, Ltd. | January 2010 |
| 291 | Crystalline lutein | Industrial Oganica S.A. de C.V. (IOSA) | November 2009 |
| 221 | Suspended lutein | Kemin Foods, L.C. | October 2007 |

## 3.8   Intended Uses and Estimated Intakes of Zeaxanthin Extract

### 3.8.1  Intended Uses of Zeaxanthin Extract

The proposed food categories and use levels for Kemin ZeaONE™, along with the maximum amounts of zeaxanthin and combined lutein and zeaxanthin provided per serving of food in each category, are shown in Table 3-8. Note that the fortification levels are *lower* than the original 2007 levels. This is due to the higher purity of the product from the revised manufacturing process. The final Kemin ZeaONE™ products are mixed with either food-grade oils or other food-grade excipients (sucrose, modified food starch) to create standardized products that meet the zeaxanthin specification (for example, 14% or 20% oil formulations and 5% or 10% beadlet formulations) and allows users to maintain a consistent formulation.

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[3] The NOAEL for the Kemin ZeaONE™ 90-d rat study is 550 mg/kg bw/day which is 14% zeaxanthin and therefore 77 mg zeaxanthin/kg bw/day (Section 5.2.1 and Appendix 6). The NOAELs for two additional zeaxanthin/lutein 90-d oral toxicity studies in the rat were 400 mg/kg bw/d (Section 5.3).

**Table 3-8. Proposed Maximum Use Levels of Chrysantis Zeaxanthin and Kemin ZeaONE™ and Corresponding Amounts of Zeaxanthin and Combined Lutein and Zeaxanthin Per Serving (µg/serving[a])**

| Food Category | 2007 Fortification Levels | Chrysantis Zeaxanthin Extract Concentrate[b] Zeaxanthin | Lutein + Zeaxanthin | 2015 Fortification Levels | Kemin ZeaONE™[c] Zeaxanthin | Lutein + Zeaxanthin |
|---|---|---|---|---|---|---|
| Baby and toddler foods | 188 | 50 | 54 | 100 | 50 | 53 |
| Candy, hard and soft | 565 | 150 | 162 | 300 | 150 | 159 |
| Cereal, instant and regular | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, high fiber | 1130 | 300 | 324 | 600 | 300 | 318 |
| Cereal, ready-to-eat, other | 565 | 150 | 162 | 300 | 150 | 159 |
| Crackers | 1130 | 300 | 324 | 600 | 300 | 318 |
| Drinks, carbonated, fruit-flavored, or energy | 565 | 150 | 162 | 300 | 150 | 159 |
| Egg substitutes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Frozen yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |
| Fruit juices and nectars | 565 | 150 | 162 | 300 | 150 | 159 |
| Gum | 565 | 150 | 162 | 300 | 150 | 159 |
| Margarine-like spreads | 1130 | 300 | 324 | 600 | 300 | 318 |
| Meal replacement beverages and mixes | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, dry | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, fermented | 1130 | 300 | 324 | 600 | 300 | 318 |
| Milk, flavored | 565 | 150 | 162 | 300 | 150 | 159 |
| Milk, soy and imitation | 1130 | 300 | 324 | 600 | 300 | 318 |
| Salad dressing | 565 | 150 | 162 | 300 | 150 | 159 |
| Soup | 1130 | 300 | 324 | 600 | 300 | 318 |
| Tea, ready-to-drink | 565 | 150 | 162 | 300 | 150 | 159 |
| Tomato-based sauces | 565 | 150 | 162 | 300 | 150 | 159 |
| Vegetable juices | 1130 | 300 | 324 | 600 | 300 | 318 |
| Water, bottled | 1130 | 300 | 324 | 600 | 300 | 318 |
| Yogurt | 1130 | 300 | 324 | 600 | 300 | 318 |

a Serving sizes correspond to Reference Amounts Customarily Consumed (21 CFR §101.12).
b Chrysantis® Purified Zeaxanthin Extract Concentrate contains approximately 27% zeaxanthin and 2% lutein by weight.
c Kemin ZeaONE™ contains a minimum of 50% zeaxanthin and 3% lutein by weight.

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## 3.9   Estimated Intakes of Zeaxanthin and Related Carotenoids From Kemin ZeaONE™

Estimates of potential intakes of the ingredient resulting from these intended uses were calculated using food consumption data reported in the United States Department of Health and Human Service's 2009-2010 and 2011-2012 National Health and Nutrition Examination Survey (NHANES). This NHANES data set provides nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S. (CDC 2012).

During the NHANES, trained dietary interviewers collect detailed information on all foods and beverages consumed by respondents in the previous 24 hour time period (midnight to midnight). A second dietary recall is administered by telephone 3 to 10 days after the first dietary interview, but not on the same day of the week as the first interview. A total of 9,043 respondents provided complete dietary intakes for the Day 1 recall, and 8,354 of the individuals provided a complete Day 2 recall. Using the list of food codes and the NHANES dietary recall data files from individuals with two complete days of dietary recall, Ramboll Environ estimated mean and 90th percentile 2-day average intakes of the ingredient.

Potential Kemin ZeaONE™, zeaxanthin, and combined lutein and zeaxanthin intakes were calculated for subpopulations of infants (< 1 y male (M) and female (F), 1 y M+F), children (2-5 y M+F, 6-11 y M and F separately), teenagers (12-18 y M and F separately) and adults (19-49 and 50+ y M and F separately). In addition, intakes were estimated for the total population 1+ y, women who were pregnant and lactating, and females of child-bearing age 14 – 45 y. The estimates were generated using survey sample weights to adjust for differences in representation of subpopulations; results therefore are representative of the U.S. population.

Estimates of potential intake of Kemin ZeaONE™, zeaxanthin, and of combined lutein and zeaxanthin from Kemin ZeaONE™ are shown in Table 3-8. The estimated mean and 90th percentile 2-day average intakes of Kemin ZeaONE™ are 3.8 and 7.2 mg per day, respectively, by the population ages 1 year and older. The estimated mean and 90th percentile 2-day average intakes of zeaxanthin by the population ages 1 year and older are 1.9 and 3.6 mg, respectively. The estimated mean and 90th percentile 2-day average intakes of combined lutein and zeaxanthin by this population are 2.0 and 3.8 mg, respectively. Across all of the subpopulations in this analysis, the maximum estimated 2-day average 90th percentile combined lutein and zeaxanthin intake is 5.0 mg; this intake was estimated for males ages 19-49 years.

**Table 3-9. Intakes from Proposed Uses: Purified Zeaxanthin Extract Concentrate, Zeaxanthin, and Combined Lutein + Zeaxanthin**

| Population | Zeaxanthin Concentrate* (mg/day) | | | | Zeaxanthin (mg/day) | | | | Lutein + Zeaxanthin (mg/day) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ZEC 2007 | | ZeaONE™ 2015 | | ZEC 2007 | | ZeaONE™ 2015 | | ZEC 2007 | | ZeaONE™ 2015 | |
| | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile | Mean | 90th %-tile |
| Infants < 12 mo | 1.0 | 2.1 | 0.6 | 1.5 | 0.3 | 0.6 | 0.3 | 0.8 | 0.3 | 0.6 | 0.3 | 0.8 |
| Infants 12-23 mo | 2.1 | 3.6 | 2.2 | 3.7 | 0.6 | 1.0 | 1.1 | 1.9 | 0.6 | 1.0 | 1.2 | 2.0 |
| Children 2-5 y | 2.7 | 4.2 | 2.9 | 5.2 | 0.7 | 1.1 | 1.5 | 2.6 | 0.8 | 1.2 | 1.5 | 2.7 |
| Males, 6-11 y | 3.3 | 5.5 | 3.3 | 6.0 | 0.9 | 1.5 | 1.7 | 3.0 | 0.9 | 1.6 | 1.8 | 3.2 |
| Females, 6-11 y | 2.8 | 4.8 | 3.3 | 5.7 | 0.8 | 1.3 | 1.6 | 2.8 | 0.8 | 1.4 | 1.7 | 3.0 |
| Males, 12-18 y | 3.8 | 6.1 | 4.1 | 7.8 | 1.0 | 1.7 | 2.1 | 3.9 | 1.1 | 1.8 | 2.2 | 4.1 |
| Females, 12-18 y | 3.1 | 5.1 | 3.7 | 7.1 | 0.8 | 1.4 | 1.9 | 3.5 | 0.9 | 1.5 | 2.0 | 3.8 |
| Males, 19-49 y | 3.9 | 6.7 | 4.9 | 9.4 | 1.0 | 1.8 | 2.4 | 4.7 | 1.1 | 2.0 | 2.6 | 5.0 |
| Females, 19-49 y | 3.1 | 5.6 | 3.9 | 7.3 | 0.8 | 1.5 | 2.0 | 3.6 | 0.9 | 1.6 | 2.1 | 3.9 |
| Males, 50+ y | 3.0 | 5.2 | 3.4 | 6.3 | 0.8 | 1.4 | 1.7 | 3.1 | 0.9 | 1.5 | 1.8 | 3.3 |
| Females, 50+ y | 2.6 | 4.8 | 3.1 | 5.9 | 0.7 | 1.3 | 1.6 | 2.9 | 0.7 | 1.4 | 1.6 | 3.1 |
| Total population, 1+ y | 3.2 | 5.6 | 3.8 | 7.2 | 0.9 | 1.5 | 1.9 | 3.6 | 0.9 | 1.6 | 2.0 | 3.8 |
| Pregnant and Lactating Females 20-59 y | NA | NA | 4.7 | 7.8 | NA | NA | 2.3 | 3.9 | NA | NA | 2.5 | 4.1 |
| Females 14-45 y | NA | NA | 4.0 | 7.4 | NA | NA | 2.0 | 3.7 | NA | NA | 2.1 | 3.9 |

*Purified Zeaxanthin Extract Concentrate (ZEC) 2007 data are based on NHANES 2003-2004 data; ZeaONE™ 2015 data are based on NHANES 2009-2010 data.

As shown in Table 3-10, the mean 2-day average intake of lutein+zeaxanthin from all naturally occurring dietary sources by American ages one year and older is 1.4 mg/day, and the 2-day average 90th percentile intake level from dietary sources is 13.4 mg/day. Consumption of foods containing Kemin ZeaONE™ is estimated to provide 2.0 mg of additional lutein+zeaxanthin for the average consumer of these products and approximately 3.8 mg of additional lutein+zeaxanthin for a "heavy" consumer of these products. A "heavy" consumer of these products is identified as a consumer at the 90th percentile intake level (FDA 2006).

Estimates of potential combined intake of lutein and zeaxanthin from dietary sources and Kemin ZeaONE™ are shown in Table 3-10. For an individual with average intakes of lutein+zeaxanthin from current dietary sources, combined lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 3.4 to 5.2 mg per day (1.4 + [2.07 or 3.8] mg/day). For an individual with lutein+zeaxanthin intakes from current dietary sources at the 90th percentile of intake, total lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 15.4 to 17.2 mg per day (13.4 + [2.0 or 3.8] mg/day) and potentially an additional 2 mg zeaxanthin from a 10 mg Kemin ZeaONE™ dietary supplement.[4]

| Table 3-10. Potential Combined Intake of Lutein+Zeaxanthin from Dietary Sources and Kemin ZeaONE™ (mg/day) | | | | | |
|---|---|---|---|---|---|
| | Source of Lutein+Zeaxanthin (L+Z) | | | | |
| | Current Diet | | Kemin ZeaONE™ | | Total |
| Population | Intake level | L+Z | Intake level | L+Z | L+Z |
| Total population, 1+ y | Mean | 1.4 | Mean | 2.0 | 3.4 |
| | | | 90th percentile | 3.8 | 5.2 |
| | 90th percentile | 13.4 | Mean | 2.0 | 15.4 |
| | | | 90th percentile | 3.8 | 17.2 |
| Data source: NHANES 2009-2012 (Calculations made by ENVIRON) | | | | | |

It is important to note that all estimates of intake presented in Table 3-10 are likely large overestimates of actual combined lutein and zeaxanthin intakes resulting from the proposed uses in the food supply. In the calculations of estimated intakes, any reported intake of a food corresponding to one of the proposed use categories (Table 3-8) was assumed to contain added Kemin ZeaONE™. Additionally, all foods were assumed to contain the maximum proposed concentration of Kemin ZeaONE™ per serving. It is likely that consumers may in fact consume only a subset of these foods containing added Kemin ZeaONE™, and not all products may contain the maximum proposed use levels.

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[4] Due to 10 mg Kemin ZeaONE™ supplementation at 20% zeaxanthin in safflower oil (10 mg x 0.02 = 2 mg).

# 4  Intended Effect

Kemin ZeaONE™ is intended to provide an additional dietary source of zeaxanthin in a variety of foods and beverages as a nutrient supplement which is necessary for the body's nutritional and metabolic processes in accordance with 21 CFR 170.3(o)(20).

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# 5 Review of Safety Data

## 5.1 Introduction

The safety of the proposed uses of Kemin's lutein and zeaxanthin extracts derived from marigold (*Tagetes erecta*) flowers was critically evaluated and found to be GRAS by an expert panel in 2007. Since 2007, numerous GRNs have been submitted to the FDA and a number of studies addressing the safety of zeaxanthin or related carotenoids became available and have been critically evaluated. In this Chapter, the updated information is presented in sections 5.2 and 5.3, and the remaining sections are from the 2007 GRAS dossier.The overall safety summary and conclusion is in section 5.13.

## 5.2 ZeaONE™ Studies

### 5.2.1 90-Day Oral Gavage Toxicity Study of Kemin ZeaONE™

**General Information**

To evaluate the toxicity of Kemin ZeaONE™, Vanta Bioscience, as commissioned by Kemin, conducted a 90-day oral toxicity study in accordance with OECD Test Guideline 408 and Good Laboratory Practice (GLP). The detailed study report is provided in Appendix 6.

**Materials and Methods**

Male and female Sprague Dawley rats (n=40) were randomly assigned to one of four groups of 10 males and 10 females and received either the control diet or the Kemin ZeaONE™ diets for 90 days. The rats were administered ZeaONE™ mixed in safflower oil once daily by oral gavage at doses of 110, 220, or 550 mg/kg bw/day.The rats in the vehicle control group received safflower oil alone.

**Results**

None of the animals died during the course of the study. The authors report no treatment-related abnormal clinical signs at any of the doses tested in either sex, except for brownish-red discoloration of the feces in all animals treated with test item.

Neurological observations included a statistically significant decrease in total and ambulatory motor activity counts in mid-dose group males. The study authors considered this variation incidental and not related to test item treatment because no such variations were observed in the high-dose group. There were no treatment-related neurological abnormalities reported for any of the doses in either sex.

Weekly mean body weights and body weight gains of the treatment groups were comparable to the concurrent controls during the course of treatment. Food consumption of treated animals of both sexes was comparable to the control groups.

The authors report no treatment-related changes in any of the hematology parameters at the end of the treatment period and no statistically significant variations in any of the urine parameters studied in either sex of the treated groups compared to the control groups.

Absolute and relative terminal body weights and organ weights of treatment groups were comparable to the concurrent vehicle controls. The authors report statistically significant

increases in absolute and relative brain weights in females of low and mid-dose groups, respectively, when compared to control groups; however, the increases were inconsistent, not dose dependent, and had no corresponding histopathological findings; therefore, these findings were not considered to be treatment-related.

**Conclusion**

The ingestion of Kemin ZeaONE™ (14% in safflower oil) was safe at doses up to 550 mg/kg bw/days in male and female rats for 90 days. The NOAEL for ZeaONE™ under the test conditions was the highest dose tested, 550 mg/kg bw/day.

## 5.2.2  Mutagenicity Study with Kemin ZeaONE™

In a bacterial reverse mutation study conducted in accordance with OECD Test Guideline 471 and Good Laboratory Practice (GLP) guidance, Vanta Bioscience, as commissioned by Kemin, evaluated ZeaONE™ (14% zeaxanthin in safflower oil) for mutagenic activity using the histidine-requiring *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, and TA 1537 in the absence and presence of metabolic activation. Concentrations up to 5000 μg/plate of ZeaONE™ dissolved in acetone were used.

No signs of cytotoxicity were reported. Statistically significant increases in revertant colony numbers were reported in strain TA98 at 0.3125 and 5 mg/plate with metabolic activation and in TA100 at 1.25 and 5 mg/plate with no metabolic activation; however, the values were deemed to be within acceptable limits of spontaneous revertants and biologically irrelevant. The authors also reported that a statistically significant decrease in the number of revertant colonies was observed in the strain TA102 at the highest dose of ZeaONE™ with metabolic activation, and at concentrations from 0.625 to 5 mg/plate in the absence of metabolic activation. These, too, were deemed to be within acceptable limits of spontaneous revertants and biologically irrelevant. A second phase of testing modified the concentration range by a factor of 2.5 and increased the metabolic activation system (S9 fraction prepared from Aroclor 1254-induced rat liver homogenate) from 5% v/v to 10% v/v. In this second confirmatory phase, only strain TA102 at 5 mg/plate with metabolic activation demonstrated a significant change (decrease) in revertants. These were also determined to be within acceptable limits and deemed biologically irrelevant.

The positive control tests showed a significant increase in the number of revertant colonies for each of the corresponding test strains and confirmed the validity of the test conditions and the sensitivity of the test system.

**Conclusion**

Kemin's ZeaONE™ was not cytotoxic or mutagenic at concentrations up to 5 mg/plate under the conditions of this study.

## 5.2.3  Chromosomal Aberration Study with Kemin ZeaONE™

In a chromosomal aberration study conducted in accordance with OECD Test Guideline 473 and GLP guidance, Vanta Bioscience, as commissioned by Kemin, evaluated ZeaONE™ (14% zeaxanthin in safflower oil) for clastogenic activity using the blood from healthy male

volunteers in the absence and presence of metabolic activation. Concentrations up to 2500 μg of ZeaONE™/mL in culture were used.

In the peripheral blood lymphocytes of one volunteer in phase 1, there was a significant decrease in the percent mitotic index at a concentration of 1250 μg/mL in the absence of metabolic activation and at 625 and 2500 μg/mL in the presence of metabolic activation (2%). In the peripheral blood lymphocytes of one volunteer in phase 3 there was a significant decrease in the percent mitotic index at concentrations of 312.5, 625, 1250, and 2500 μg/mL in the presence of metabolic activation (4%). These values, although statistically significant, are biologically non-significant since structural aberrations observed at the respective concentrations were comparable to the vehicle control group. Otherwise, no significant changes in the percent aberrant cells and in the percent mitotic index were observed in the peripheral blood lymphocytes from either volunteer treated up to a concentration of 2500 μg test article/mL in culture both in the absence and presence of metabolic activation system when compared with concurrent negative controls. There was no evidence of concentration-dependent polyploidy and although various structural aberrations such as deletion, exchange, fragment, dicentric, chromatid break, ring and gaps were reported, these were seen in all treatment groups including positive and negative controls.

**Conclusion**

Kemin's ZeaONE™ was not clastogenic at concentrations up to 2500 μg ZeaONE™/mL in culture medium under the conditions of this study.

## 5.3 Zeaxanthin and Related Carotenoid Studies Since 2007

The following studies have been published in the scientific literature since the 2007 GRAS determination.

### 5.3.1 Human Studies with Zeaxanthin

In order to determine if adding lutein + zeaxanthin, docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA), or both to the Age-Related Eye Disease Study (AREDS) formulation decreases the risk of developing advanced age-related macular degeneration (AMD), Aronow and Chew (2014) conducted a five-year, multicenter, randomized, double-masked, placebo-controlled study from 2006-2012. Lutein (10 mg) + zeaxanthin (2 mg), DHA (350 mg) + EPA (650 mg), or both, or placebo were added to the AREDS supplement, and 4203 participants aged 50 to 85 years at high risk for progression to advanced AMD were randomly assigned to one of the four study supplements daily. The median dose of lutein + zeaxanthin was 2.6 mg/day with a range from 0.043 to 39.8 mg/day. No statistically significant reduction in progression to advanced AMD was reported in the groups receiving lutein + zeaxanthin, DHA + EPA, or both when compared with the placebo group. The authors reported that no adverse effects were observed. Due to the limitation in study design and a highly selected (not generalized) study population, the authors suggested that lutein+zeaxanthin requires further investigation for potential inclusion in the AREDS supplements.

In Bone et al. (2007), in order to determine the effectiveness of a meso-zeaxanthin supplement with lutein and zeaxanthin in raising macular pigment density in human subjects, a 120-day supplementation study was conducted in which 10 subjects (8 male and 2 female, aged 21 to

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58 years) were given gel caps that provided 20 mg/day of predominantly meso-zeaxanthin, with smaller amounts of lutein and zeaxanthin, while a second group of 9 subjects (5 male and 4 female, aged 19 to 31 years) were given gel caps containing a placebo. Reported results indicated that the macular pigment optical density (MPOD) was significantly higher in supplementation group than in placebo group. It was concluded that a supplement containing predominantly meso-zeaxanthin is generally effective at raising macular pigment density, and may be a useful addition to the defenses against AMD. Although the authors said that no bias was introduced into the results, it should be noted this study was not the standard, double blind, placebo-controlled trial in which subjects are randomly assigned to the treatment and placebo groups, and it did not run the supplementation and placebo groups concurrently. Also, the difference in the mean ages of the two groups was significant ($p<0.05$) and it was a small population (n = 19).

A double-blind, placebo-controlled study to evaluate the effects of lutein and zeaxanthin on visual processing speed and efficiency was reported by Bovier et al. (2014). For a duration of four months, healthy young subjects (18-32 years old) were provided supplementation with either placebo (n = 10), zeaxanthin only (20 mg/day; n = 29) or a mixed formulation containing 26 mg/day zeaxanthin, 8 mg/day lutein, and 190 mg/day mixed omega-3 fatty acids (n = 25). Results indicated that supplementation with zeaxanthin and the mixed formulation produced significant ($p<0.01$) increases in critical flicker fusion thresholds (about 12%) and visual motor reaction time (about 10%) compared to placebo. The authors concluded that supplementation with lutein and zeaxanthin resulted in significant improvements in visual processing speed even in young healthy subjects. No adverse health effects were reported for any subject in this study.

A randomized, placebo-controlled study to evaluate the effects of lutein and zeaxanthin on the visual processing speed was reported by Bovier and Hammond (2015). For a duration of four months, healthy young subjects (18-32 years old) were provided supplementation with either placebo (n = 15), zeaxanthin only (20 mg/day; n = 29) or a mixed formulation containing 26 mg/day zeaxanthin, 8 mg/day lutein, and 190 mg/day mixed omega-3 fatty acids (n = 25). It is not clear if this is the same population as Bovier et al. (2014). Results indicated that supplementation with zeaxanthin and the mixed formulation produced significant ($p<0.05$) increases in temporal contrast sensitivity function (about 20%) and MPOD (about 20%) compared to placebo. The authors concluded that supplementation with lutein and zeaxanthin can increase visual processing speed even in young healthy subjects. No adverse health effects were reported for any subject in this study.

A double-blind, randomized, placebo-controlled study to evaluate the effects of daily supplementation with a proprietary milk-based formulation of goji berry (containing zeaxanthin) on macular characteristics and plasma ZEA and antioxidant capacity levels, was reported by Bucheli et al. (2011). Healthy elderly subjects (65 to 70 years old) received either 13.7 g/day of goji berry containing 10 mg/day zeaxanthin (n = 75) or placebo (n = 75) for 90 days. The placebo group demonstrated hypopigmentation and soft drusen accumulation in the macula, whereas the goji berry group remained stable. Both plasma zeaxanthin level and antioxidant capacity increased significantly in the goji berry group, by 26% and 57%, respectively, but did not change in the placebo group. No adverse events were reported in the placebo group. One adverse event, one subject with vomiting and fever of 6-day duration, was reported in the goji

berry group but this event was classified as mild and unrelated to the study product by the principal investigator. The study has several limitations including a narrow age range of study subjects and the fact that the optical density of the preretinal pigment was not directly assessed. No significant relationship was reported between change in plasma zeaxanthin level and change in macular characteristics to support the effect of zeaxanthin on macula.

A randomized, placebo-controlled, clinical trial to investigate serum and macular response to, and safety of supplementation with, meso-zeaxanthin, lutein, and zeaxanthin was reported by Connolly et al. (2011). Forty-four (44) healthy subjects consumed one tablet per day containing 10.6 mg meso-zeaxanthin, 5.9 mg lutein, and 1.2 mg zeaxanthin or a placebo for six months. Subjects were assessed at baseline and at three and six months. Clinical pathology was performed at baseline and six months and tested for changes in renal and liver function, lipid profile, hematologic profile, and markers of inflammation. Results indicated that subjects supplemented with meso-zeaxanthin, lutein, and zeaxanthin exhibited significant increases in serum concentrations of these carotenoids and a subsequent increase in central MPOD. No adverse effects from consuming these carotenoids were reported.

Graydon et al. (2012) conducted two intervention studies to compare the effect of lutein- and zeaxanthin-rich foods and supplements on macular pigment level and serological markers of endothelial activation, inflammation and oxidation in healthy volunteers. Study 1 subjects were randomized and received either 131 mL/day of carrot juice (15 mg/day of β-carotene, n=25) or 10.4 g/day of spinach powder (15 mg/day lutein and zeaxanthin, n=27) for 8 weeks. Study 2 subjects received supplements containing 10 mg/day lutein and 5 mg/day zeaxanthin (n=25), 15 mg/day β –carotene (n=25), or placebo (n=25) for 8 weeks in a randomized, double-blind, placebo-controlled trial. The authors reported that results of both studies indicated that supplementation with lutein and zeaxanthin, whether as foods or as supplements, had no significant effect on macular pigment level or serological markers of endothelial activation, inflammation and oxidation, but "may improve macular pigment level in the highest serum responders and in those with initially low macular pigment level". No adverse health effects were reported for any subject in both studies. The authors acknowledged that these were pilot studies, and that further investigations are required with better study design in terms of doses and duration of supplementation, sensitivity of measurement, and study subjects.

A double-blind, randomized, placebo-controlled study to evaluate the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast was reported by Hammond et al. (2014). For one year, 56 healthy subjects (18-30 years old) were provided daily supplementation with placebo and 53 healthy subjects (18-40 years old) were provided daily supplementation with 2 mg/day zeaxanthin and 10 mg/day lutein. Significant increases in macular pigment optical density, serum lutein and zeaxanthin levels and improvements in chromatic contrast and photostress recovery time were reported in the group supplemented with lutein and zeaxanthin when compared to the placebo group. Glare disability was correlated with macular pigment optical density but did not increase significantly in the group supplemented with lutein and zeaxanthin. The authors concluded that supplementation with lutein and zeaxanthin leads to improved visual performance. Twenty adverse events occurred during the entire study period though the authors note that none of the adverse events were directly attributable to the study product. The adverse events were not described.

In a randomized, placebo-controlled study to examine the effect of oral supplementation of omega-3 long-chain polyunsaturated fatty acids on changes in serum levels of lutein/zeaxanthin, 40 subjects (64-86 years old) with or without AMD received daily dose of 10 mg lutein and 2 mg zeaxanthin and either omega-3 long-chain polyunsaturated fatty acids (350 mg docosahexaenoic acid and 650 mg eicosapentaenoic acid, n=20) or placebo (n=20) for 6 months (Huang et al. 2008). Results indicated that serum levels of lutein/zeaxanthin increased compared with baseline, but did not differ by omega-3 long-chain polyunsaturated fatty acids. Subjects with AMD had a lower increase in serum lutein concentration than did those without AMD. No serious adverse effects resulting from study products were reported.

A randomized, double-blinded, placebo-controlled trial to examine serum and macular responses to supplemental lutein and zeaxanthin in 108 Chinese patients (50-81 years old) with early was reported by Huang et al. (2013). Twenty-seven patients per group were provided with daily dose of 10 mg/day lutein, 20 mg/day lutein, 10 mg/day zeaxanthin plus 10 mg/day lutein, or placebo for 48 weeks. Results indicated that supplementation with lutein and/or zeaxanthin significantly increased serum concentrations and macular pigment optical density. No adverse health effects were reported for any subject in this study and no subject developed or reported carotenodermia (occasional skin pigmentation). The authors indicated, however, that the lack of significant side effects of supplementation need to be confirmed in larger populations, and specifically in elderly patients with kidney and liver diseases.

A randomized, double-blind, placebo-controlled trial to evaluate the effect of supplemental lutein and zeaxanthin on serum, macular pigmentation, and visual performance in patients with early age-related macular degeneration was reported by Huang et al. (2014, 2015). A total of 112 patients (50+ years old) with early age-related macular degeneration were randomly assigned to receive 10 mg lutein, 20 mg lutein, lutein (10 mg) plus zeaxanthin (10 mg), or a placebo daily for two years. Results indicated that serum lutein concentration, MPOD, and visual sensitivity significantly increased in all the treated groups. No adverse health effects were reported for any subject in this study. Limitations of this study include highly selective criteria for study subjects, no zeaxanthin control group and no discussion about the effects of zeaxanthin and meso-zeaxanthin and their interactions with lutein supplementation, measurement sensitivity.

A randomized, double-blind, placebo-controlled trial to evaluate the effect of supplemental lutein and zeaxanthin on macular pigment and visual function in patients with early AMD was reported by Ma et al. (2012a, b). A total of 108 patients (50-79 years old) with early age-related macular degeneration were randomly assigned (n=27 per group) to receive 10 mg lutein, 20 mg lutein, lutein (10 mg) plus zeaxanthin (10 mg), or placebo daily for 48 weeks. Thirty-six age-matched controls without AMD were also enrolled to compare baseline data with patients with early AMD. Significant increase in N1P1 response densities and macular pigment optical density and improvement in best-corrected visual acuity and contrast sensitivity were observed in all the treated groups. No adverse health effects were reported for any subject in this study. Limitations of this study include single-site study design and the influence of dietary intake fluctuation.

A randomized, double-blind, placebo-controlled trial with 31 patients with Alzheimer's disease (mean age of 80 years old) and 31 control subjects (mean age of 76 years old) to investigate

the impact of supplemental macular carotenoids on macular pigment, vision, and cognitive function in patients with Alzheimer's disease was reported by Nolan et al. (2015). The 31 patients were supplemented for six months with either Macushield (n=16, 10 mg meso-zeaxanthin, 10 mg lutein, 2 mg zeaxanthin) or placebo (n=15, sunflower oil). The 31 control subjects were similarly allocated to Macushield (n=15) or placebo (n=16). Significant increases in serum concentrations of lutein, zeaxanthin, meso-zeaxanthin, macular pigment, and contrast sensitivity were observed in the groups with supplement for both patients with Alzheimer's disease and control subjects. No significant changes were reported in any of the cognitive function outcome variables measured. The authors concluded that supplementation with the macular carotenoids provided "clinically meaningful improvements in visual function." No adverse health effects were reported for any subject in this study.

A randomized, double-blind, placebo-controlled study to evaluate whether dietary supplementation with zeaxanthin raises MPOD and improves driving ability for patients with AMD was reported by Richer et al. (2011, 2012). A total of 60 patients (57 males and three females, mean age at 74.9 years old) with mild to moderate AMD were randomly assigned to one of two dietary supplement carotenoid pigment intervention groups for one year: 8 mg zeaxanthin (n=25) and 8 mg zeaxanthin plus 9 mg lutein (n=25) or 9 mg lutein (control group, n=10). Results indicated that zeaxanthin-induced foveal MPOD elevation mirrored that of lutein and provided complementary distinct visual benefits by improving foveal cone-based visual parameters. No adverse health effects were reported for any subject in this study.

Two randomized, double-blind, placebo-controlled studies to investigate macular pigment optical density responses to supplementation with lutein and zeaxanthin were reported by Schalch et al. (2007). In the first study, 92 subjects (male, 18-45 years old) were randomized to receive one of the following four supplementations for six months: 10.7 mg/day lutein, 12.6 mg/day zeaxanthin, 11.9 mg/day zeaxanthin plus 10.2 mg/day lutein, or placebo. Then, 20 subjects from the first study joined the second study, and 10 additional subjects were recruited as a new placebo group. These subjects received one of the following four supplementations for another six months: 21.4 mg/day lutein (n=3), 25.2 mg/day zeaxanthin (n=6), 11.9 mg/day zeaxanthin plus 10.2 mg/day lutein (n=11), or placebo (n=10). Results for both studies indicated that macular pigment optical density and plasma concentrations increased with supplementation with lutein and zeaxanthin. The authors found that "lutein is predominantly deposited in the fovea while zeaxanthin deposition appears to cover a wider retinal area." No adverse health effects were reported for any subject in both studies.

Stringham and Hammond (2008) reported a study conducted to evaluate the effect of supplementation with lutein and zeaxanthin on macular pigment and improvements in glare disability and photostress recovery. Forty healthy subjects (17 male and 23 female, mean age at 23.9 years old) received a daily dose of 10 mg lutein and 2 mg zeaxanthin for six months. Results indicated that supplementation with lutein and zeaxanthin significantly increased MPOD and reduced the deleterious effects of glare when compared to the baseline measurement for the same subjects. No adverse health effects were reported for any subject in this study. This study was not placebo-controlled, and no statistical analysis was provided.

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Tanito et al. (2012) reported a randomized, double-blind study with 22 healthy subjects (10 male and 12 female, 23-58 years old) conducted to determine whether either lutein or zeaxanthin supplementation affects macular pigment concentration/optical density in healthy Japanese individuals. Subjects were randomized to receive either 10 mg of orally administered lutein (n = 11) or zeaxanthin (n = 11) daily for up to 3 months. Results indicated that in normal healthy Japanese individuals without high myopia, lutein supplementation increased MPOD levels within the fovea more effectively than zeaxanthin. No adverse health effects were reported for any subject in this study. This study was not placebo-controlled.

Van de Kraats et al. (2008) reported a study conducted to measure the baseline optical densities of lutein or zeaxanthin in 23 subjects (5 male and 18 female, mean age of 24.3 years old). Three other male subjects took 20 mg/day zeaxanthin for 6 months, and their optical densities of lutein or zeaxanthin were measured approximately monthly for 18 months. Results indicated that zeaxanthin supplementation caused a significant increase in the optical densities of zeaxanthin, and no or minor changes in the optical densities of lutein. No adverse health effects were reported for any subject in this study. This study was not placebo-controlled.

## 5.3.2  Human Studies with Other Carotenoids

Capeding et al. (2010) reported the effect of lutein-fortification on the growth of infants through a 16-week prospective, randomized, controlled, and double-blind study with parallel groups of healthy term infants fed either control formula (Wyeth S-26 Gold, designated as Gold) or experimental formula (Wyeth S-26 Gold fortified with lutein at 200 mg/L, designated as Gold + Lutein). Two hundred thirty-two (232) infants ≤ 14 days postnatal age were randomized and 220 (94.8%) completed the study. Weight, head circumference, and length were measured at weeks 4, 8, 12, and 16, and clinical chemistries were performed at Week 16. The authors reported no differences in growth or clinical chemistries between the two groups and all parameters evaluated were within normal ranges. The authors concluded that infants fed lutein-fortified S-26 Gold demonstrated growth equivalent to that of infants fed unfortified lutein formula.

## 5.3.3  Animal Studies with Zeaxanthin

Ravi et al. (2014) reported on potential adverse effects of OmniXan™, a RR-zeaxanthin (65%) enriched product obtained from paprika (*Capsicum annum*) fruits in acute, subchronic toxicity and mutagenicity studies. Four female Wistar rats were orally (gavage) administered a single dose of zeaxanthin concentrate at 2000 mg/kg bw and observed for 14 days in the acute toxicity study conducted according to OECD Test Guideline 420. The oral $LD_{50}$ was determined to be greater than 2000 mg/kg bw/day.

Wistar rats (10/sex/group) were gavaged daily with OmniXan™ at doses of 0, 4, 40 or 400 mg/kg bw/day for 90-days in a subchronic toxicity study conducted according to OECD Test Guideline 408. There were no consistent, statistically significant, dose-dependent adverse effects reported in any parameter evaluated and there were no deaths. The NOAEL was determined to be 400 mg/kg bw/day, the highest dose tested.

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The mutagenicity assay was conducted using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without metabolic activation according to OECD Guidelines 471. No mutagenicity was reported.

Ravikrishnan et al. (2011) reported on the potential adverse effects of lutein and zeaxanthin (Lutemax 2020™ obtained from *Tagetes erecta L.*) in acute, subchronic toxicity and mutagenicity studies. Three female Wistar rats were orally (gavage) administered a single dose of lutein/zeaxanthin concentrate at 2000 mg/kg bw and observed for 14 days in the acute toxicity study conducted according to OECD Test Guideline 423. The oral $LD_{50}$ was determined to be greater than 2000 mg/kg bw/day.

Wistar rats (10/sex/group) were gavaged daily with lutein/zeaxanthin concentrate (OmniXan™) at doses of 0, 4, 40 or 400 mg/kg bw/day for 90-days in the subchronic toxicity study conducted according to OECD Test Guideline 408. No treatment related clinical signs and mortalities were reported, and the NOAEL was determined to be 400 mg/kg bw/day, the highest dose tested.

The mutagenicity assay was conducted using strains *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with metabolic and without activation according to OECD Guidelines 471. No mutagenicity was reported.

Thurnham and Howard (2013) reported on the potential toxicity and genotoxicity of meso-zeaxanthin. Han Wistar rats (10/sex/group) were gavaged daily with meso-zeaxanthin at doses of 0, 2, 20 or 200 mg/kg bw/day for 13 weeks. No treatment related clinical signs and mortalities were reported, and the NOAEL was determined to be 200 mg/kg bw/day, the highest dose tested.

The mutagenicity assay was conducted using the Ames test method with strains of *S. typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 and *E. coli* tryptophan auxotroph WP2uvrA with and without microsomal enzymes. No genotoxicity was reported.

### 5.3.4  Animal Studies with Other Carotenoids

Nidhi and Baskaran (2013) reported on acute and subacute toxicity tests in lutein-deficient male mice to investigate potential adverse effects of lutein from *Tagetes erecta*. In the acute test, male albino mice with lutein-deficiency (10/group) were orally administered single doses of lutein at 0, 0.57 (physiological dose), 100, 1000, or 10,000 mg/kg bw and observed for 14 days. In the subacute test, male albino mice with lutein-deficiency (10/group) were orally administered daily doses of lutein at 0, 100, or 1000 mg/kg bw for four weeks. Results indicated that plasma lutein levels increased dose-dependently (p < 0.01) after acute and subacute feeding of lutein in mice. No treatment-related toxicologically significant adverse effects were reported. The oral $LD_{50}$ was determined to be greater than 10,000 mg/kg bw/day. The NOAEL in the subacute study was determined to be 1000 mg/kg bw/day, the highest dose tested. The authors concluded that currently recommended doses of lutein as a dietary supplement (250 µg to 20 mg/person/day) are approximately 4000-fold lower than the NOAEL.

Beppu et al (2009) reported on the safety evaluation of fucoxanthin including single and repeated oral dose toxicity testing. In the single dose study, male and female ICR mice (10/sex/group) were orally administered doses of fucoxanthin at 0, 1000, or 2000 mg/kg and

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observed for 14 days. In the repeated doses study, male and female ICR mice (10/sex/group) were orally administered daily doses of fucoxanthin at 0, 500, or 1000 mg/kg for 30 days. In both studies, no mortalities were reported. The authors report that significantly increased total cholesterol concentrations were found in all fucoxanthin-treated groups. The authors stated that this may due to the interference from a major metabolite of fucoxanthin, and to further ascertain the safety of fucoxanthin, the mechanism by which fucoxanthin induces hypercholesterolemia in mice should be elucidated.

A toxicological evaluation of carotenoids from the red yeast *Rhodotorula glutinis* was reported by Latha and Jeevaratanm (2012). Experiments were conducted with Wistar rats (8/sex/group) fed with repeated doses via gavage of red yeast pigment at 0, 100, or 300 mg/kg bw/d for 13 weeks. No major histological changes were reported among different groups. The authors concluded that the extract from red yeast pigment may be used safely in food preparations as a food colorant with an added benefit of antioxidant activity.

### 5.3.5  In Vitro Studies

Chiste et al. (2014) evaluated the potential of several carotenoids (including β-Carotene, zeaxanthin, lutein, β-cryptoxanthin, and lycopene) to inhibit hemolysis of human erythrocytes, as mediated by the toxicity of peroxyl radicals through an optimized *in vitro* cellular antioxidant assay. Six tested carotenoid concentrations ranged from 0.1 to 3 µM, and the study duration was three hours. Reported results indicated that lycopene ($IC_{50}$ = 0.24 ± 0.05 µM) was the most efficient at preventing the hemolysis, followed by β-carotene (0.32 ± 0.02 µM), lutein (0.38 ± 0.02 µM), and zeaxanthin (0.43 ± 0.02 µM). β-Cryptoxanthin did not present any erythroprotective effect, but rather induced a hemolytic effect at the highest tested concentration (3 µM). The authors concluded that selected carotenoids may have the potential to act as important erythroprotective agents by preventing peroxyl radical-induced toxicity in human erythrocytes.

### 5.3.6  Summary of Recent Studies

A critical evaluation of the studies published regarding zeaxanthin, lutein and related carotenoids demonstrate that no adverse effects of zeaxanthin or lutein have been reported.

### 5.4   2007 GRAS Dossier Review of the Safety Data

The safety determinations of the extracts were based on reviews of published studies conducted in humans and animals regarding the absorption, distribution, metabolism, excretion, bioavailability, toxicity and mutagenicity of both the lutein and lutein ester products, and their primary carotenoid components, lutein and zeaxanthin (unesterified and esterified forms). No toxic or adverse effects from the consumption of the predominantly lutein products or lutein and zeaxanthin from other sources were identified, and consumption of the xanthophylls was reported to be well tolerated. The only adverse effect noted in the safety reviews was the occurrence of carotenodermia in subjects consuming 15 or 30 mg/person/day of a mixed lutein esters product. Carotenodermia is a reversible condition considered to be harmless that is characterized by a yellowish discoloration of the skin (IOM 2000).

In the evaluation of the safety of ingestion of ZEC under its intended conditions of use, ENVIRON reviewed information relevant to the safety of ingestion of zeaxanthin, other

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components identified in the product, and safety of ingestion of the whole product. Given that the safety of lutein extracts from marigold (in combination with zeaxanthin) as a dietary ingredient has been established, this review focused specifically on studies addressing the safety of consumption of zeaxanthin.

Information pertaining to the safety of ingestion of zeaxanthin was identified in searches using its CAS Number, 144-68-3, in conjunction with search terms including safety, toxicity, absorption, metabolism and excretion. The searches included the Toxicology and Medicine categories within the DIALOG® database. Potentially relevant articles were identified and reviewed. The review also included some human studies in which combinations of lutein+zeaxanthin were investigated if the amount of zeaxanthin could be identified. The safety review was corroborated by critical review of toxicity studies conducted by Chrysantis on the ZEC, and publicly available toxicity studies on other sources of purified zeaxanthin extracts.

## 5.5   Absorption, Transport, Metabolism and Excretion of Zeaxanthin

### 5.5.1   Absorption and Transport

Approximately 600 carotenoids have been identified, with approximately 60 found in the human diet (Yonekura and Nagao 2007). Not all of these carotenoids are efficiently absorbed by humans, only a subset of dietary carotenoids has been found in human blood or tissues (Boileau et al. 1999; Yonekura and Nagao 2007; Rao and Rao 2007).

Carotenoids are fat-soluble compounds and consequently are absorbed like dietary fat. After consumption of carotenoid-containing foods, carotenoids are released from their food matrix and incorporated into mixed micelles, which consist of bile acids, free fatty acids, monoglycerides, and phospholipids. The amount of carotenoid incorporated into micelles depends on the polarity of the carotenoid and on micellar fatty acid composition and saturation. Carotenoids appear to be absorbed by the mucosa of the small intestine, mainly in the duodenum, via passive diffusion. They then are packaged into triacylglycerol-rich chylomicrons and secreted into lymph for delivery to the blood stream, where the chylomicrons are degraded by lipoprotein lipase. The resulting chylomicron remnants containing carotenoids are rapidly taken up by the liver. The liver secretes carotenoids associated with hepatic very low density lipoprotein (VLDL), but in the fasting state most plasma carotenoids are associated with low density lipoproteins (LDL) and high density lipoproteins (HDL). In fasting blood up to 75% of hydrocarbon carotenoids such as β-carotene and lycopene are found in LDL, and the remaining carotenoids are associated with HDL and to a lesser degree with VLDL. The more polar carotenoids such as lutein and zeaxanthin are more evenly distributed between LDL and HDL fractions in fasting blood, with greater amounts in HDL (Chopra et al. 2000; Cardinault et al. 2005; Wang et al. 2007).
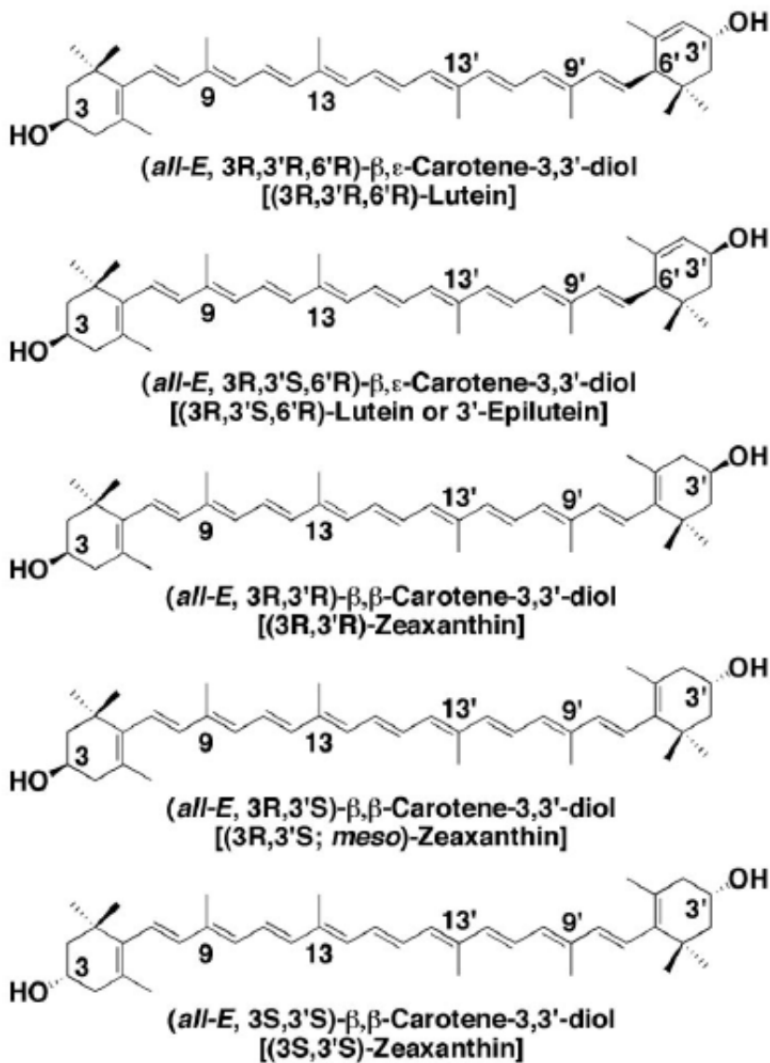
## 5.6   Metabolism and Excretion

Ingestion of zeaxanthin or lutein typically results in increases in blood concentrations of the ingested carotenoid and increased concentrations of their respective metabolites. In metabolic reactions of non pro-vitamin A carotenoids such as zeaxanthin and lutein, the general skeleton of the polyene chain of the carotenoids remains intact. Chemical transformations of the carotenoid end-group, however, can result in the formation of many metabolites. These metabolites are likely excreted in the urine as polar conjugates (Olson 1994).

The metabolism of lutein and zeaxanthin has been detailed in Khachik et al. (1995, 1997, 1999, 2002, 2006b). Metabolites of zeaxanthin and lutein can result from four types of reactions involving the end-groups of these carotenoids. These reactions include allylic oxidation at C-3, reduction with epimerization at C-3, reversible double-bind migration, and acid-catalyzed dehydration. All of these transformations occur through a series of oxidation-reduction and double-bond isomerization reactions. Potential pathways for the transformation of zeaxanthin and lutein into their respective metabolites are presented in Figure 10. Figures 11 and 12 present the chemical structures of the compounds identified in Figure 10.

The formation of lutein and zeaxanthin metabolites is accompanied by one or a combination of reactions. For example, dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin (also known as all-*trans*-lutein and all-*trans*-zeaxanthin) can exist in an equilibrium involving an intermediate carotenoid known as *3'*-epilutein. *3'*-Epilutein and zeaxanthin are believed to exist in equilibrium through reversible double bond migration. The presence of *3'*-epilutein in human serum/plasma therefore may be due to metabolic conversion of both lutein and/or zeaxanthin to this compound.

Khachik and colleagues (2002) have found the stereochemistry of carotenoids in human ocular tissue to differ from that found in human plasma and liver. Through high-performance liquid chromatography (HPLC) analysis, (*3R,3'R,6'R*)-lutein, (*3R,3'R*)-zeaxanthin, (*3R,3'S; meso*)-zeaxanthin, (*3R,3'S,6'*)-lutein (*3'*-epilutein), 3-hydroxy-β,ε-caroten-3'-one, and 5*Z*- and *all-E*-lycopene were detected in nearly all ocular tissue examined. However, *meso*-zeaxanthin was not detected in human plasma or liver. Given the high levels of dietary lutein and zeaxanthin present in the liver, it was speculated that *meso*-zeaxanthin was formed in the ocular tissue and not formed elsewhere and transported to this tissue (Khachik et al. 2002). Results from more recent research in monkeys provide additional evidence to indicate that conversion of lutein to *meso*-zeaxanthin occurs exclusively in the retina (Johnson et al. 2005).

**Figure 10. Chemical Structures of Zeaxanthin and Lutein and Their Non-Dietary Stereoisomers**



Source: Khachik et al. 2006a.

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**Figure 11. Proposed Metabolic Pathways for Transformation of Zeaxanthin and Lutein into Their Respective Metabolites in Humans**
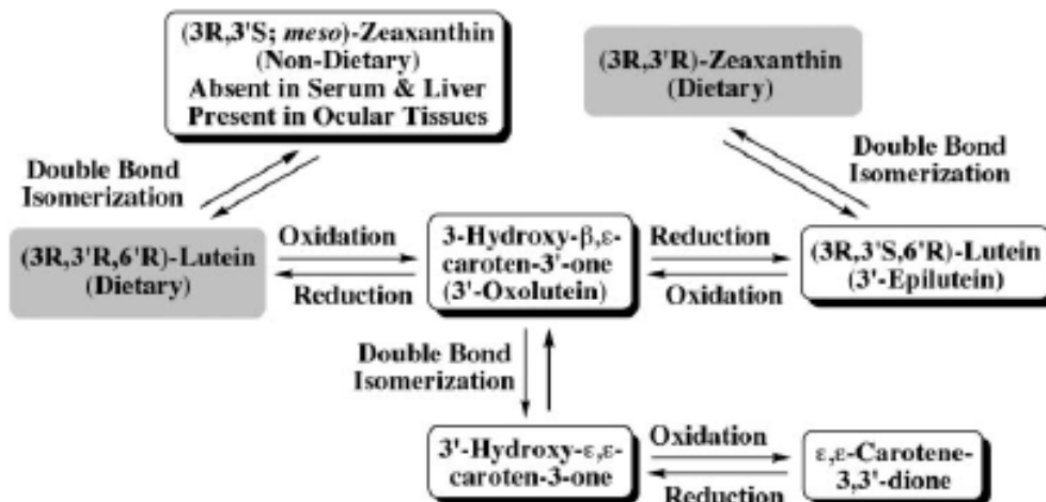


Source: Khachik et al. 2006a.

**Figure 12. Chemical Structures of the Oxidative Metabolites of Zeaxanthin and Lutein**
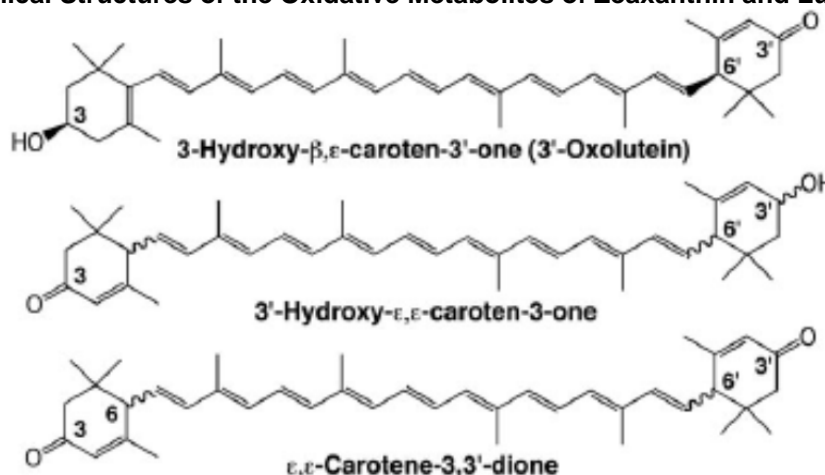


Source: Khachik et al. 2006a.

## 5.7 Plasma Response and Tissue Concentrations Following Ingestion of Zeaxanthin and Lutein

Studies in which the plasma and tissue responses to zeaxanthin and lutein intake have been monitored in humans and animals provide information on the fate of these carotenoids and their metabolites following ingestion. Results from these studies indicate that ingestion of pure lutein or pure zeaxanthin appears to affect plasma levels only of the ingested carotenoid and its metabolites. Intake of zeaxanthin does not appear to affect plasma concentrations of other dietary carotenoids.

The macula lutea is located in the center of the retina and functions to maintain acute central vision. Macular pigment absorbs blue-light prior to its reaching the underlying the underlying photoreceptor cell layer, protecting it from light and presumably oxidative damage (Krinsky et al.

2003). Macular pigment is composed solely of lutein and zeaxanthin. Zeaxanthin (in the *trans*-form) is the predominant carotenoid in the retina, particularly in the central region, with the Z:L ratio decreasing further out into the peripheral region of the retina. Increased ingestion of foods rich in lutein and zeaxanthin or ingestion of lutein or zeaxanthin supplements has been reported to increase macular pigment density.

Results from intervention and observational studies in humans and studies in animals regarding plasma and tissue responses are reviewed below.

### 5.7.1  Humans

### 5.7.1.1  Intervention Studies

Studies in which the effects of consumption of zeaxanthin (alone or in combination with lutein) on plasma and tissue levels of carotenoids were assessed were reviewed and are summarized in Table 5-1. The plasma lutein and zeaxanthin levels ranged from approximately 0.130 to 0.428 µmol/L and 0.032 to 0.101 µmol/L, respectively, at baseline. The ratio of plasma lutein to zeaxanthin ranged from 2.8 to 8.8 µmol/L in these studies, with a mean (unweighted) average of 4.7.

The ranges of lutein and zeaxanthin doses reported in studies summarized in Table 5-1 are 0 up to 30 mg/day and 0.1 up to 30 mg/day, respectively. Following the periods of supplementation, plasma lutein and zeaxanthin levels ranged from 0.164 to 5.167 µmol/L and 0.057 to 0.92 µmol/L, respectively. In many of these studies, dietary intervention with foods high in lutein and zeaxanthin or consumption of supplements containing lutein or zeaxanthin resulted in significantly increased plasma lutein and zeaxanthin levels. Increases in macular pigment optical density were also observed in several studies. Findings from some of the studies summarized in Table 5-1 are reviewed in more detail below.

Khachik and colleagues (1995) investigated plasma responses in humans consuming 10 mg of purified zeaxanthin for 21 days or 10 mg lutein for 18 days. During the zeaxanthin period of administration, plasma levels of the carotenoid increased from slightly less than 2 µg/dL to approximately 6 to 8 µg/dL after one week of supplementation. In addition, plasma levels of *3′*-epilutein and the oxidation products of these products (ketocarotenoids) increased significantly. After one week of lutein supplementation, blood levels of lutein increased from approximately 10 to 20 µg/dL to approximately 40 to 50 µg/dL (mean serum lutein concentration increased from 280 to 1400 nM). Increases in blood concentrations of zeaxanthin and lutein oxidation products, i.e., monoketocarotenoids and diketocarotenoids, also were observed. The plasma concentration of *3′*-epilutein did not increase significantly during lutein supplementation. Results from this study demonstrate that *in vivo* oxidation of lutein and zeaxanthin is a key reaction in the metabolism of these carotenoids.

The plasma kinetics of zeaxanthin were also investigated by Hartmann and colleagues (2004). In this study, 10 healthy adults consumed 1 mg zeaxanthin daily and another 10 healthy adults consumed 10 mg zeaxanthin daily. The synthetic zeaxanthin beadlet formulation, which contained 80% *all*-E, 0.4% 9-Z, 17.5% 13-Z, and 2.2% 15-Z zeaxanthin, was incorporated into hard gelatin capsules. Following a 3-day run-in period, the zeaxanthin capsules were consumed once daily for a period of 42 days. Blood samples were drawn in the morning on three

consecutive days during the baseline period. On days 1 and 42, after the subjects had fasted overnight, blood specimens were drawn with an indwelling canula before and 2, 4, 6, 8, 12, 15 and 24 hours after zeaxanthin administration. Additional fasting blood samples were collected during the dosing period and on days 7, 14, 21, 28, 35, 38, 39, 40 and 41 (dosing period) and on days 44, 48, 53, 58, 64, 70 and 76 (post-dosing period). Plasma samples were analyzed for *all*-E-zeaxanthin and for the sum of 3-Z-zeaxanthin isomers (13-*Z*,-, 9-*Z*- and 15-*Z*-zeaxanthin), for *E*- and *Z*-isomers of lutein, α-carotene, β-carotene, lycopene and β-cryptoxanthin, and for *all-E*-3'-dehydro-lutein, retinol, α-tocopherol and lipids.

Hartman et al. (2004) reported that "*all-E*-zeaxanthin concentrations in plasma at baseline and on day 1 accounted for 95% of the total zeaxanthin concentrations but decreased to 86% on day 42. The remaining concentrations were determined as the sum of *3-Z*-zeaxanthin isomers (typically 85% *13-Z*-zeaxanthin in addition to minor amounts of *9-Z*-zeaxanthin and *15-Z*-zeaxanthin)." Plasma *all-E*-zeaxanthin concentrations in subjects consuming 1 mg zeaxanthin per day rose from 0.051 ± 0.034 µmol/L to 0.20 ± 0.07 µmol/L, while plasma concentrations of *all-E*-zeaxanthin increased from 0.045 ± 0.016 µmol/L to 0.92 ± 0.28 µmol/L. The dose-normalized bioavailability of *all-E*-zeaxanthin after the 10 mg dose was 40% lower than after the 1 mg dose. Because relevant disposition parameters such as the accumulation factor, time to reach ≥90% of steady state concentrations and terminal elimination half-life (R, $t_{ss}$, and $t_{1/2}$, respectively) did not significantly differ from each other, the observed nonlinearity was not related to dose-dependent disposition kinetics for *all-E*-zeaxanthin. The investigators speculated that intestinal absorption of zeaxanthin may decrease with increasing doses.

For both dose groups, maximum plasma concentrations of *all-E*-zeaxanthin were reached on average at a $t_{max}$ of 10-12 hours after dosing. After 17 days of dosing, >90% of steady state concentrations were reached, which was compatible with an effective half-life for accumulation of 5 days. The terminal elimination half-life for the two groups was 12 ± 7 days.

Plasma levels of *all-E*-3'-dehydro-lutein were observed to increase in parallel with levels of *all-E*-zeaxanthin, indicating that the increase in *all-E*-3'-dehydro-lutein was related to *all*-E-zeaxanthin dosing. *all-E*-3'-Dehydro-lutein was previously identified in human plasma (Khachik et al. 1992; as cited in Hartmann et al. 2004), and formation of the compound was assumed to be the result of lutein oxidation. Based on the current study, the investigators speculated that under normal dietary conditions, *all-E*-3'-dehydro-lutein is predominantly formed from other dietary sources, most likely from lutein, rather than from dietary zeaxanthin. Plasma lutein, β-carotene, lycopene, and β-cryptoxanthin concentrations were unaffected by zeaxanthin dosing. Zeaxanthin was well tolerated and no clinically relevant abnormalities in laboratory indices, vital signs or electrocardiograms related to the compound were observed.

In a subsequent study, Thurmann and colleagues (2005) investigated the kinetics of lutein in a multiple-dose trial. Eight healthy subjects consumed 4.1 mg lutein per day, eight healthy subjects consumed 20.5 mg lutein per day, and three subjects served as controls. The lutein supplement was derived from a marigold extract and provided 0.34 and 1.7 mg zeaxanthin for the low and high dose groups, respectively. Participants consuming the lutein + zeaxanthin supplements were instructed to avoid lutein- and zeaxanthin-rich vegetables and fruits during the study. Fasting blood samples were collected at baseline on three consecutive days and at

weekly intervals thereafter until day 35. Additional blood samples were taken on days 38, 39, 40 and 41. On day 42, blood samples were obtained before dosing and 2, 4, 6, 8, 12 and 24 hours after dosing to establish a 24 hour kinetic profile. Additional blood samples were drawn during the post-dosing period up to day 67. Plasma samples were analyzed for the E- and Z- isomers of lutein, *all*-E-3'-dehydro-lutein, and *all*-E-zeaxanthin and for the sums of Z-zeaxanthin and Z-lutein isomers. β-carotene, lycopene, β -cryptoxanthin, α-carotene and retinol concentrations also were measured in the blood samples.

Plasma *all*-E-zeaxanthin concentrations rose from 0.052 ± 0.018 to 0.080 ± 0.016 µmol/L in subjects consuming 4.1 mg lutein and 0.34 mg zeaxanthin per day, while plasma concentrations of all-E-zeaxanthin increased from 0.035 ± 0.028 µmol/L to 0.143 ± 0.074 µmol/L in subjects consuming 20.5 mg lutein and 1.7 mg zeaxanthin daily. Plasma *all-E*-lutein concentrations increased from 0.140 ± 0.031 µmol/L to 0.517 ± 0.129 µmol/L in subjects consuming the lower dose of lutein + zeaxanthin, and from 0.148 ± 0.120 µmol/L to 1.452 ± 0.689 µmol/L in the higher dose group. Results from the study suggest that lutein dosing impaired zeaxanthin bioavailability, though the lutein+zeaxanthin supplements had no effects on plasma concentrations of other carotenoids and retinol. An increase in plasma 3'-dehydro-lutein was observed in response to administration of the lutein+zeaxanthin supplements, and lutein was determined to represent the major source of 3'-dehydro-lutein formation. Based on the findings from this study and the previous study (Hartmann et al. 2004), the investigators concluded that the pharmacokinetics of the xanthophylls lutein and zeaxanthin are similar.

In a double-blind, randomized controlled trial of 45 adults ages 60 and older, lutein+zeaxanthin supplements were consumed daily for 6 months (Khachik et al. 2006b; Rosenthal et al. 2006). Lutein doses were 2.5, 5.0 or 10 mg per day, and the corresponding zeaxanthin intakes from the supplements were 0.13, 0.25 or 0.5 mg per day. Mean serum lutein concentrations increased in a dose-dependent manner. Plasma lutein levels in the 5.0 and 10 mg groups at the end of the study were significantly higher than levels in the 2.5 mg lutein group. Serum zeaxanthin concentrations of all subjects increased from a baseline mean of 0.057 ± 0.006 µmol/L to 0.095 ± 0.009 µmol/L, though no differences among doses were observed. Mean serum concentrations of the metabolites 3'-oxolutein, 3'-hydroxy- ε,ε-caroten-3′-one and ε,ε-carotene-3,3'-dione also significantly increased in the three test groups within 1 month of supplementation and remained elevated until the end of the supplementation period. Serum levels of zeaxanthin and the metabolites declined within 6 months after supplementation, but stayed significantly higher than baseline levels. Serum lutein levels returned to baseline levels 6 months after supplementation. The serum concentrations of other carotenoids were not affected by the lutein+zeaxanthin supplementation. No apparent toxicity or side effects were observed throughout the study.

Serum and macular pigment concentrations of zeaxanthin were monitored in two individuals consuming supplements containing 30 mg crystalline, unesterified zeaxanthin produced from a commercial culture of Flavobacteria (Bone et al. 2003). One subject consumed the zeaxanthin supplements daily for 60 days and the other subject consumed the supplements for 120 days. The supplements were consumed in addition to the participants' normal, self-selected diets. In one subject, serum zeaxanthin rose from a baseline level of 0.097 ± 0.007 µmol/L to 0.56 ± 0.070 µmol/L after approximately 30 days of supplementation. In the second subject, serum

zeaxanthin was 0.086 ± 0.009 µmol/L at baseline and plateaued at 0.480 ± 0.050 µmol/L after approximately 10 days of supplementation. Macular pigment optical density increased approximately 8-10% above baseline levels following daily intake of zeaxanthin for 120 days.

Schalch and colleagues (2007) measured plasma and macular pigment optical density (MPOD) responses to daily intakes of approximately 10 to 20 mg doses of lutein, zeaxanthin or a combination of lutein+zeaxanthin over 6-12 months. After the first 6 months of daily ingestion of 12.6 mg pure synthetic zeaxanthin, mean plasma zeaxanthin concentrations rose from 0.040 ± 0.030 µmol/L to approximately 0.850 ± 0.320 µmol/L; following another 6 months of daily intake of 25.3 mg zeaxanthin, mean plasma zeaxanthin concentrations rose to 1.09 ± 0.41 µmol/L. The mean plasma lutein concentration in subjects consuming 10.7 mg lutein and 0.8 mg zeaxanthin from a marigold extract daily for 6 months plateaued at approximately 0.99 ± 0.39 µmol/L, and mean plasma lutein levels reached approximately 1.35 ± 0.87 µmol/L after daily intake of 21.4 mg lutein and 0.16 mg zeaxanthin; the mean baseline lutein concentration in these subjects was 0.16 ± 0.07 µmol/L. Subjects consuming a combination of 10.2 mg lutein and 11.9 mg zeaxanthin had baseline lutein and zeaxanthin plasma concentrations of 0.17 ± 0.07 and 0.06 ± 0.03 µmol/L, respectively; after the 6-month period of supplementation, lutein and zeaxanthin plasma levels plateaued at approximately 0.55 ± 0.12 and 0.61 ± 0.16 µmol/L, respectively. After an additional 6-month period of supplementation, mean lutein and zeaxanthin plasma levels were approximately 0.53 ± 0.24 and 0.52 ± 0.30 µmol/L, respectively. MPOD increased approximately 15% in subjects consuming lutein supplements or the combined lutein+zeaxanthin supplement. In subjects consuming pure zeaxanthin, MPOD increased in both the fovea and parafovea regions, with a total increase of approximately 14% based on calculations that accounted for the wider retinal deposition.

The effects of a dietary supplement containing 14.9 mg *meso*-zeaxanthin, 1.4 mg zeaxanthin, and 5.5 mg lutein on plasma levels of the xanthophylls and MPOD were measured in 10 adults consuming the supplements daily for a period of 120 days (Bone et al. 2007). The mean baseline level of plasma zeaxanthin + *meso*-zeaxanthin was 0.097 ± 0.048 µmol/L, while the mean value from week 6 through the end of the supplementation was 0.264 ± 0.065 µmol/L. At baseline and after supplementation (week 6 through 120 days), the mean plasma lutein levels were 0.305 ± 0.125 and 0.380 ± 0.120 µmol/L, respectively. Changes in MPOD were evaluated in a total of 20 eyes; the rate of increase of MPOD was significant in 12 eyes, no changes occurred in seven eyes, and a significant decrease was observed in one eye.

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**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| **Zeaxanthin** | | | | | |
| Aronow & Chew 2014 | Randomized, double-blind, placebo-controlled trial evaluating the risks and benefits of adding carotenoids (including ZEA) to the AREDS formulation for the treatment of progression to advanced AMD. | m & f, 50 - 85 y at high risk of AMD progression (n=4203) | LUT (10 mg) +ZEA (2 mg), DHA (350 mg)+EPA (650 mg), or both or placebo (base AREDS formula). Median LUT+ZEA = ~ 2.6 mg/d; range = 0.043 — 39.8 mg/d. The study duration was 5 years. | **Key outcomes:** No effect on progression to AMD or changes in visual acuity.<br><br>**Adverse effects:** No adverse effects reported for LUT+ZEA group. | The authors acknowledged that the study design had some limitations and may not be generalizable. LUT+ZEA may require further investigation for potential inclusion in the AREDS supplements. |
| Bone et al. 2007 | Supplementation study to determine the effectiveness of a meso-ZEA supplement with LUT and ZEA in raising macular pigment density in human subjects. | 10 subjects (8 m & 2 f, 21 to 58 y) were given gel caps containing meso-ZEA supplement with LUT and ZEA, while 9 subjects (5 m & 4 f, 19 to 31 y) were given gel caps containing a placebo. | The dose was 20 mg/d of predominantly meso-ZEA, with smaller amounts of LUT and ZEA for a period of 120 days. | **Key outcomes:** The MPOD was significantly higher in supplementation group than in placebo group.<br><br>**Adverse effects:** No adverse effects reported. | This study was not the standard, double blind, placebo-controlled trial in which subjects are randomly assigned to the treatment and placebo groups, and it did not run the supplementation and placebo groups concurrently. Also, the difference in the mean ages of the two groups was significant (p<0.05). However, the authors said these did not introduce a bias in to the study results. |

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**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Bovier et al. 2014 | Double-blind, placebo-controlled study to evaluate the effects of LUT and ZEA on the visual processing speed and efficiency. | 10 subjects (4 m & 6 f, 18-32 y) for placebo, 29 subjects (14 m & 15 f, 18-32 y) for ZEA only, and 25 subjects (9 m & 16 f, 18-32 y) for a mixed formulation containing ZEA, LUT, and omega-3 fatty acids. | Dose of placebo, ZEA at 20 mg/d, or a mixed formulation containing 26 mg/day ZEA, 8 mg/d LUT, and 190 mg/d mixed omega-3 fatty acids. The duration is four months. | **Key outcomes**: Supplementation with ZEA and the mixed formulation produced significant increases in critical flicker fusion thresholds and visual motor reaction time compared to placebo.<br><br>**Adverse effects**: No adverse effects reported. | Not a toxicological study. |
| Bovier and Hammond 2015 | Randomized, placebo-controlled study to evaluate the effects of LUT and ZEA on the visual processing speed. | 15 subjects (18-32 y) for placebo, 29 subjects (18-32 y) for ZEA, and 25 subjects (18-32 y) for a mixed formulation containing ZEA, LUT, and omega-3 fatty acids. | Dose of placebo, ZEA at 20 mg/d, or a mixed formulation containing 26 mg/d ZEA, 8 mg/d LUT, and 190 mg/d mixed omega-3 fatty acids. The duration is four months. | **Key outcomes**: Supplementation with ZEA and LUT produced significant increases in temporal contrast sensitivity function and MPOD compared to placebo.<br><br>**Adverse effects**: No adverse effects reported. | Not a toxicological study. |

**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Bucheli et al. 2011 | Double-blind, randomized, placebo-controlled study to evaluate the effects of daily supplementation with a proprietary milk-based formulation of goji berry (containing ZEA) on macular characteristics and plasma ZEA and antioxidant capacity levels. | 75 subjects (30 f & 45 f, 65-70 y) for placebo and 75 subjects (28 m & 47 f, 65-70 y) for goji berry | Daily dose of placebo or 13.7 g/d of goji berry (10 mg/d ZEA). The duration is 90 d. | **Key outcomes**: The placebo group demonstrated hypopigmentation and soft drusen accumulation in the macula, whereas the goji berry group remained stable. Both plasma ZEA level and antioxidant capacity increased significantly in the goji berry group, but did not change in the placebo group.<br><br>**Adverse effects**: No adverse events were reported in the placebo group. One adverse event, vomiting and fever of 6-day duration, was reported in the goji berry group. This adverse event was classified as mild and unrelated to the study product. | Not a toxicological study. Study subjects were within a narrow age range. The optical density of the preretinal pigment was not directly assessed. No significant relationship was observed between change in plasma ZEA level and change in macular characteristics to support the effect of ZEA on macula. |
| Chiste et al. 2014 | Practical and optimized cellular antioxidant assay to evaluate the potential of carotenoids (including ZEA) to inhibit hemolysis of human erythrocytes induced by peroxyl radicals. | *In vitro* assay at $30 \times 10^6$ cells/mL | Six tested carotenoid concentrations from 0.1 to 3 µM. The study duration was 3 h. | Lycopene (IC50 = 0.24 ± 0.05 µM) was the most efficient to prevent the hemolysis, followed by β-carotene (0.32 ± 0.02 µM), LUT (0.38 ± 0.02 µM), and ZEA (0.43 ± 0.02 µM). | Not a standardized toxicological study. Unknown relevance *in vivo*. |

**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Connolly et al. 2011 | Randomized, placebo-controlled, clinical trial to investigate serum and macular response to, and safety of supplementation with, meso-ZEA, LUT, and ZEA. | Forty-four healthy subjects | Daily dose of 10.6 mg meso-ZEA, 5.9 mg LUT, and 1.2 mg ZEA (intervention, I group) or placebo (P group). The study duration was 6 months. | **Key outcomes:** Subjects supplemented with meso-ZEA, LUT, and ZEA exhibited significant increases in serum concentrations of these carotenoids and a subsequent increase in central MPOD.<br><br>**Adverse effects:** No adverse effects reported. | |
| Graydon et al. 2012 | Randomized, double-blind, placebo-controlled trial to compare the effect of LUT- and ZEA-rich foods and supplements on macular pigment level and serological markers of endothelial activation, inflammation and oxidation. | Study 1: 25 subjects (10 m & 15 f, 35.6 y) for spinach powder and 27 subjects (9 m & 18 f, 33.3 y) for carrot juice Study 2: 25 subjects (11 m & 14 f, 35.5 y) for LUT and ZEA, 25 subjects (8 m & 17 f, 36.6 y) for β-carotene, and 25 subjects (12 m & 13 f, 39.6 y) for placebo | Study 1: Subjects received a daily dose of either 131 mL/d of carrot juice (15 mg/d of β-carotene) or 10.4 g/d of spinach powder (15 mg/d of LUT and ZEA) for 8 weeks. Study 2: Subjects received supplements containing 10 mg/d of LUT and 5 mg/d of ZEA, 15 mg/d of β-carotene, or placebo for 8 weeks. | **Key outcomes:** Supplementation with LUT and ZEA, whether as foods or as supplements, had no significant effect on macular pigment level or serological markers of endothelial activation, inflammation and oxidation, but may improve macular pigment level in the highest serum responders and in those with initially low macular pigment level.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. The authors acknowledged that these were pilot studies, and further investigations are required with better study design in terms of doses and duration of supplementation, sensitivity of measurement, and study subjects. |

| Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies | | | | | |
|---|---|---|---|---|---|
| **Reference** | **Study Description** | **Subjects (n)** | **Dose - Duration** | **Observed Effects** | **Ramboll Environ Remarks** |
| Hammond et al. 2014 | Double-blind, randomized, placebo-controlled study to evaluate the effects of LUT and ZEA on photostress recovery, glare disability, and chromatic contrast. | 56 subjects (22 m & 34 f, 18-30 y) for placebo and 53subjects (22 m & 30 f, 18-40 y) for LUT and ZEA | Daily dose of placebo or 2 mg/d of ZEA and 10 mg/d of LUT. The duration is 1 y. | **Key outcomes:** Significant increase in MPOD, serum LUT and ZEA levels and improvements in chromatic contrast and photostress recovery time were observed in the group supplemented with LUT and ZEA when compared to the placebo group. Glare disability was correlated with MPOD but did not increase significantly in the group supplemented with LUT and ZEA.<br><br>**Adverse effects:** Twenty adverse events occurred during the entire study period. However, no adverse events occurred were directly attributable to the study product. | Not a toxicological study. |

| Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies | | | | | |
|---|---|---|---|---|---|
| **Reference** | **Study Description** | **Subjects (n)** | **Dose - Duration** | **Observed Effects** | **Ramboll Environ Remarks** |
| Huang et al. 2008 | Randomized, placebo-controlled study to examine the effect of oral supplementation of omega-3 long-chain polyunsaturated fatty acids on changes in serum levels of LUT/ZEA in persons 60 years of age and older, with or without AMD. | 40 subjects (17 m & 23 f, 64-86 y) with or without AMD (20 per group) | Subjects received daily dose of LUT (10 mg) and ZEA (2 mg) and either omega-3 long-chain polyunsaturated fatty acids (350 mg DHA and 650 mg EPA) or placebo for 6 months. | **Key outcomes:** Serum levels of LUT/ZEA increased compared with baseline, but did not differ by omega-3 long-chain polyunsaturated fatty acids. Subjects with AMD had a lower increase in serum LUT concentration than did those without AMD. <br><br> **Adverse effects:** No adverse effects reported. | Not a toxicological study. The authors acknowledged that a long-term large clinical trial is necessary to investigate the benefits and adverse effects of omega-3 long-chain polyunsaturated fatty acids for the treatment of AMD. |
| Huang et al. 2013 | Randomized, double-blinded, placebo-controlled trial to examine serum and macular responses to supplemental LUT and ZEA in Chinese subjects with early AMD. | 108 patients (46 m & 62 f, 50-81 y) with early AMD (27 per group) | Subjects received daily dose of 10 mg/d LUT, 20 mg/d LUT, 10 mg/d ZEA+10 mg/d LUT, or placebo for 48 weeks. | **Key outcomes:** Supplementation with LUT and/or ZEA significantly increased serum concentrations and MPOD. <br><br> **Adverse effects:** No adverse effects reported. No subjects developed or reported occasional skin pigmentation (carotenodermia). | Not a toxicological study. The authors indicated that the side effects of supplementation need to be confirmed in larger populations, particularly in elderly patients with kidney and liver diseases. |

**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Huang et al. 2014 | Randomized, double-blind, placebo-controlled trial to investigate functional and macular pigment changes in patients with early AMD after multiple supplementation with LUT and ZEA. | 112 patients (46 m & 66 f, > 50 y) with early AMD | Patients received 10 mg LUT, 20 mg LUT, 10 mg LUT+10 mg ZEA, or placebo daily for 2 y. | **Key outcomes:** Supplementation with LUT and/or ZEA significantly increased MPOD and supplementation with LUT significantly increased retinal sensitivity.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. Limitations of this study include highly selective criteria for study subjects, no ZEA control group and no discussion about the effects of ZEA and meso-ZEA and their interactions with LUT supplementation, and no evaluation of the effects of LUT/ZEA supplementation on reducing the progression of AMD due to limited sample size and intervention period. |
| Huang et al. 2015 | Randomized, double-blinded, and placebo-controlled trial to evaluated the effect of supplemental LUT and ZEA on serum, macular pigmentation, and visual performance in patients with early AMD. | 112 patients (46 m & 66 f, > 50 y) with early AMD | Patients received 10 mg LUT, 20 mg LUT, 10 mg LUT+10 mg ZEA, or placebo daily for 2 y. | **Key outcomes:** Serum LUT concentration, MPOD, and visual sensitivity significantly increased in all the treated groups.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. Limitations of this study include highly selective criteria for study subjects, measurement sensitivity, and no evaluation of the effects of LUT/ZEA supplementation on reducing the progression of AMD due to limited sample size and intervention period. |

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| Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies | | | | | |
|---|---|---|---|---|---|
| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
| Ma et al. 2012a | Randomized, double-masked, placebo-controlled trial to evaluate the effect of LUT and ZEA on macular pigment and visual function in patients with early AMD. | 108 patients (45 m & 63 f, 50-79 y) with early AMD (27 per group) | Patients received 10 mg LUT, 20 mg LUT, 10 mg LUT+10 mg ZEA, or placebo daily for 48 weeks. | **Key outcomes:** Significant increase in MPOD and improvement in best-corrected visual acuity and contrast sensitivity were observed in all the treated groups. **Adverse effects:** No adverse effects reported. | Not a toxicological study. Limitations of this study include single-site study design, influence of dietary intake fluctuation, and no evaluation of the effects of LUT/ZEA supplementation on reducing the progression of AMD due to limited sample size and intervention period. |
| Ma et al. 2012b | Randomized, double-masked, placebo-controlled trial to examine the effects of LUT and ZEA supplementation on retinal function in patients with early AMD. | 108 patients (45 m & 63 f, 50-79 y) with early AMD (27 per group) 36 subjects (15 m & 21 f, 50-79 y) without AMD (only for a baseline examination) | Patients received 10 mg LUT, 20 mg LUT, 10 mg LUT+10 mg ZEA, or placebo daily for 48 weeks. | **Key outcomes:** Significant increases in N1P1 response densities and MPOD were observed in all the treated groups. **Adverse effects:** No adverse effects reported. | Not a toxicological study. Limitations of this study include highly selective criteria for study subjects, influence of dietary intake fluctuation, and no evaluation of the effects of LUT/ZEA supplementation on reducing the progression of AMD due to limited sample size and intervention period. |
| Nolan et al. 2015 | Randomized, double-blind, placebo-controlled trial to investigate the impact of supplemental macular carotenoids on macular pigment, vision, and cognitive function in patients with Alzheimer's | 31 patients (13 m &18 f, mean age 80 y) with Alzheimer's disease and 31 age-similar control subjects (18 m & 13 f, mean age 76 y) | Subjects received dose of either 10 mg meso-ZEA, 10 mg LUT and 2 mg ZEA or placebo (sunflower oil) for 6 months. | **Key outcomes:** Significant increases in serum concentrations of LUT, ZEA, meso-ZEA, macular pigment, and contrast sensitivity were observed in the groups with supplement for both patients with Alzheimer's disease and control | Not a toxicological study. |

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**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| | disease. | | | subjects. No significant changes were found in any of the cognitive function outcome variables measured. **Mortalities:** No effect on mortalities. **Adverse effects:** No adverse effects reported. | |
| Richer et al. 2011 | Randomized, double-blind, placebo-controlled study to evaluate whether dietary supplementation with ZEA raises MPOD and has unique visual benefits for patients with atrophic AMD. | 60 patients (57 m & 3 f, mean age 74.9 years) with AMD, 25 subjects for ZEA, 25 subjects for ZEA+LUT, and 10 subjects for "faux placebo" 9 mg LUT. | Subjects received dose of 8 mg ZEA, 8 mg ZEA plus 9 mg LUT, or "faux placebo" 9 mg LUT for 1 y. | **Key outcomes:** ZEA-induced foveal MPOD elevation mirrored that of LUT and provided complementary distinct visual benefits by improving foveal cone-based visual parameters, whereas LUT enhanced those parameters associated with gross detailed rod-based vision, with considerable overlap between the two carotenoids. The equally dosed ZEA plus LUT group fared worse in terms of raising MPOD, presumably because of duodenal, hepatic-lipoprotein or retinal carotenoid competition.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. |

**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Richer et al. 2012 | Randomized controlled clinical trial to evaluates the relationship between carotenoid supplementation, contrast sensitivity and glare recovery and the relationship between driving ability and retinal macular pigmentation. | 60 patients (predominantly male, mean age 74.9 years) with AMD, 25 subjects for ZEA, 25 subjects for ZEA+LUT, and 10 subjects for "faux placebo" 9 mg LUT. | Subjects received daily dose of 8 mg ZEA, 8 mg ZEA plus 9 mg LUT, or "faux placebo" 9 mg LUT for 1 y. | **Key outcomes:** Self-described driving ability was notably associated with baseline pre-supplementation macular pigmentation. Linear regression modeling suggests that self-described ability to safely drive a car was strongly associated with final macular re-pigmentation post supplementation. Moreover, the greatest effect was found with ZEA even though LUT had greater effects than ZEA with respect to contrast sensitivity and glare recovery.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. |
| Schalch et al. 2007 | Randomized, double-blind, placebo-controlled study to investigate MPOD responses to supplementation with LUT and ZEA. | Study 1: 92 subjects (male, 18-45 y), 23 per group<br>Study 2: 30 subjects (male, 18-45 y), 3 subjects for LUT, 6 subjects for ZEA, 11 subjects for LUT+ZEA, and 10 subjects for placebo. | Study 1: Subjects received daily dose of 10.7 mg LUT, 12.6 mg ZEA, 11.9 mg ZEA plus 10.2 mg LUT, or placebo for 6 months. Study 2: Subjects received daily dose of 21.4 mg LUT, 25.2 mg ZEA, 11.9 mg ZEA plus 10.2 mg LUT, or placebo for 6 months. | **Key outcomes:** MPOD and plasma concentrations increased with supplementation with LUT and ZEA. LUT is predominantly deposited in the fovea while ZEA deposition appears to cover a wider retinal area.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. |

**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| Stringham and Hammond 2008 | Study to evaluate the effect of supplementation with LUT and ZEA on macular pigment and improvements in glare disability and photostress recovery. | 40 healthy subjects (17 m & 23 f, mean age 23.9 y) | Subjects received a daily dose of 10 mg LUT and 2 mg ZEA for 6 months. | **Key outcomes:** Supplementation with LUT and ZEA significantly increased MPOD and reduced the deleterious effects of glare when compared to the baseline measurement for the same subjects.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. Not a placebo-controlled study. No statistical method was provided. |
| Tanito et al. 2012 | Randomized, double-blind study to determine whether either LUT or ZEA supplementation affects MPOD in healthy Japanese individuals. | 22 healthy subjects (10 m & 12 f, 23-58 y), 11 per group | Subjects received either 10 mg of LUT or ZEA daily for 3 months. | **Key outcomes:** In normal healthy Japanese individuals without high myopia, LUT supplementation increased MPOD levels within the fovea more effectively than ZEA.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. Not a placebo-controlled study. |
| van de Kraats et al. 2008 | Study to separately measure the optical densities of LUT and ZEA in the human retina in vivo. | 23 subjects (5 male & 18 female, mean age 24.3 y) for baseline, 3 subjects (3 male, 28-64 y) for ZEA supplementation | The daily dose for ZEA supplementation was 20 mg/d for 6 months. | **Key outcomes:** ZEA supplementation caused a significant increase in the optical densities of ZEA, and no or minor changes in the optical densities of LUT.<br><br>**Adverse effects:** No adverse effects reported. | Not a toxicological study. Not a placebo-controlled study. |

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**Table 5-1 (2015). Toxicological Data Summary of Zeaxanthin and Related Carotenoids from Human Studies**

| Reference | Study Description | Subjects (n) | Dose - Duration | Observed Effects | Ramboll Environ Remarks |
|---|---|---|---|---|---|
| **Other Carotenoids** | | | | | |
| Capeding et al. 2010 | Prospective, randomized, controlled, and double-blind study with parallel groups of healthy term infants fed either control formula or experimental formula to evaluate the effect of LUT-fortification on the growth of infants. | 232 infants ≤ 14 days postnatal age were randomized and 220 completed the study | LUT at 200 µg/L and ZEA at 15 µg/L. The study duration was 16 weeks. | **Key outcomes**: No differences in growth or blood chemistry parameters were found between the two groups. All measured growth and blood chemistry parameters fell within the normal ranges.<br><br>**Adverse effects:** No adverse effects reported for the group fed LUT-fortified infant formula. | Not a toxicological study. |

Abbreviations:
AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; d = day; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; f = female; g = gram(s); h = hour(s); IC50 = inhibition concentration for 50% of the test organisms; L = liter; LUT = lutein; m = male; mg = milligram(s); mL = milliliter(s); MPOD = macular pigment optical density; µM = micromolar; y = year; ZEA = zeaxanthin

**Table 5-1 (2007). Human Plasma and Macular Pigment Response to Zeaxanthin/Lutein Supplementation**

| Reference | Study Design | Group Details | Daily Dose L (mg) | Daily Dose Z (mg) | Baseline Plasma L level (µmol/L) | End Plasma L level (µmol/L) | Change in Plasma L | Baseline Plasma Z level (µmol/L) | End Plasma Z level (µmol/L) | Change in Plasma Z | Additional Endpointse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bone et al. 2003 | 2 M; subject (1) was 53 y, consumed a crystalline Z supplement in a culture of flavobacteria suspended in oil for 120 d; subject (2) was 21 y, consumed the supplement for 60 d | 1 | 0 | 30 | NR | NR | NA | 0.097 ± 0.007a | 0.56 ± 0.07 a | NA | -plasma plateau after ~30 days<br>-MPOD rate of increase:<br>Left eye 0.71± 0.08 mAU/d<br>Right eye 0.56 ± 0.09 mAU/d |

| Reference | Study Design | Group Details | Daily Dose L (mg) | Daily Dose Z (mg) | Baseline Plasma L level (µmol/L) | End Plasma L level (µmol/L) | Change in Plasma L | Baseline Plasma Z level (µmol/L) | End Plasma Z level (µmol/L) | Change in Plasma Z | Additional Endpointse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | | | | | | 0.086 ± 0.009 a | 0.48 ± 0.05 a | | -plasma plateau after ~10 days -MPOD rate of increase: Left eye 0.35 ± 0.08 mAU/d Right eye 0.31 ± 0.08 mAU/d |
| Bone et al. 2007 | 8 M and 2 F, mean age 30.5±10.9 y, consumed supplements extracted from marigolds, suspended in oil and provided in gel-caps for 120 d | | 5.5 | 1.4 Z, 14.9 MZ | 0.305 ± 0.125 | 0.380 ± 0.120 | NR | 0.097 ± 0.048 Z + MZ | 0.264 ± 0.065 Z + MZ | NR | -increase in MPOD in 12 eyes (0.27-2.22 mAU/d); no significant changes in 7 eyes; decrease in one eye (-0.46 ± 0.18 mAU/d) |
| Cheng et al. 2005 | 13 MF, mean 24.7 y, consumed 15 g wolfberries/d for 28 d | | <0.1 | 3 | 0.164 ± 0.021 b | 0.164 ± 0.022 b | ns | 0.0382 ± 0.0034 b | 0.0960 ± 0.0096 b | sig | |
| Goodrow et al. 2006 | 7 M and 26 F, mean age 79 y, consumed 1 egg/d for 5 wk | | 0.143 ± 0.028 | 0.094 ± 0.018 | 0.168 ± 0.015 b | 0.211 ± 0.020 b | sig | 0.041 + 0.004 b | 0.057 + 0.005 b | sig | |
| Hammond et al. 1997 | 13 MF, 30-65 y, consumed 60 g/d spinach and/or 150 g/d corn for 15 wk | Spinach + Corn | 11.2 | 0.6 | NR | NR | 33% ± 22% | NR | NR | ns | Retinal responders: (8) MPOD change: 19% ± 11% NS at 8 weeks Sig elevation at 12, 14, 15 wk, and 1-6 months post. |

**Table 5-1 (2007). Human Plasma and Macular Pigment Response to Zeaxanthin/Lutein Supplementation**

| Reference | Study Design | Group Details | Daily Dose L (mg) | Daily Dose Z (mg) | Baseline Plasma L level (µmol/L) | End Plasma L level (µmol/L) | Change in Plasma L | Baseline Plasma Z level (µmol/L) | End Plasma Z level (µmol/L) | Change in Plasma Z | Additional Endpointse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | NR | NR | 31% | NR | NR | | Retinal nonresponders: (2) MPOD change: -11% |
| | | | | | NR | NR | 0 | NR | NR | 0 | Serum & retinal nonresponders: (1) |
| | | Spinach | 10.8 | 0.3 | NR | NR | NR | NR | NR | NR | |
| | | Corn | 0.4 | 0.3 | NR | NR | 11% | NR | NR | 70% | Subject 1: MPOD change 25% |
| | | | | | NR | NR | -6% | NR | NR | 6% | Subject 2: 7% |
| Handelman et al. 1999 | 6 M and 5 F, mean age 62 y, consumed 1.3 egg yolks/d as part of a corn oil- or tallow-based diet for 4.5 wk | Corn oil diet | ~0.38 | ~0.28 | 0.269 ± 0.083 a | 0.403 ± 0.114 a | ns | 0.049 ± 0.012 a | 0.105 ± 0.023 a | sig | |
| | | Tallow diet | | | 0.333 ± 0.089 a | 0.427 ± 0.114 a | sig | 0.048 ± 0.012 a | 0.116 ± 0.027 a | sig | |
| Hartmann et al. 2004 | 10 M and 10 F, 5 in each group, consumed synthetic all-E-zeaxanthin gelatin beadlets for 42 d | Low | 0 | 1 | 0.23 ± 0.16 | 0.24 ± 0.15 | ns | 0.054 ± 0.038 | 0.20 ± 0.07 | sig | -plasma steady state concentrations reached after 17 d |
| | | High | 0 | 10 | 0.19 ± 0.05 | 0.23 ± 0.08 | ns | 0.047 ± 0.020 | 0.92 ± 0.28 | sig | |
| Johnson et al. 2000 | 4 F and 3 M, 33–54 y, consumed 60 g/d spinach and 150 g/d corn for 15 wk | Spinach + corn | 11.2 | 0.6 | 0.37 ± 0.05 | 0.67 ± 0.11 | sig | .058 ± 0.12 | NR | ns | -increase in MPOD 0.399 ± 0.045 to 0.469 ± 0.059 |
| Khachik et al. 1995 | 3 M, 42-59 y, consumed a purified Z supplement for 21 d | | 0 | 10 | NR | NR | sig | NR | NR | sig | -4-fold increase in plasma Z after 1 wk |
| Khachik et al. 2006b<br><br>Rosenthal | 45 MF with or without AMD, mean age 71y, consumed 1 of 3 supplements containing commercially | Low | 2.5 | 0.13 | 0.280 ± 0.034 | 0.500 ± 0.110 | ns | 0.057 ± 0.006 | 0.095 ± 0.009 | sig, ns by dose | |
| | | Medium | 5 | 0.25 | 0.210 ± 0.034 | 0.720 ± 0.108 | sig | | | | |

**Table 5-1 (2007). Human Plasma and Macular Pigment Response to Zeaxanthin/Lutein Supplementation**

| Reference | Study Design | Group Details | Daily Dose L (mg) | Daily Dose Z (mg) | Baseline Plasma L level (µmol/L) | End Plasma L level (µmol/L) | Change in Plasma L | Baseline Plasma Z level (µmol/L) | End Plasma Z level (µmol/L) | Change in Plasma Z | Additional Endpointse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| et al. 2006 | available lutein for 6 mo | High | 10 | 0.5 | 0.210 ± 0.034 | 1.000 ± 0.111 | sig | | | | |
| Schalch 2007<br><br>Kvansakul et al. 2006<br><br>Rodriguez-Carmona et al. 2006 | 18 MF, consumed L and Z supplements containing nonesterified lutein extracted from marigold and/or synthetic zeaxanthin formulated into beadlets for 12 mo (dosages was doubled after 6 mo) | L (n=3) | 10.7; 21.4 d | 0.8; 1.6 d | 0.16 ± 0.07 | 1.35 ± 0.87 | NR | 0.05 ± 0.02 | 0.14 ± 0.04 | NR | -MPOD % Change (vs. placebo): Z: 2.7, L: 14.5, C and PC: 15.1 -foveal MPOD (vs. placebo): Placebo 0.42 ± 0.06 Z: 0.52 ± 0.05 (p<0.07), L: 0.53 ± 0.04 (p<0.07), C: 0.54 ± 0.06 PC: 0.55 ± 0.03 |
| | | Z (n=5) | 0 | 12.6; 25.2 d | 0.13 ± 0.08 | 0.17 ± 0.07 | | 0.04 ± 0.03 | 1.09 ± 0.41 | | |
| | | L+Z (n=5) | 10.2 | 11.9 | 0.17 ± 0.07 | 0.53 ± 0.24 | | 0.06 ± 0.03 | 0.52 ± 0.30 | | |
| | | Placebo, L+Z (n=5) | 0; 10.2 d | 0; 11.9 d | 0.13 ± 0.04 | 0.32 ± 0.25 | | 0.04 ± 0.03 | 0.29 ± 0.32 | | |
| Triesch-mann et al. 2007 | 98 MF, mean age 71.5±7.1 y, consumed L+Z ester supplement (Ocuvite Lutein) for 6 mo | | 12 | 1 | 0.28 ± 0.026 | 1.042 ± 0.0738 | sig | 0.032 ± 0.002 | 0.039 ± 0.002 | sig | -mean (± SEM) difference in MPOD at 0.5º eccentricity between baseline and visit 6, 3 months following discontinuation of the supplement, was 0.1 (±0.009) ODU |
| Thurmann et al. 2005 | 16 MF, approx. 29 y, consumed 1 of 2 dosages of lutein beadlets extracted from marigold in hard gelatin capsules for 42 d | Low | 3.76 | 0.34 | 0.140 ± 0.031 | 0.517 ± 0.129 | sig | 0.052 ± 0.018 | 0.080 ± 0.016 | sig | -dose-normalized maximum concentrations of plasma Z did not differ between groups. |
| | | High | 18.8 | 1.7 | 0.148 ± 0.120 | 1.452 ± 0.689 | sig | 0.035 ± 0.028 | 0.143 ± 0.074 | sig | |
| Wang et al. 2007 | 12 MF, (7 AMD cases, 5 controls), consumed | Controls | 8.936 | 1.311 | 0.225 ± 0.029 | 0.458 ± 0.083 | sig | 0.037 ± 0.007 | 0.063 ± 0.015 | sig | |

**Table 5-1 (2007). Human Plasma and Macular Pigment Response to Zeaxanthin/Lutein Supplementation**

| Reference | Study Design | Group Details | Daily Dose L (mg) | Daily Dose Z (mg) | Baseline Plasma L level (µmol/L) | End Plasma L level (µmol/L) | Change in Plasma L | Baseline Plasma Z level (µmol/L) | End Plasma Z level (µmol/L) | Change in Plasma Z | Additional Endpointse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | prepared meals containing high levels of L+Z for 4 wk | AMD | | | 0.174 ± 0.029 | 0.452 ± 0.070 | sig | 0.034 ± 0.006 | 0.063 ± 0.012 | sig | |
| Wenzel et al. 2006 | 16 MF, 24-59 y, consumed 6 eggs/wk for 12 wk. Two brands of eggs were used, each provided different levels of L+Z | Brand 1 | 0.20 | ~0.13 | 0.389 ± 0.07 b | 0.477 ± 0.06 b | sig | 0.101 ± 0.02 b | 0.15 ± 0.025 b | sig | -MPOD 0.18 ± 0.02 to 0.27 ± 0.02 |
| | | Brand 2 | 0.60 | ~0.36 | 0.428 ± 0.09 b | 0.540 ± 0.01 b | ns | 0.057 ± 0.01b | 0.11 ± 0.02b | sig | -MPOD 0.37 ± 0.06 to 0.42 ± 0.05 |
| Wenzel et al. 2007 | 2 M, 24 and 31 y, and 1 F, 52 y, consumed lutein diester supplement for 120 d | | 30 | 2.7 | 0.400 | 5.167 | sig | 0.098 | 0.347 | sig | -peak plasma L+Z at 30 d -MPOD increased in all three subjects with a mean change of approximately 0.09 log units at 20' eccentricity and 0.08 log units at 30' eccentricity -MPOD increased in two subjects at 60' eccentricity, and in one subject at 120' eccentricity |

a standard deviation
b standard error of the mean
c The units for plasma level in the text, µg/dL, were converted to µmol/L by multiplying by 10/molecular weight of L or Z ($C_{40}H_{56}O_2$) = 10/568.88 daltons.
d First value corresponds to the dose during the first 6 months of supplementation; the second value is the dose during the second 6 months of supplementation.
e Values significant unless otherwise specified.

Abbreviations:
AMD = age-related macular degeneration; NA = not applicable; NR = not reported; ns = not significant; sig = significant.

## 5.7.1.2 Observational Studies

Data collected in observational studies have been examined to correlate lutein and zeaxanthin intakes and plasma levels with reduction of disease risk. There is also much evidence that the amount of macular pigment is inversely associated with the incidence of age-related macular degeneration. The potential role of carotenoids in the prevention of AMD has been comprehensively reviewed (Snodderly 1995). In addition, studies have documented that the highest levels of combined lutein and zeaxanthin intake (up to 6 mg/d and 12 mg/d) are associated with a decreased risk of AMD and cataracts, respectively (Seddon et al. 1994; Chasen-Taber et al. 1999). Results from observational studies demonstrating an association of higher intakes of lutein and zeaxanthin with an increase in macular pigment ocular density and decreased risk of cataracts and AMD are summarized in Table 5-2.

In addition to their role in eye health, lutein and zeaxanthin intake may also reduce the risk of certain types of cancers due to their antimutagenic and anticarcinogenic properties (Ribaya-Mercado and Blumberg 2004). There is a growing body of evidence supporting their protective role against breast and lung cancer as well as evidence that they may also protect against colorectal, prostate, upper aerodigestive, ovarian, endometrial, and cervical cancer. Suggested mechanisms of protection include selective modulation of apoptosis, inhibition of angiogenesis, enhancement of gap junctional intercellular communication, induction of cell differentiation, prevention of oxidative damage and modulation of the immune system. Emerging evidence also suggests that lutein and zeaxanthin may protect against heart disease and stroke by scavenging peroxynitrite radicals, slowing the progression of atherosclerosis.

| Table 5-2. Observational Studies of Lutein and Zeaxanthin (L+Z) Intake and Health Outcomes | | | |
|---|---|---|---|
| **Study** | **Study Population** | **L+Z Intake (µg/d) [a]** | **Results** |
| **Age-related Macular Degeneration (AMD)** | | | |
| Age-Related Eye Disease Study Research Group 2007 | MF, 60-80 y; participants in the Age-Related Eye Disease Study (AREDS) (n=4519) | **Median/1000 calories, by quintile** | **Odds Ratio for Neovascular AMD [b]** |
| | | 521 | 1.0 |
| | | 763 | 0.74 |
| | | 1000 | 0.54[c] |
| | | 1333 | 0.68[c] |
| | | 2095 | 0.65[c] |
| | | | p-trend = 0.08 |
| | | Dietary lutein+zeaxanthin intake was also inversely associated with geographic atrophy (OR, 0.45; 95% CI, 0.24-0.86) and large or extensive intermediate drusen (OR, 0.73; 95% CI, 0.56-0.96) in comparisons of highest vs. lowest quintiles of adjusted intakes. | |
| Seddon et al. 1994 | MF, 55-80 y; participants in the Eye Disease Case-Control Study (n=356 with AMD, n=520 controls) | **Median, by quintile** | **Odds Ratio for AMD [b]** |
| | | 561 | 1.0 |
| | | 1211 | 1.14 |
| | | 1708 | 0.82 |
| | | 2487 | 0.74 |
| | | 5757 | 0.40 |
| | | | p-trend <0.001 |
| **Cataracts** | | | |
| Brown et al. 1999 | M, 40-75 y in 1986; participants in the Health Professionals Follow-up Study (n=36644) | **Median, by quintile** | **Relative Risk of Cataract Extraction [b]** |
| | | 1300 | 1.0 |
| | | 2279 | 1.0 |
| | | 3182 | 0.98 |
| | | 4342 | 0.83 |
| | | 6871 | 0.81 |
| | | | p-trend = 0.03 |
| Chasen-Taber et al. 1999 | F, 45-71 y; participants in the Nurses' Health Study (n=77446) | **Median, by quintile** | **Relative Risk of Cataract Extraction [b]** |
| | | 1172 | 1.0 |
| | | 2064 | 1.01 |
| | | 2817 | 0.95 |
| | | 6047 | 0.81 |
| | | 11685 | 0.88 |
| | | | p-trend = 0.04 |
| Vu et al. 2006 | Adults, average age ~62 y; (n=2322) | 796 | -OR (95% CI) [b] for every 1-mg increase in daily LZ intake: Cortical Cataract 0.74 (0.46-1.19) Nuclear Cataract 0.60 (0.40–0.90) Posterior Subscapular Cataract 0.81 (0.51-1.28) |

| Table 5-2. Observational Studies of Lutein and Zeaxanthin (L+Z) Intake and Health Outcomes | | | |
|---|---|---|---|
| **Study** | **Study Population** | **L+Z Intake (µg/d)** [a] | **Results** |
| **Macular Pigment Ocular Density (MPOD)** | | | |
| Burke et al. 2005 | MF, 45–73 y; (n=98) | F: 1832 ± 166<br>M: 1474 ± 109 | -Serum L+Z concentrations were correlated with MPOD at the 3 most central loci (10', 30' and 60').<br>-Dietary intakes of L+Z were associated with MPOD at all loci evaluated. |
| Curran-Celantano et al. 2001 | MF, 18-50 y; average age 36.0±7.9 y; (n=280) | 1101 ± 838 | -Serum L, Z, and dietary L+Z correlated with MPOD.<br>-Serum L 0.28 ± 0.13 µmol/L + Z 0.091 ± 0.044 µmol/L |
| Nolan et al. 2007 | Adults (n=754) | Not specified | -Serum L and Z and dietary L and Z correlated with MPOD.<br>-Dietary L correlated with serum L (0.286, p < 0.01)<br>-Dietary Z correlated with serum Z (0.249, p < 0.01) |
| a Food Frequency Questionnaires were used to assess dietary L+Z intakes in all studies.<br>b Adjusted for health and/or demographic factors.<br>cp ≤ 0.05 | | | |

## 5.7.2  Animals

Studies in primates, quail and rodents have been conducted to evaluate changes in tissue and plasma concentrations of lutein, zeaxanthin, and their metabolites as a result of supplementation with the xanthophylls. In primate studies, dosages ranged from 0.5 to 9.34 mg lutein/kg-bw/day and 0.5 to 10 mg zeaxanthin/kg-bw/day. Study periods lasted from 6 weeks to 24 months. Quail were administered 3.5 to 5.04 mg zeaxanthin/kg-bw/day in conjunction with 0.02 to 0.03 mg lutein/kg-bw/day lutein for a period of 6 months. In a rodent study, 144 mice were administered 72.2 to 623 mg lutein + zeaxanthin/kg-bw/day of for a period of 28 days.

## 5.7.2.1  Primates

Non-human primates have a macular structure closely resembling that of humans (Neuringer et al. 2004). Therefore, primates are generally considered a valuable animal model when researching human ocular-related conditions. Studies have been conducted on monkeys in order to determine ocular tissue deposition and plasma response as a result of supplementation of zeaxanthin and/or lutein in these animals. Four studies are discussed below.

In a study of female rhesus monkeys (*Macaca mulatta*), Khachik and colleagues (2006a) measured changes in the mean levels of zeaxanthin, lutein and their metabolites in the retina, ciliary body, iris and lens of the eye following a 12-month supplementation period with synthetic zeaxanthin and/or marigold-derived lutein. Each of the 3 intervention groups had 5 monkeys; 3 additional monkeys were used as controls. In both the lutein (L-fed) and zeaxanthin (Z-fed) fed groups, 2 monkeys were sacrificed at the end of the 12 month intervention period. One eye from each monkey was used for carotenoid analysis; the other eye was examined for retinal histopathology. The remaining 3 monkeys from each group were sacrificed after 18 months for

post-intervention carotenoid analysis. All monkeys were evaluated using fundus photography, histopathology of the retinas and urine samples to identify any safety concerns or abnormalities resulting from the long-term supplementation of lutein and zeaxanthin.

Changes in plasma concentrations of lutein, zeaxanthin, *3'*-oxolutein and *3'*-epilutein were measured at baseline and months 6, 12 and 18 (post-supplementation monkeys only). *Meso*-Zeaxanthin was absent in plasma.

The L-fed monkeys received 9.34 mg lutein/kg-bw/day and 0.66 mg zeaxanthin/kg-bw/day. Mean plasma lutein levels increased 2.6-fold compared to baseline at 6 months and had increased further by 12 months. In the 3 monkeys evaluated at 6 months post-supplementation, plasma lutein levels had returned to baseline. The mean plasma level of *3'*-epilutein did not significantly change after 6 months compared to baseline, but by 12 months was 3.2- and 4.7-fold higher compared to baseline and control plasma levels, respectively. Mean plasma concentrations *3'*-oxolutein were significantly higher than control group plasma concentrations by 6 months of supplementation and were 2-fold higher than control by 12 months. Since the L-fed animals also received small amounts of zeaxanthin, mean plasma zeaxanthin levels were also measured. No increase over baseline levels was observed at 6 months, but at 12 months, plasma zeaxanthin levels were 1.4-fold higher than baseline. At 6-months post-supplementation, zeaxanthin levels had returned to baseline (Khachik et al. 2006a).

The Z-fed monkeys received 10 mg zeaxanthin/kg-bw/day. Mean plasma zeaxanthin levels increased 3.6-fold over baseline at 6 months and had increased further by 12 months. In the 6 month post-supplementation monkeys, plasma zeaxanthin levels were 1.8-fold lower than they had been at baseline. Plasma *3'*-epilutein concentrations were unaffected by zeaxanthin supplementation. Mean plasma *3'*-oxolutein concentrations, on the other hand, were 2.4-fold higher than that observed in the control monkeys by 12 months. Mean plasma lutein concentrations did not differ across the different time points.

The L/Z-fed monkeys received 0.5 mg lutein/kg-bw/day and 0.5 mg zeaxanthin/kg-bw/day. Mean plasma lutein levels were 2.4-fold higher compared to baseline and 2.5-fold higher than control animals at 6 months. No statistical significance remained at 12 months. Mean plasma zeaxanthin levels increased 3-fold compared to baseline and 5-fold higher compared to the control monkeys at 6 months. As observed with lutein, these differences were no longer present at 12 months. The mean plasma level of *3'*-epilutein did not significantly change from baseline levels at 6 or 12 months. However, at 6 months, *3'*-epilutein levels were 4.3-fold higher than in the control monkeys. The mean plasma concentration of *3'*-oxolutein was 2-fold higher than that of the control monkeys at 6 months, with this difference no longer present at 12 months. The investigators proposed that the observed decreases in lutein and zeaxanthin may be due to interactions of the metabolites formed from the conversion of lutein and zeaxanthin.

The mean plasma levels of *3'*-epilutein and *3'*-oxolutein were compared among groups. At 12 months, mean plasma *3'*-epilutein levels in the L-fed monkeys were 4.7-fold and 2-fold higher than the Z-fed and L/Z-fed monkeys, respectively. The mean plasma *3'*-oxolutein levels among the three groups did not differ at 6 months. At 12 months, the level of this metabolite in the L- and Z-fed monkeys was 2.8-fold higher than that of the L/Z-fed monkeys (Khachik et al. 2006a).

Mean lutein levels in the retina of L-fed monkeys were 3.7-fold higher than levels in the control monkeys. These elevated lutein levels were not maintained at 6 months post-supplementation. Mean retina *meso*-zeaxanthin levels were not significantly different from those of the control animals at 12 months. Mean *3'*-oxolutein levels were 3.2-fold higher than levels in the control animals at 12 months, and 6-fold higher than the levels observed in the monkeys evaluated 6 months post-supplementation. Mean iris lutein levels at 12 months were 4.2-times higher than levels in control monkeys. Mean lutein levels in the ciliary body of the eyes were not different compared to either the control animals or in the animals evaluated at 6 months post-supplementation. Mean lens lutein levels did not differ from levels in the control animals.

In the group of Z-fed monkeys, mean concentrations of zeaxanthin, meso-zeaxanthin, and 3'-oxolutein increased 2.4-fold compared to the control monkeys at 12 months. Retina lutein concentrations did not increase. In the monkeys evaluated 6 months after cessation of supplementation, no differences in carotenoid levels compared to the controls were reported. Zeaxanthin was detected in the iris of the Z-fed monkeys, while no measurable amount was detected in the control group. Mean zeaxanthin levels in the ciliary body of the eye were 7.7-fold higher than levels in controls and 3.7-fold higher than levels in the monkeys analyzed 6 months post-supplementation. Mean levels of lutein and *3'*-oxolutein in the ciliary body in Z-fed monkeys did not differ from levels in the control animals. Mean lens zeaxanthin levels in Z-fed monkeys also did not differ from levels in the control animals.

Monkeys in the L/Z-fed group were all sacrificed at 12 months. No retina histopathology was performed in this group and both eyes from all animals were analyzed for carotenoid content. Mean concentrations of Z, L, *meso*-Z and *3'*-oxolutein in the retinas of monkeys in the L/Z-fed group did not differ from levels observed in the control monkeys. In addition, no significant differences were observed in the ciliary body or lens for either carotenoid or their corresponding metabolite. Only significant increases in mean iris levels of lutein and zeaxanthin were reported at 12 months in comparison with the control animals.

Fundus photography and histopathological examination of the retinas revealed no abnormalities in any of the animals used in this study. The retinal pigment epithelium (RPE) of all the monkeys appeared normal and showed a normal distribution of melanosomes and lipofucsin granules. Bruch's membranes were normal and free of deposition. The choroids and their cellular components were also normal. No inflammation or an abnormal number of circulating leukocytes in retinal or choroidal blood vessels was reported. Furthermore, urine samples taken at baseline and months 6, 12 and 18 underwent creatinine analysis and total protein assay. The data did not suggest the production of clinical renal damage to the monkeys. The investigators concluded that long-term supplementation with lutein and or zeaxanthin did not result in ocular or kidney toxicity.

Changes in ocular tissue and plasma levels of zeaxanthin and lutein as a result of supplementation with these carotenoids appear to be transient, with concentrations returning to baseline levels within 6 months after the termination of supplementation. Both lutein and zeaxanthin alter ocular tissue composition, with the greatest impact occurring in the retina. The investigators noted that when lutein and zeaxanthin were fed to the monkeys separately at a high dose (~10 mg/kg-bw/day), zeaxanthin was slightly better absorbed by the retina than lutein,

as the increase in the mean levels of these carotenoids over baseline were 4.3-fold and 3.7-fold, respectively. *meso*-Zeaxanthin was not present in the plasma. Finally, as suggested by the studies above, supplementation of low doses of zeaxanthin in conjunction with relatively high doses of lutein (as represented by the L-fed monkeys) appears to hinder zeaxanthin absorption, allowing for only a 1.4-fold increase in plasma zeaxanthin levels after 12 months. Alternatively, equivalent levels of zeaxanthin and lutein (as represented by the L/Z-fed monkeys) produced a 3-fold increase in plasma zeaxanthin levels within 6 months (Khachik et al. 2006a).

In a separate primate study, the effects of lutein or zeaxanthin supplementation on the serum, macular pigment, adipose tissue and retina of xanthophyll-free rhesus monkeys were investigated (Neuringer et al. 2004, Johnson et al. 2005). In the study, 18 monkeys were fed xanthophyll-free semipurified diets containing adequate levels of all known nutrients including vitamin A and vitamin E from birth until 7 to 16 years of age. These diets were also fed to the mothers of the animals throughout pregnancy. All monkeys also received limited amounts of low-carotenoid or carotenoid-free foods including bananas, cereals and gelatin. The monkeys were fed 3 times per day and had fresh water available at all times. They were maintained on a 12:12-hour light-dark cycle. Animals in this experiment were compared to a reference group of 17 age-matched rhesus monkeys from the same colony consuming a diet with normal levels of carotenoids. This standard stock diet included *trans*-lutein, *trans*-zeaxanthin and β-carotene.

Six monkeys were then assigned to a purified lutein beadlet group (L-fed), 6 to a synthetic zeaxanthin beadlet group (Z-fed); the remaining 6 served as the control group and remained exclusively on the semipurified diet. The lutein beadlets contained all-*trans*-lutein and no detectable zeaxanthin. The zeaxanthin beadlets contained approximately 90% all-*trans*-zeaxanthin and 10% *cis*-zeaxanthin with no detectable lutein. Four monkeys from the L-fed group and 4 monkeys from the Z-fed group were also assigned to receive low levels of n-3 fatty acids, while the remaining 2 animals in each group received adequate levels. All monkeys in the two treatment groups received 2.2 mg/kg-bw/day of their respective carotenoid, which was 7.7-higher than daily xanthophyll intakes in the normal reference group. Supplementation began in three cohorts, at different times of the year, with each cohort including two L-fed and two Z-fed monkeys.

Neuringer and colleagues (2004) followed the L-fed and Z-fed monkeys for up to 56 weeks of supplementation and measured changes in serum and macular pigment content. Supplements were initially given 7 d/wk; the frequency was reduced to 4 d/wk after week 52 for cohort 1, week 44 for cohort 2, and week 15 for cohort 3. Cohorts 1 and 2 continued to receive the supplements 4 d/wk until the end of 56 weeks. Cohort three continued the 4 d/wk supplementation until 24 to 34 weeks. The decrease in frequency of xanthophyll supplementation was due to limited supplies of pure zeaxanthin and lutein.

For all treatment group monkeys, fasting blood samples were obtained from the saphenous or femoral vein before carotenoid supplementation, 2 and 4 weeks after the beginning of supplementation, and every 4 weeks thereafter. For control and reference group monkeys, single samples were taken.

In the normal rhesus monkey reference group, the mean serum lutein concentration was 0.074 μmol/L, all in the *trans* form. Serum zeaxanthin concentration was 0.081 μmol/L, with 72% in the *trans* form and 28% in the *cis* form. Serum lutein in the L-fed monkeys reached a mean concentration of 1.14 μmol/L within the first 4 weeks of supplementation. All serum lutein was in the all-*trans* form and no zeaxanthin was detected. In the same timeframe, serum zeaxanthin in the Z-fed monkeys reached a mean concentration of 0.65 μmol/L. Two-thirds (approximately 67%) of the serum zeaxanthin was in the all-*trans* form and the remaining one-third (approximately 33%) was in the *cis* form. Small amounts of *3'*-didehydrolutein (DDL) were also measured in the serum of some of the members of the Z-fed monkeys. In both treatment groups, serum xanthophyll concentrations remained higher than baseline at all points from 4 weeks onwards. No significant interactions between supplement type and duration between the two treatment groups were observed. Control (xanthophyll-free) monkeys had no measurable serum lutein or zeaxanthin levels (Neuringer et al. 2004).

Macular pigment optical density of the monkeys in the two carotenoid treatment groups was measured by two-wavelength monochromatic fundus reflectometry at baseline and every 4 weeks after the beginning of supplementation until 56 weeks. Macular pigment of the control and reference group monkeys was measured in the same manner on one or two occasions.

Macular pigment optical density in the carotenoid free monkeys (the control group) was zero or very low. In the L-fed monkeys, macular pigment optical density increased over the first 24 weeks of supplementation. In the Z-fed monkeys, macular pigment optical density increased over the first 32 weeks of supplementation. After these periods, no additional consistent increases were observed. No overall differences between the L- and Z-fed monkeys were observed. High levels of lutein or zeaxanthin supplementation did not lead to deposition of crystals within the retina.

No correlation between serum xanthophyll concentrations and macular pigment optical density was found. The investigators theorized that this "may be due to a saturation effect caused by the high serum levels of lutein or zeaxanthin, so that uptake of xanthophylls into the retina, rather than the circulating blood level, was the limiting factor in the accumulation of macular pigment." Furthermore, the only significant correlation found was between the serum concentration of all-*trans*-zeaxanthin and macular pigment density within the Z-fed monkeys. These findings suggest that all-*trans*-zeaxanthin may be a more effective source for macular pigment than *cis*-zeaxanthin (Neuringer et al. 2004).

Johnson and colleagues (2005) analyzed adipose tissue and retina carotenoid levels in the same experimental group of monkeys. Since the monkeys in each treatment group were paired into 3 cohorts, the monkeys analyzed by Johnson et al. (2005) were supplemented for total periods of 24, 14 or 8 months in the Z-fed monkeys and 15, 13 or 6 months in the L-fed monkey group.

Adipose tissue of the control monkeys had no measurable levels of lutein or zeaxanthin. In the Z-fed and L-fed monkeys, samples of subcutaneous tissue were taken from the subscapular region of the back at 2, 4, 8, 12, 16, 20, 24, 36 and 48 weeks of supplementation. In both the L-fed and Z-fed monkeys, adipose tissue carotenoid concentrations increased following 2 weeks of supplementation compared to baseline. Thereafter, concentrations were highly variable,

resulting in no significant differences between the two groups at any time point. When the Z-fed and L-fed monkey values were combined, total xanthophyll concentrations were greater at the end of the study than at the end of the 7 d/wk supplementation period. By the end of the study, total xanthophyll concentrations in the Z-fed and L-fed monkeys were similar to levels found in the normal reference group monkeys.

Retinal samples were taken at either a 4-mm central punch, 8-mm annulus or the periphery of the retina. Extracted samples were analyzed with a reversed-phase, gradient HPLC system. Results from the samples had to be corrected for the interference of an unidentified compound that appeared near the zeaxanthin peak.

No detectable lutein or zeaxanthin was found in the retinas of the control (xanthophyll-free) monkeys. In the 4-mm sample of the normal reference monkeys, the mean total xanthophyll level was 2.91 ± 0.84 pmol/mg. 88% of this was in the all-*trans*-zeaxanthin or *meso*-zeaxanthin form, and the remainder was lutein. Only one monkey had a small amount of detectable *cis*-zeaxanthin. In the 8-mm annulus samples, the mean total xanthophyll level was 0.27 ± 0.04 pmol/mg; only 10% of the concentration found in the 4-mm sample. 63% of this was all-*trans*-zeaxanthin, with the remainder being lutein. No *meso*- or *cis*-zeaxanthin was detected. The peripheral retina of the normal reference monkeys had a mean total xanthophyll concentration of 0.22 ± 0.04 pmol/mg, very similar to the total concentration in the 8-mm annual sample. In the periphery, lutein was the predominant carotenoid, making up 64% of the total xanthophylls. 36% was all-*trans*-zeaxanthin and 1% *cis*-zeaxanthin (Johnson et al. 2005).

In the L-fed monkeys, the mean lutein concentration of the 4-mm macular area was 2.44 ± 0.34 pmol/mg; 8-times that found in the normal reference monkeys. Lutein levels in the 8-mm and periphery samples were 8- and 5-times that of the normal reference monkeys, respectively. Zeaxanthin in the L-fed monkeys was found only in the 4-mm retina samples and only in the *meso*-zeaxanthin form.

In the Z-fed monkey group, no lutein or *meso*-zeaxanthin was identified in any of the retina samples. Ninety-five percent of the zeaxanthin detected was all-*trans*-zeaxanthin. *Cis*-Zeaxanthin constituted the 5% of the remaining xanthophyll content. It was only identified in the 4-mm samples. As with the L-fed monkeys, Z-fed monkeys had significantly higher levels of total xanthophylls in the 8-mm and peripheral samples compared to the normal reference monkeys. Total xanthophyll concentrations in each of the 3 retinal regions did not differ between the Z-fed and L-fed monkeys (Johnson et al. 2005).

Leung and colleagues (2001) supplemented the diets of 3 rhesus monkeys with a Chinese red berry called gou qi zi (*Fructus lycii*). The berry contains naturally high amounts of zeaxanthin and negligible amounts of lutein (300 μg/g and <3 μg/g, respectively). The investigators studied the distribution of zeaxanthin and lutein in the serum, liver, spleen, brain and retina of the monkeys after 6 weeks. Three monkey received a daily supplementation equivalent to 0.5 mg zeaxanthin/kg-bw/day in olive oil through a nasogastric tube. Three monkeys received a vehicle control of olive oil and the other 3 were assigned to a normal reference group. All nine monkeys were fed a normal diet providing approximately 2.1 mg of zeaxanthin + lutein (Leung et al. 2001).

Basal serum levels of lutein in all monkeys were approximately 10-fold higher than those of zeaxanthin. In the 3 Z-fed monkeys, mean serum zeaxanthin levels increased approximately 2-fold over baseline levels by the end of the 6-week treatment period (3.0 ± 1.6 ng/ml to 6.9 ± 4.0 ng/ml). Mean serum lutein levels increased approximately 1.5-fold compared to baseline levels in these monkeys (31.1 ± 12.8 ng/ml to 50.1 ± 26.9 ng/ml).

In both liver and spleen, lutein levels were consistently higher than zeaxanthin. The Z-fed animals appeared to have elevated levels of zeaxanthin, but the differences in zeaxanthin or lutein between the treated and the control groups were not statistically significant because of a large calculated standard deviation. After the retina, the liver was found to have the highest concentration of zeaxanthin (66.6 ± 34.2 ng/g; Z-fed monkeys).

Evaluation of the macular pigment of the retina at 3-mm punch revealed Z-fed monkeys to have almost 2-fold higher zeaxanthin levels than vehicle control monkeys by the end of treatment (1.16 ± 0.34 ng vs. 0.55 ± 0.19 ng, respectively). A significant increase in the density of zeaxanthin was also observed compared to the vehicle control monkeys at the end of treatment (0.041 ± 0.012 ng/mm$^2$ vs. 0.020 ± 0.006 ng/mm$^2$, respectively). Lutein levels among all monkeys were comparable. Peripheral and equatorial samples of the retina showed undetectable levels of lutein and zeaxanthin in all study groups (Leung et al. 2001).

The diet received by all the monkeys in this experiment contained lutein, which may have affected the lutein serum and tissue response observed in the Z-fed monkeys. The investigators noted that zeaxanthin metabolites may have made up part of the measured lutein levels.

Squirrel monkeys (*Saimiri sciureus*) are another type of monkey known to have a macula lutea similar to humans. Snodderly and colleagues (1997) used this primate to monitor changes in plasma carotenoid concentrations. Prior to the supplementation period, two monkeys were first subjected to a 6 week depletion period; another monkey had been on a life-long carotenoid-depleted diet. The supplemental beadlets of zeaxanthin were ≥ 99% all-*trans*-zeaxanthin. Supplementation was gradually increased, beginning with approximately 0.55 mg zeaxanthin/kg-bw/day for 9 weeks and increasing to approximately 2.0 mg zeaxanthin/kg-bw/day for an additional 7 weeks, resulting in a total supplementation period of 16 weeks.

Plasma was analyzed from weekly fasting blood samples drawn from the femoral vein of each monkey. Plasma concentrations responded quickly to alterations in diet. During the 6-week depletion period, plasma all-*trans*-lutein dropped to approximately 20% of baseline concentrations. All-*trans*-zeaxanthin concentrations also dropped rapidly. Rapid changes were also observed during the zeaxanthin supplementation period, with increases in zeaxanthin concentrations reaching relatively stable levels within 2 weeks. Plasma levels of the 13-*cis*-zeaxanthin isomer were also found to increase or decrease with increases or decreases in zeaxanthin supplementation. Plasma lutein was not altered by zeaxanthin supplementation. Furthermore, even during the periods of highest zeaxanthin intake, 2 of the monkeys maintained higher plasma levels of lutein than zeaxanthin (10-20% more) despite ingesting approximately 5-fold more zeaxanthin than lutein. The researchers concluded that plasma's preferential absorbance of lutein was maintained, despite considerable dietary alterations in zeaxanthin intake (Snodderly et al. 1997).

## 5.7.2.2 Quail

Quail are also considered a good animal model for carotenoid research because they appear to possess the appropriate enzymes for conversion of dietary all-*trans*-lutein and all-*trans*-zeaxanthin to the same non-dietary isomers and metabolites observed in humans (Khachik et al. 2002). In a study conducted by Toyoda and colleagues (2002), carotenoid composition of retina, serum, liver and fat in Japanese quail (*Coturnix japonica*) were studied after 6 months of supplementation with biosynthesized all-*trans*-zeaxanthin from *Sphingobacterium multivorum*. Eight quail were in the treatment group and received a carotenoid-deficient diet and zeaxanthin supplementation. Sixteen other quail were equally divided between a normal reference group consuming a commercial turkey diet and a control group consuming the carotenoid-deficient diet.

Males in the Z-fed group consumed 3.5 mg zeaxanthin/kg-bw/day and females consumed 5.04 mg zeaxanthin/kg-bw/day. The male and female Z-fed quails also consumed 0.02 and 0.03 mg lutein/kg-bw/day, respectively. Male and female quail in the normal reference group consumed an average of 0.1 and 0.16 mg zeaxanthin/kg-bw/day, respectively, and 0.29 and 0.45 mg lutein/kg-bw/day, respectively. Quail in the control group consumed 0.02 or 0.04 mg zeaxanthin/kg-bw/day (males and females) and 0.03 or 0.06 mg lutein/kg-bw/day (males and females).

Z-fed quail had a zeaxanthin serum concentration that was 75-fold higher than in the control birds. Serum zeaxanthin and lutein concentrations were directly correlated with the amount of each consumed ($\rho = 0.92$; $p < 0.0001$ and $\rho = 0.74$; $p < 0.004$, respectively, $\rho = 1$ indicates perfect correlation). Moreover, serum lutein tended to correlate with dietary zeaxanthin, but serum zeaxanthin did not correlate with lutein consumption.

The pattern of zeaxanthin concentrations in the liver also closely patterned the differences in dietary intake of this carotenoid. Overall, supplementation with zeaxanthin significantly increased mean zeaxanthin concentrations in each tissue. Zeaxanthin concentrations in fat, liver, and retina correlated significantly and positively with serum zeaxanthin concentrations and with those in other tissues.

The retina of the quail in the Z-fed group preferentially absorbed zeaxanthin. In the Z-fed males and females, zeaxanthin concentrations increased 3-fold and 4-fold from baseline, respectively. In the control and reference quail groups, zeaxanthin concentrations were more than double those of lutein. In the Z-fed quail, however, retina zeaxanthin levels increased to concentrations 20-fold higher than retina lutein levels, with lutein levels actually decreasing in the retina. Overall, the concentrations of lutein and zeaxanthin in the retinas fed the different diets varied far less than the differences in the serum or diet concentrations.

## 5.7.2.3 Mice

Park and colleagues (1998) studied the uptake of dietary lutein and zeaxanthin derived from marigold into the plasma, liver and spleen of mice. A semisynthetic diet was fed to 8-week old female BALB/c mice for 28 days at dosages of 72.7, 145, 295 or 623 mg lutein + zeaxanthin/kg-bw. Lutein was the predominant carotenoid, with zeaxanthin constituting only approximately 1.4% of these intake values. A control group of mice receiving no supplemental lutein or

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zeaxanthin was also studied. A total of 180 mice were included in the study with 36 mice in each group. Thus, 144 received one of the 4 dosages of lutein + zeaxanthin.

Food and water were available *ad libitum* and body weight and food intake was measured weekly. Six mice from each treatment group were sacrificed on days 0, 3, 7, 14, 21 and 28 of the study. Blood plasma, liver and spleen were collected.

Lutein and zeaxanthin were the only carotenoids found in the plasma of the treatment group mice. The control mice had no detectable levels of any carotenoids. Plasma lutein and zeaxanthin carotenoid concentrations increased rapidly through day 3 of supplementation. By day 7, concentrations did not differ among the lutein + zeaxanthin-treated mice (2.58 ± 0.2 µmol/L).

Similar to the plasma results, concentrations of lutein + zeaxanthin in the liver of the treated mice increased rapidly in a dose-dependent manner by day 3 of the study (ranging from 0.62 nmol/L to 0.95 nmol/L). At day 3, the highest dose lutein + zeaxanthin group had the highest concentrations of lutein + zeaxanthin and was significantly different from the other treatment groups and the control. Liver lutein + zeaxanthin concentrations generally continued to increase throughout the study, with the highest-dose group liver concentrations reaching a plateau after day 14.

Spleen lutein + zeaxanthin concentrations also increased rapidly by day 3 but then continued to gradually increase throughout the remainder of the study. This increase was dose- and time-dependent, with the highest dose treatment group having significantly higher concentrations than the lower treatment and control groups throughout the entire study.

None of the animal studies presented identified any adverse events, such as kidney toxicity or retinal crystallization as a result of zeaxanthin and/or lutein supplementation. No reports of clinical illness or abnormalities were reported by any of the researchers.

## 5.8   Bioavailability of Zeaxanthin from Food and Supplemental Sources

### 5.8.1  Factors Affecting Bioavailability

The bioavailability of xanthophylls is affected by several factors, including the food matrix and processing, structure of the carotenoid molecule, interactions with other nutrients (mainly dietary fat) and nutritional status (Zaripheh and Erdman 2002). Studies also suggest that lutein diester is more bioavailable than unesterified lutein (Bowen et al. 2002), and that the bioavailability of zeaxanthin dipalmitate is greater compared to the non-esterified form of zeaxanthin (Breithaupt et al. 2004), although Chung et al. (2004) found no difference in the bioavailability of lutein vs. lutein esters. Little research has been conducted specifically regarding the bioavailability of zeaxanthin.

## 5.8.2 *In Vitro* Studies

A step in the absorption process of carotenoids that may affect their bioavailability involves the incorporation of released carotenoids into mixed micelles. Micellarization of carotenoids is dependent on the ingestion of fat and its presence in the intestine. *In vitro* studies have been conducted to assess efficiency of micellarization of carotenoids and differences in transport and absorption. Results from these studies suggest that micellarization and absorption of lutein and zeaxanthin are comparable, the micellarization from green leafy vegetables may be lower than micellarization from yellow and orange fruits and vegetables, and that micellarization of xanthophylls in oil-based supplements may be higher as compared to xanthophylls in a food matrix.

The bioaccessibility of Chrysantis ZEC was compared to the bioaccessibility of zeaxanthin purified from wolfberry in an *in vitro* digestion study using a Caco-2 model system (Appendix 4). In this study, oil enriched with zeaxanthin from the Extract Concentrate or purified zeaxanthin from wolfberry was mixed with yogurt. Two samples of zeaxanthin from the Extract Concentrate were used; sample number two was micronized prior to the testing. The percent recovery of zeaxanthin in digesta from the Extract Concentrate was 84 and 105% in the two samples respectively, and the percent recovery from wolfberry was 92.5%. The percent micellarization did not differ between the Extract Concentrate and wolfberry sources, with values in all samples ranging from 71.6 to 76.9%. The percent uptake of micellarized zeaxanthin by Caco-2 cells also did not vary by source. The percent uptake from the two Extract Concentrate samples was 10.9 and 10.4%, respectively, and the percent uptake from wolfberry was 12.2%. Results from this study indicate that the bioaccessibility of zeaxanthin from Chrysantis ZEC is comparable to the bioaccessibility of zeaxanthin purified from wolfberry.

Chitchumroonchokchai and colleagues (2004) assessed the efficiency of micellarization of carotenoids from spinach and a lutein supplement after simulated digestion. In this *in vitro* experiment, the efficiency of micellarization measurement represented the percentage of the carotenoid in the test food that was transferred to the filtered aqueous fraction during simulated digestion. The percent micellarization of trans-lutein and trans-zeaxanthin in spinach puree was slightly more than 50% for each carotenoid, and greater than the percent micellarization of cis-lutein or β-carotene (approximately 25-40%). The percent micellarization of trans-lutein and trans-zeaxanthin from the oil-containing lutein supplement was in the range of 70-80%, which was significantly higher than the percent micellarization from the spinach puree. The results suggest that differences in the efficiency of micellarization of ingested carotenoids in the small intestine may have a marked effect on the extent to which the compounds are absorbed.

In another *in vitro* study, foods rich in zeaxanthin, including wolfberry, orange pepper, red pepper and squash were subjected to simulated gastric and small intestinal digestion and the efficiency of micellarization of zeaxanthin was measured (Chitchumroonchokchai and Failla 2006). The combined mean micellarization of the free zeaxanthin, zeaxanthin monoesters, and zeaxanthin diesters from the digested foods were 81 ± 8, 44 ± 5, and 11 ± 4%, respectively. When exposed to micelles generated during digestion of the test foods, zeaxanthin uptake by Caco-2 cells was proportional to the medium content. Free zeaxanthin was the most abundant form in Caco-2 cells, although zeaxanthin monoesters also were detected.

The *in vitro* bioaccessibility of carotenoids from various foods was assessed by measuring the micellarization of carotenoids in a gastrointestinal model (Granado-Lorencio et al. 2007). In this study, approximately 7% of the zeaxanthin measured in fresh spinach and 6% of the zeaxanthin in lettuce were recovered in the micellar phase after *in vitro* digestion, while approximately 54, 48 and 39% of zeaxanthin measured in sweet corn, red peppers and oranges, respectively were recovered in the micellar phase. The percent recovery of lutein from spinach and lettuce were 5 and 14%, respectively, and recovery of lutein from sweet corn, red peppers and oranges was 59, 66 and 26%, respectively. Results from this *in vitro* study suggest that the accessibility of carotenoids from food varies by type of food. In general, the micellarization of xanthophylls from leafy green vegetables was considerably lower than micellarization from yellow and orange vegetables and fruits.

O'Sullivan and colleagues (2007) examined the percentage transport of various carotene and xanthophyll supplements through a differentiated Caco-2 cell monolayer culture and compared the differences in absorption of individual carotenoids at varying amounts (0.5, 1, 1.5, 2, and 2.5 µg). The percentage transfer of lutein to the basolateral chamber ranged from a mean (± SEM) of 2.3 ± 1.4 to 10.5 ±2.4 %. When the percentage transfer to the basolateral chamber of lutein was compared at all amounts added, the 0.5 µg amount differed significantly from all others. The percentage transfer of zeaxanthin to the basolateral chamber ranged from 4.5 ± 2.3 to 11.8 ± 3.9 %. The 0.5 µg amount had a significantly higher percentage transfer than 2.5 µg zeaxanthin. The total cellular uptake and secretion of lutein ranged from 5.1 ± 1.2 to 20.2 ± 3.3 %; this range was similar to the range of 5.5 ± 2.5 to 13.4 ± 4% observed for zeaxanthin. At each concentration, there was no significant difference in the percent transfer or the total uptake and secretion between lutein and zeaxanthin. These data indicate that lower amounts of carotenoids were absorbed and transferred more efficiently than higher amounts, which suggests a saturation effect at higher exposure.

### 5.8.3   *In Vivo* Studies

The relative bioavailability of lutein from foods compared to supplements and lutein from foods compared to other carotenoids has been assessed in *in vivo* studies. Results from some of these studies indicate that lutein provided in a supplement is more bioavailable as compared to lutein in a food matrix, though other studies have shown no difference between these two forms in lutein bioavailability. The available evidence also indicates that xanthophylls in eggs are highly bioavailable.

In a crossover design study, serum lutein responses were compared following ingestion of approximately 6 mg lutein from spinach, lutein supplements, lutein ester supplements, or lutein-enriched eggs each for a period of 10 days (Chung et al. 2004). All test meals contained approximately 20 g fat (55-60% of energy) to ensure optimal lutein absorption. Baseline and dose-adjusted lutein responses were significantly higher after egg consumption than after consumption of the other forms of lutein. The lutein bioavailability from lutein supplements, lutein ester supplements or spinach did not differ. Results from this study suggest that lutein from lutein-enriched eggs is highly bioavailable.

van het Hof et al. (1999) reported that the relative bioavailability of lutein from vegetables compared to FloraGLO® crystalline lutein was 67%. Similarly, Castenmiller et al. (1999) reported that the relative bioavailability of lutein was 52% from whole leaf, minced, or enzymatically liquefied spinach as compared to the bioavailability from FloraGLO® crystalline lutein. Mean plasma lutein concentrations following ingestion of a single supplement containing 9 mg lutein (lutein vs. lutein ester content not specified) were reported to be higher as compared to the plasma lutein concentration following ingestion of a single dose of spinach containing 9.2 mg lutein (Riso et al. 2003).

## 5.9 Safety of Related Carotenoids and Impurities in the Zeaxanthin Purified Extract Concentrate

The primary carotenoids in Chrysantis ZEC are zeaxanthin and lutein. Other carotenoids including antheroxanthin, violaxanthin, canthaxanthin, mutatoxanthin, β-cryptoxanthin, and β-carotene also have been identified in the purified concentrate extract (Table 2-2 and Table II-4 of the 2007 dossier).

### 5.9.1 Antheraxanthin and Violaxanthin

Antheraxanthin and violaxanthin are epoxy analogs of lutein and zeaxanthin. Antheraxanthin (5,6-epoxyzeaxanthin) is a metabolic intermediate in plants that is reversibly formed from zeaxanthin or violaxanthin depending on the presence of light and/or nitrogen or oxygen. A schematic of this is presented in Figure 9. Violaxanthin (5,6,5',6'-diepoxyzeaxantin) is a diepoxide derivative of zeaxanthin. In photosynthetic tissues of plants, violaxanthin is converted in the presence of light and a source of nitrogen via an enzymatically catalyzed reduction of the epoxide groups to form antheraxanthin and then zeaxanthin (Bamji and Krinsky 1965; Latowski et al. 2000). In the dark, and with the presence of free oxygen, the same sequence of reactions is reversible and zeaxanthin can be converted first to antheraxanthin and then to violaxanthin.

**Figure 13. Xanthophyll Cycle (Violaxanthin Cycle)**
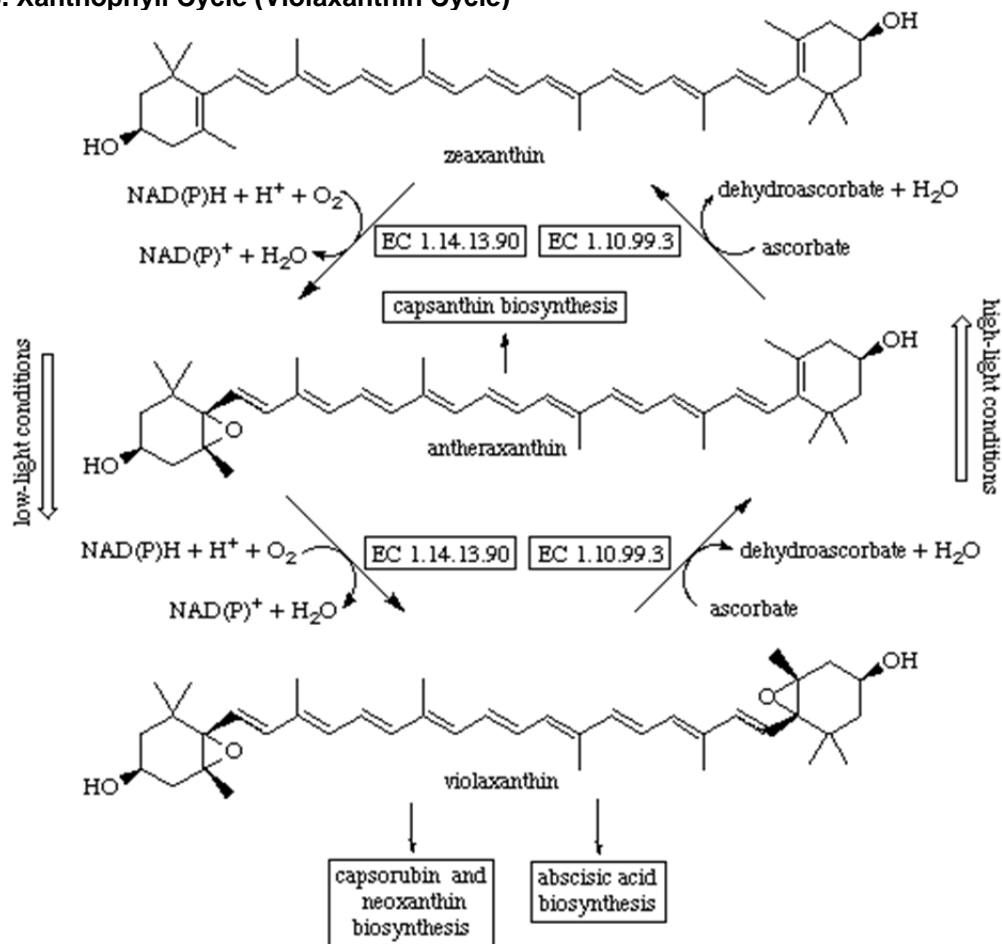


Source:International Union of Biochemistry & Molecular Biology (IUBMB) (2004).

Epoxy-xanthophylls that commonly occur in edible plant tissues have not been identified in human blood or milk (Khachik et al. 1997; Kostic et al. 1995). In a study in which three individuals consumed a single 16.6 µmol dose of pure violaxanthin in oil, analysis by HPLC of plasma samples collected 3, 6 and 9 hours after ingestion showed no traces of violaxanthin or any of its metabolites (Barua and Olson 2001). Additionally, the area of peaks existing at baseline did not increase significantly in any of the plasma extracts. In the same study, there was no plasma response following ingestion of lutein 5,6-epoxide (taraxanthin), another xanthophyll epoxide.

As previously discussed in 3.1.1, antheraxanthin and violaxanthin are found in foods at concentrations ranging from 2 to 3863 µg/100 g and 5 to 7400 µg/ 100 g, respectively (Table 3-2). Based on the detected levels of antheraxanthin and violaxanthin in Chrysantis ZEC from Table II-4 of the 2007 dossier, it can be assumed that individuals consuming the concentrate at 90th percentile daily intake levels would ingest 18 µg and 12 µg of antheraxanthin and violaxanthin, respectively. These levels of intake are considerably lower than intakes provided by naturally occurring dietary sources of these carotenoids. Additionally, based on the findings presented above, the antheraxanthin and violaxanthin consumed from use of the Chrysantis ZEC in foods are not likely absorbed or metabolized.

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### 5.9.2 Mutatoxanthin

Mutatoxanthin is another carotenoid present in some fruits and vegetables. It is believed to be biosynthesized via epoxidefuranoxide rearrangement of antheraxanthin (Rodriguez-Amaya 2001). In certain types foods such as mangos, concentrated orange juice and asparagus, it can be present at concentrations of 8 to 4448 µg/100 g (Table 3-2). Based on the detected levels of mutatoxanthin in Chrysantis ZEC from Table II-4 of the 2007 dossier, it can be assumed that individuals consuming the concentrate at 90th percentile daily intake levels would ingest 9 µg of mutatoxanthin. This amount of mutatoxanthin is a small fraction of the amount consumed in a typical serving of orange juice or mango.

### 5.9.3  β- Carotene and β- Cryptoxanthin

β-carotene and β-cryptoxanthin are commonly present in many foods consumed by humans. The two carotenoids are related through a biosynthetic pathway in which β-carotene can convert to β-cryptoxanthin via a hydroxylation reaction (Rodriguez-Amaya 2001). Based on the detected levels of β-carotene and β-cryptoxanthin in Chrysantis ZEC from Table II-4 of the 2007 dossier, it can be assumed that individuals consuming the concentrate at 90th percentile daily intake levels would ingest 2 µg and 5 µg of β-carotene and β-cryptoxanthin, respectively. These are trivial amounts of the carotenoids as compared to intakes from naturally occurring dietary sources as shown in Table 3-5.

### 5.9.4 Canthaxanthin

Canthaxanthin is a carotenoid that is present in many plants and has been reported to cause a crystalline retinopathy in animals and humans that consume this compound as a dietary supplement (Goralczyk et al. 1997, 2000; Kopcke et al. 1995). In monkeys administered 5.4, 16.2 or 48.6 canthaxanthin mg/kg-bw/day for 2.5 years, inclusions were found in the peripheral retina of all monkeys although the presence of these deposits did not interfere with morphology or retinal function (Goralczyk et al. 1997). In a subsequent dose-response study, one group of monkeys received 0.2, 0.6, 1.8, 5.4, 16.2, or 48.6 mg canthaxanthin/kg-bw/day for 2.5 to 3 years; a second group consumed 200 and 500 mg canthaxanthin/kg-bw/day for 5 years (Goralczyk et al. 2000). The threshold level of canthaxanthin crystals was determined to be 0.6 mg canthaxanthin/kg-bw/day. The grade of crystals increased up to a dose of 16.2 mg canthaxanthin/kg-bw/day. No adverse effects on visual function as measured by electroretinography (ERG) were observed. In a biostatistical evaluation of 411 human subjects with canthaxanthin deposition, the minimum dosage at which crystals were seen in any reported case was at least 30 mg canthaxanthin/day, with no crystals appearing in patients ingesting levels below this dosage (Kopcke 1995).

Canthaxanthin is used in the U.S. as a color additive. Canthaxanthin may be safely used in foods and ingested drugs in amounts consistent with Good Manufacturing Practices (GMP) (21 CFR §73.75; 21 CFR §73.1075). Canthaxanthin is permitted to be used at levels not exceeding 66.7 mg/kg of solid or semisolid food, 4.41 mg/kg of broiler chicken feed, and 80 mg/kg of fish feed (21 CFR §73.75).

Based on existing biological and toxicological data, the World Health Organization (WHO) has set the Acceptable Daily Intake (ADI) of canthaxanthin for humans at 0 to 0.03 mg/kg-bw (FAO/WHO 2000). For a 60 kg adult, intake based on this ADI corresponds to a maximum of

1800 µg canthaxanthin/day. The Scientific Committee on Food has established an ADI of 0.025 mg canthaxanthin/kg-bw/day, rounded up to 0.03 mg canthaxanthin/kg-bw/day (EC 1997). This ADI also corresponds to a daily intake of 1800 µg canthaxanthin/day.

Based on the detected levels of canthaxanthin in Chrysantis ZEC from Table II-4 of the 2007 dossier, it can be assumed that individuals consuming the concentrate at 90th percentile daily intake levels would ingest approximately 9 µg of canthaxanthin, or 0.16 µg/kg-bw for a 60 kg person. This level of intake is considerably lower than the current ADI established for canthaxanthin.

## 5.9.5  Thiophenes

*Tagetes* is part of the *Compositae* family of plants. This group of plants contains naturally occurring compounds, including thiophenes, that are believed to be responsible for the insecticidal activity of the plant (Wells et al. 1993). Thiophenes have been identified in several species of *Tagetes*, including *Tagetes erecta.* Thiophenes exhibit phototoxic properties (Wells et al. 1993), and thiophenes present in marigold extracts have been reported to have sensitizing capacity in animals and to cause contact dermatitis in humans (Rampone et al. 1986; Hausen and Helmke 1995; Towers et al. 1979; Chan et al. 1977).

In a study conducted by Rampone et al. (1986), $\alpha$-terthienyl isolated from marigolds was intradermally applied to guinea pig skin at concentrations of 0.1% and 1% in a 3% azone gel vehicle. Cutaneous phototoxicity was observed at topical application concentrations of 1%. Through an *in vitro* analysis, it was determined that although the thiophene affected cell membranes at these concentrations, it did not appear to induce cytogenetic damage.

Hausen and Helmke (1995) reported sensitizing experiments in guinea pigs with thiophenes from a marigold extract of flowers and stems. Testing at three different concentrations (1%, 3% and 10%), the investigators determined the irritation threshold to be higher than 3%, as no animals responded to this concentration of the raw extract. A second group of animals was sensitized several months later and became more strongly sensitized than the first group. In sensitized animals, $\alpha$-terthienyl was noted to be an irritant at 1%. A 1% solution of $\alpha$-terthienyl in white petroleum produced no reactions in patch tests on humans. Results from this study therefore show that $\alpha$-terthienyl from marigold extracts possesses sensitizing capacity in addition to phototoxic effects in animals.

Towers and colleagues (1979) demonstrated that the thiophene $\alpha$-terthienyl (from an unspecified plant source) causes contact dermatitis in humans. When patch tests were conducted using the compound on skin, it was found to be phototoxic by evoking erythema, blistering and hyperpigmentation.

Similar results were found when three people (1 female and 2 males) were exposed to $\alpha$-terthienyl (Chan et al. 1977). The compound was derived from *Tagetes* and a 1% extract in petrolatum album was applied to the skin in two locations. Both areas were covered in patches, with one serving as the control and the other as the test area. After allowing the compound to remain on the skin for 24 hours, the test patch was removed and the site was exposed to UVA for up to 20 minutes. Burning pain and erythema were noted between 7 and 17 minutes. Histopathological examination of a skin biopsy specimen from one of the male subjects showed dermal inflammation with polymorphonuclear invasion but no epidermal changes. However, epidermal changes were found in a skin biopsy specimen from the female subject.

As shown in Table II-4 of the 2007 dossier, Chrysantis ZEC was analyzed for the presence of thiophene 2,2′5′2″ terthiophene, and in all three samples tested, the concentration of 2,2′5′2″ terthiophene was below the limit of detection (LOD) of 50 mg/kg.

### 5.9.6 Waxes

Waxes are defined as esters formed between long chain fatty acids and long chain alcohols. As shown in Table II-4 of the 2007 dossier, Chrysantis ZEC contains waxes of chain lengths $C_{29}$, $C_{31}$ and $C_{33}$. Analysis of the Extract Concentrate showed that levels of $C_{30}$, $C_{32}$, $C_{34}$ and $C_{35}$ were below the limit of detection.

Waxes are relatively abundant in cereal grains, bran, germ, leaves, seeds, nuts and unrefined oil. In a review conducted by Hargrove and colleagues (2004), wheat, oats and rice were reported to contain 0.1-0.9 g waxes/100 g plant (dry weight).

Based on the detected levels of waxes in Chrysantis ZEC from Table II-4 of the 2007 dossier, approximately 1 µg of waxes are present in each 100 µg of Chrysantis ZEC. Individuals consuming Chrysantis ZEC at the 90th percentile of intake are estimated to consume 5562 µg of the ZEC, which contains approximately 56 µg of waxes. This amount is far less than that obtained from other common food sources. For example, waxes of similar chain length to those found in the Chrysantis ZEC are naturally present on the surface of apples, and constitute approximately 0.1% of the total fruit weight of Washington State apples (Kolattukudy 1984). Consumption of a medium size (138 g) apple therefore results in intake of about 137 mg wax.

### 5.9.7 Other Substances

Chrysantis ZEC contains a number of additional substances, including cellulose, lignin, protein, fat, moisture, and ash (Table II-4 of the 2007 dossier). These constituents are present in high concentrations in most foods consumed by humans, and therefore are not safety concerns.

## 5.10 Corroborative Safety Data: Toxicology Studies With Chrysantis ZEC

Chrysantis provided ENVIRON toxicological studies conducted on their purified zeaxanthin extract concentrate (Appendix 5). These studies are discussed in detail below and summaries are presented in Table 5-1. Results from these unpublished studies provide corroborative evidence of the safety of the ingestion of Chrysantis ZEC.

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## 5.10.1 Genotoxicity

There were two genotoxicity studies conducted with Chrysantis ZEC by Next Century Incorporated (Stirparo 2005a, 2005b). Both studies were conducted in compliance with US FDA and US EPA Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice published in ENV/MC/CHEM(98)17. The studies met or exceeded FDA Redbook 2000 and OECD guidelines with minor exceptions that the authors report do not affect study validity. The first study reported a positive result in a reverse mutation assay with a single strain (TA98) of *Salmonella typhimurium*, however, because there were uncertainties about the validity of this result, a second reverse mutation assay was conducted. The second study reported a negative result in this particular strain. The results of both genotoxicity studies are described below.

In an initial test for mutagenic activity, Chrysantis ZEC (batch number 257-MAY04-001) was tested in the Ames reverse mutation assay using *Salmonella typhimurium* strains TA1535, TA97a, TA98 and TA100 and *E. coli* strain WP2 *uvr*A (3287) (Stirparo 2005a). Tests were conducted both in the presence and absence of an Aroclor 1254-induced, rat liver "S9" homogenate metabolic activation system using both a standard plate-incorporation bioassay and a confirmatory preincubation test protocol. Eight concentrations of the purified extract: 5, 10, 50, 100, 500, 1000, 2500, and 5000 μg/plate were tested in the plate incorporation assay and concentrations of 100, 500, 1000, 2500, and 5000 μg/plate were tested in the preincubation confirmatory test using dimethyl sulfoxide (DMSO) as the solvent. Negative control cultures treated with DMSO alone were included in each test. Toxicity from the test article, as would be evident by a concentration-related reduction in mean number of revertants per plate and/or the reduction of the background growth of the bacterial "lawns," was not reported in any of the cultures. The mean number of revertants observed in the negative controls (DMSO) for each of the tester strains was within acceptable historical negative control ranges. All tester strains demonstrated appropriate phenotypic characteristics.

The results of the assays showed no positive increases in the incidence of mutants in any strain except for strain TA98. In that strain, in both the plate incorporation assay and in the preincubation test, increases in mutants of approximately 2 to 3 times the mean control value were observed. This was considered to be indicative of a positive result in this single strain, according to New Century's scoring criteria for this test system, but there was no indication of a dose-related increase in mutation frequency, and most of the responses were within the normal historical control range for the TA98 strain (5-50 revertants/plate), thus rendering the responses equivocal. The indications of mutagenic activity in strain TA98 were seen both in the presence and absence of a metabolic activation system in the plate incorporation test but only *without* metabolic activation in the preincubation test protocol, adding to the equivocal nature of the observed responses, as the preincubation assay is intended to provide a more extensive involvement of the bacterial culture with the test article and metabolic activation system.

Because of the equivocal nature of the responses in the first assay, a repeat Ames assay was performed in which another batch of Chrysantis ZEC, prepared by the same purification process as the batch evaluated in the first assay. The test substance was evaluated for mutagenicity using only *Salmonella typhimurium* (Ames) strains TA98 and TA100 (Stirparo 2005b). Tests were conducted both in the presence and absence of Aroclor® induced rat liver "S9"

homogenate metabolic activation system using both a standard plate-incorporation bioassay and a confirmatory preincubation test protocol. As in the first Ames test, eight concentrations of the purified extract from 5 to 5000 µg/plate were tested in the plate incorporation assay and concentrations from 100 to 5000 µg/plate were tested in the preincubation confirmatory test using DMSO as the solvent. Negative control cultures treated with DMSO alone were included in each test.

The results showed slight toxicity, as evident by reduced background microcolony lawns, in strain TA100 at concentrations of 1000 µg/plate and above in the assay without activation, and at 5000 µg/plate with metabolic activation. However, no toxicity was reported with strain TA98 at any concentration. No indication of positive mutagenic activity was reported, either in the plate incorporation or the suspension mutagenicity assays in either tester strain as defined by New Century's criterion of a doubling of the solvent control values, or by the more scientific criterion of a dose-related increase in mutant frequency. The investigators concluded that, under the conditions of this second Ames assay, "zeaxanthin purified concentrate from marigold is…negative for the induction of mutagenicity in the bacterial reverse mutation test."

Based on the equivocal nature of the responses in the first study and the definitively negative results of the second study, it is reasonable to conclude that Chrysantis ZEC is not mutagenic.

## 5.10.2 Acute Mammalian Studies

Chrysantis ZEC (lot number: 257-MAY04-001) was tested for acute oral toxicity in 3 female Sprague-Dawley derived albino rats using the "up and down" protocol by Product Safety Laboratories (PSL 2004). This study was conducted in compliance with Good Laboratory Practice (GLP) regulations as defined in US FDA GLP Standards (21 CFR § 58), US EPA Health Effects Test Guidelines (OPPTS 870.1100, 2002), and OECD Guidelines for Testing of Chemicals (Procedure 425, 2001). ENV/MC/CHEM(98)17. The rats were individually housed in suspended stainless steel cages with mesh floors and acclimated for a period of 17 to 21 days. Room temperature ranged from 21-25ºC throughout the study and rats were kept on a 12-hour light/dark cycle. The rats were fed Purina Rodent Chow #5012 feed and filtered tap water was supplied *ad libitum*. An initial limit dose of 5000 mg/kg-bw of the test substance dissolved in corn oil was administered to one female by gavage. Due to the absence of mortality in this rat, 2 additional females were treated with the same dose. The 5000 mg/kg-bw zeaxanthin concentrate was administered by gavage as a 30% w/w suspension in corn oil in two doses given approximately 2 hours apart. The test substance contained approximately 356 g xanthophylls per kg (dry basis), with a carotenoid profile of approximately 85% zeaxanthin, 9% lutein and 4% epoxides.

All rats were observed for mortality, signs of gross toxicity, and behavioral or neurological changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on days 7 and 14 of the study. Necropsies were performed on all rats at terminal sacrifice and tissues and organs of the thoracic and abdominal cavities were examined.

No mortality or signs of toxicity were observed and all rats gained weight during the 14-day observation period. Two rats exhibited a brown oily stain on the base of their tails; this finding

disappeared by day 2 of observation. No gross abnormalities were observed in tissues or organs at necropsy. The acute oral $LD_{50}$ for the Chrysantis ZEC was concluded to be >5000 mg/kg-bw in female rats.

## 5.10.3 Subacute Mammalian Studies

A 10-day dietary pilot study in Sprague Dawley rats (PSL 2005a) was conducted to identify a suitable dose of Chrysantis ZEC (lot number: 257-MAY04-001) for testing in a subsequent 28-day study by Product Safety Laboratories (PSL 2005b). Twenty-four healthy rats (12 males and 12 females) were selected for the test and equally distributed into 8 groups (3/sex/group). Rats were individually housed in suspended stainless steel caging with mesh floors. The room temperature and humidity were controlled and the rats were maintained on a 12-hour light/dark cycle. During the 6-day acclimation period, rats were fed Purina Certified Rodent Meal #5002 and filtered water *ad libitum*. During the study, rats received the test substance or control vehicle in their feed; both feed and filtered water continued to be supplied *ad libitum* throughout the study.

Dietary levels of 0, 3200, 4600 or 6500 ppm of the test substance were administered to the rats. The carotenoid profile of the concentrate was approximately 78% zeaxanthin, 4% lutein and 15% epoxides. The dietary concentration of zeaxanthin was adjusted for a concentrate purity of approximately 33% zeaxanthin. The mean overall daily intake of Chrysantis ZEC in male rats fed dietary concentrations of 0, 3200, 4600 and 6500 ppm was 0, 355, 490 and 673 mg zeaxanthin/kg-bw/day, respectively. For the same dietary concentrations, the mean overall daily intake of Chrysantis ZEC in female rats was 0, 340, 451, and 604 mg/kg-bw/day, respectively.

Individual food consumption was measured and recorded on days 3, 7, and 10 (adjusting for spillage). Mean food consumption was calculated for each group during each interval and the overall testing period. Mean food efficiency was also calculated for each group based on mean daily body weight gain and mean daily food consumption data. Individual body weights were recorded twice during the acclimation period, and at days 0, 3, 7, and 10 of the study. Mean body weight gains were calculated for each group at each interval and for the overall testing period.

All rats were observed twice daily for mortality. Clinical observations were made daily during the study. Observations included, but were not limited to, gross evaluation of skin, fur, eyes, mucous membranes, occurrences of secretions and excretions, respiration, circulation, autonomic and central nervous systems, somatomotor activity and behavior patterns. Particular attention was directed to changes in gait, posture, and salivation.

On day 10, all rats were euthanized via $CO_2$ inhalation. All euthanized rats were subjected to a full necropsy, which included examination of the external surface of the body, all orifices and the thoracic, abdominal and cranial cavities and their contents.

There were no mortalities or clinical signs of toxicity observed in any of the test-group rats throughout the study. No differences were reported in body weights, clinical observations, nutritional status, or gross alterations at necropsy between the treated and control groups. Due to the color of the test substance, the intestines of all rats in the treated groups appeared to be

stained slightly red. Under the conditions of the study, investigators established a No Observed Adverse Effect Level (NOAEL) of 673 and 604 mg Chrysantis ZEC /kg-bw/day for males and females, the highest dose tested..

In a 28-day study conducted by Product Safety Laboratories (PSL 2005b), 40 Sprague-Dawley derived rats (20 males and 20 females) were selected, randomized, and distributed (5/group; males or females only) into test groups and were administered Chrysantis ZEC or control substance in their feed. Housing and feeding conditions were similar to those described in the 10-day study. This study was conducted in compliance with Good Laboratory Practice (GLP) regulations as defined in OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17, OECD, Paris, 1998 and in accordance with OECD Guidelines for the Testing of Chemicals, Section 4 (Part 407): Health Effects (1987).

Dietary concentrations of 0, 65, 650 and 6500 ppm of Zeaxanthin Chrysantis ZEC (batch number: 257 AGO-08) were administered to the rats. Study target doses of the test substance were set at 6, 60 and 600 mg/kg-bw/day. The tested extract was 26.5% zeaxanthin, which comprised 84% of the 31.7% of total carotenoids. The amount of test substance added to the feed was adjusted for the 26.6% zeaxanthin purity, resulting in actual mean dietary intakes of zeaxanthin purified concentrate during the study, based on food consumption data, of 0, 5, 54 and 548 mg/kg-bw for males and 0, 6, 55 and 549 mg/kg-bw for females. Therefore, the rats received doses of approximately 19, 204, and 2068 mg Chrysantis ZEC/kg-bw for males and 23, 207, and 2072 mg Chrysantis ZEC/kg-bw for females.

Cage-side observations, food consumption and body weight and weight gain were monitored as discussed in the 10-day study. Detailed clinical observations were also conducted weekly on all rats. For these evaluations, each rat was removed from its cage so that its reaction to handling and its behavior in an open field could be assessed. The observations recorded for each rat included, but were not limited to, changes in skin, fur, eyes and mucus membranes, occurrence of secretions and excretions and autonomic activity (such as lacrimation, piloerection, pupil size, unusual respiratory pattern), changes in gait and posture. Response to handling, as well as the presence of clonic or tonic movements, repetitive activities (such as excessive grooming or repetitive circling), difficult or prolonged parturition or bizarre behavior (such as self-mutilation or walking backwards) was also monitored.

Blood samples for hematology and clinical biochemistry were collected from all rats after overnight fasting on day 28 of the study period. Samples were taken via orbital sinus bleeding under isoflurane anesthesia. Blood samples were collected via the inferior vena cava under isoflurane anesthesia prior to terminal sacrifice on day 30 to determine the prothrombin time and partial thromboplastin time. Upon completion of clinical chemistry, remaining serum samples were pooled for serology.

The hematological parameters examined included: erythrocyte count, hematocrit, mean corpuscular hemoglobin, absolute reticulocyte count, total white blood cell, prothrombin, hemoglobin concentration, mean corpuscular volume, red cell distribution width, platelet count, differential leukocyte count, and activated partial thromboplastin time. Mean corpuscular hemoglobin concentration was also calculated. If platelet clumps were observed on blood smears, these findings were recorded.

The clinical chemistry parameters examined included: serum aspartate aminotransferase, sorbital dehydrogenase, total bilirubin, blood creatinine, triglycerides, total serum protein, globin, inorganic phosphorus, potassium, serum alanine aminotransferase, alkaline phosphatase, urea nitrogen, total cholesterol, fasting glucose, albumin, calcium, sodium and chloride.

At terminal sacrifice, all rats were euthanized and subjected to a full necropsy that included examination of the external surface of the body, all orifices, and the thoracic, abdominal and cranial cavities and their contents. The organ-to-body-weight ratio of the following organs was determined: liver, brain, uterus, kidneys, heart, ovaries or testes, adrenals, spleen, and the epididymides.

Organs preserved in 10% neutral buffered formalin for possible future histopathological examination included: all gross lesions, lungs, trachea, brain, spinal cord, thymus, heart, sternum with bone marrow, adrenals, liver, spleen, kidneys, thyroid/parathyroid, urinary bladder, ovaries, testes, uterus, vagina, esophagus, ileum, cecum, accessory genital organs, peripheral nerve (sciatic), stomach, duodenum, jejunum, colon, rectum, lymph node, pancreas and salivary gland. Histopathological examination was performed on the preserved organs and tissues of the rats from the control and high-dose groups.

Mean and standard deviations were calculated for all quantitative data. If warranted by sufficient group sizes, the treated and control groups were compared using a One-Way Analysis of Variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's Multiple Comparisons test. Data was evaluated for homogeneity of variances and normality by the Bartlett's test and male and female rats were evaluated separately.

There were no mortalities during the study. One rat in the mid-dose test group exhibited red ocular discharge on test days 7 and 8. This finding was isolated and investigators considered it "not to be of toxicological importance." Orange staining of the fur was noted in the mid- and high-dose males and in the high-dose females. Investigators found this observation to be the "result of the test diet in contact with the rat's fur during feeding…[and] thus…considered unrelated to systemic exposure and not to be of toxicological significance."

Mean body weights and mean daily body weight gains for all groups of female rats were comparable to control values. The same was reported for all groups of male rats. However, there was a statistically significant decrease in high-dose male mean daily body weight gain during week 1. This finding was isolated and not dose-related. Therefore, investigators considered this finding "not to be of toxicological importance." Overall mean daily food consumption and mean daily food efficiency for all groups of male and female rats were comparable to control values.

No treatment-related effects in hematology parameters were reported. A statistically significant decrease in the average mean cell hemoglobin concentration in rats fed mid-dose levels [give the dose!] of the test substance was "considered to be unrelated to treatment because it was not dose related." No adverse effects in coagulation or clinical chemistry parameters were reported.

No gross abnormalities of toxicological significance were reported for any of the rats necropsied at the conclusion of the 29-day observation period. Mean absolute and relative organ weights were comparable to control with the exception of high-dose female rats that had a statistically significant increase in adrenal-to-brain weight ratio. Given the absence of a dose-related event or correlation to any histomorphological changes in the adrenals, investigators concluded this finding to have "no toxicological significance."

No histomorphological tissue changes associated with test substance ingestion were reported in the high-dose rats. Common microscopic findings similarly distributed among high-dose and control rats included focal inflammatory cellular infiltrate in liver parenchyma or tracheal submucosa. Other microscopic findings for tissues from high-dose and control rats were also considered "incidental and unrelated to zeaxanthin."

The results of the study included no mortalities, no adverse effects on clinical observations, body weight gain, food consumption or food efficiency, organ weights, gross necropsy findings or clinical chemistry values. Incidental indications of statistically significant differences from control values were not found to be dose-related and were considered random events not related to effects from the test material. Histopathology findings did not reveal any changes that were considered related to the treatment with zeaxanthin-purified concentrate. Under the conditions of this study, the NOAEL of Chrysantis ZEC was determined to be the highest doses tested, 6,500 ppm or approximately 548 mg zeaxanthin/kg-bw/day and 549 mg/kg-bw/day for males and females, respectively. This dose corresponds to a NOAEL of 2068 mg Chrysantis ZEC/kg-bw for males and 2072 mg Chrysantis ZEC/kg-bw for females.

No publications were identified in our search of the scientific literature on subchronic or chronic dosing toxicological studies with pure zeaxanthin derived from marigold. However, a 13-week rat dietary study with a crystalline lutein product that contained >75% lutein and between 2 and 8% zeaxanthin by weight has been published (Kruger et al. 2002). Dietary consumption data showed that the mean daily intake of zeaxanthin by animals receiving the highest dose tested would be equivalent to a daily dose of up to 208 mg zeaxanthin/kg-bw/day. The results of the study showed no statistically significant adverse effects on food intake, body weights, clinical chemistry, or hematological parameters and no significant dose-related differences were observed in mean absolute or relative organ or on gross or microscopic pathology examinations.

### 5.10.4 Conclusion (2007)

The available toxicological studies that have been conducted provide compelling evidence for the safety of the ingestion of Chrysantis ZEC. The only indication of any toxicological potential was from a study in which the mutagenicity of Chrysantis ZEC was evaluated by the Ames reverse mutation assay with several *Salmonella typhimurium* strains that reported a positive result in a single strain (TA98) (Stirparo 2005a). However, because of the equivocal nature of the response in the first assay, a repeat assay with this strain was conducted. The second assay was negative (Stirparo 2005b). Based on the equivocal nature of the responses in the first study and the definitively negative results of the second study, it is reasonable to conclude that Chrysantis ZEC is not mutagenic. In addition, published studies on the mutagenicity of zeaxanthin provide additional support for this conclusion (FAO/WHO 2006, FDA 2001). In an

acute oral toxicity study and a 10-day dose range-finding study with rats, there were no mortalities or signs of toxicity observed in rats. In the 10-day study, the investigators established a NOAEL of 673 and 604 mg zeaxanthin/kg-bw/day for males and females, respectively for Chrysantis ZEC. In addition, there were no adverse effects on clinical observations, body weight gain, food consumption or food efficiency, organ weights, gross necropsy findings, or clinical chemistry values in the 28-day dietary rat study with Chrysantis ZEC. This study reported that the highest doses tested were the NOAELs for zeaxanthin of 548 mg zeaxanthin/kg-bw/day and 549 mg/kg-bw/day for male and female rats, respectively. These doses correspond to NOAELs of 2068 mg Chrysantis ZEC/kg-bw for males and 2072 mg Chrysantis ZEC/kg-bw for females.

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**Table 5-3. Summary of Toxicological Studies with Chrysantis Purified Zeaxanthin Extract Concentrate**

| Reference | Study Objective & Design | Test Substance | Effect |
|---|---|---|---|
| **Genotoxicity** | | | |
| Stirparo 2005a | Test the mutagenicity of Z-purified concentrate in a bacterial reverse mutation assay of *S. typhimurium* TA97a, TA98, TA100, TA1535 AND *E. coli* WP2 uvrA (328) in the presence and absence of an exogenous metabolic activation system. | 5, 10, 50, 100, 500, 1000, 2500 or 5000 µg/plate (incorporation)<br><br>100, 500, 1000, 2500 or 5000 µg/plate (preincubation)<br><br>Batch #: 257-MAY04-001 | -Equivocal mutagenic activity observed in *S. typhimurium* tester strain TA98. |
| Stirparo 2005b | Test the mutagenicity of Z-purified concentrate in a bacterial reverse mutation assay of *S. typhimurium* TA98 and TA100. | 5, 10, 50, 100, 500, 1000, 2500 or 5000 µg/plate (incorporation)<br><br>100, 500, 1000, 2500 or 5000 µg/plate (preincubation)<br><br>Batch #: Not Specified, material processed exactly the same as batch 257-MAY04-001 | -Negative findings for the induction of mutagenicity in the bacterial reverse mutation test. |
| **Acute Studies** | | | |
| PSL Product Safety Laboratories 2004 | Assess acute toxicity of Z-purified concentrate via oral route in rats using the Up and Down Procedure.<br><br>n=3F Sprague-Dawley albino Rats<br><br>1-time administration; 14-day observation period. | 5000 mg/kg-bw<br><br><br><br>Lot #: 257-MAY04-001 | -No mortalities.<br>-All rats gained normal weight.<br>-Rats appeared active and healthy for 14-day observation period.<br>-Necropsy revealed no gross abnormalities. |
| **Subacute Studies** | | | |
| PSL Product Safety Laboratories 2005a | Evaluate the palatability and general toxicity of Z-purified concentrate s in rats. | Male: 0, 355, 490 or 673 mg/kg-bw<br><br>Female: 0, 340, 451 or | -No mortalities.<br>-No adverse clinical observations, body weight changes, nutritional effects or gross alterations |

**Table 5-3. Summary of Toxicological Studies with Chrysantis Purified Zeaxanthin Extract Concentrate**

| Reference | Study Objective & Design | Test Substance | Effect |
|---|---|---|---|
| | n=12M/12F, 6 per group of CRL:CD® (SD)IGS BR VAF/Plus® rats<br><br>Range finding study.<br><br>10 days | 604 mg/kg-bw<br><br>Lot #: 257-MAY04-001 | identified as related to treatment.<br>-NOAEL determined to be 673 mg/kg-bw/day for male rats and 604 mg/kg-bw/day for female rats. |
| PSL Product Safety Laboratories 2005b | Determine the potential of Z to produce toxicity in Crl:CD® (SD) IGS BR rats<br><br>n=30<br><br><br>28 days | Male: 5, 54, and 548 mg zeaxanthin/kg-bw/d<br>Female: 6, 55, and 549 mg zeaxanthin/kg-bw/d<br><br><br>Batch #: 257 AGO-08 | -No mortalities.<br>-No clinical observations, body weight, food consumption or food efficiency adverse effects.<br>-No organ weight changes, gross findings, clinical pathology or histopathology alterations attributable to test substance.<br>-Investigators established NOAEL of 548 mg/kg-bw/d for males and 549 mg/kg-bw/d for females. |
| | Source: Chrysantis Inc. (presented in Appendix 5) | | |

## 5.11 Corroborative Safety Data: Toxicological Studies of Synthetic Zeaxanthin

Roche Vitamins®, Inc. submitted a New Dietary Ingredient (NDI) Notification to the FDA in March of 2001 summarizing unpublished safety studies conducted on the use of their synthetically-derived zeaxanthin product (FDA 2001). These studies were also reviewed in a safety evaluation of zeaxanthin conducted by the World Health Organization (FAO/WHO 2006). ENVIRON summarized the information presented in these two publicly available documents and this information can be used to provide corroborative evidence of the safety of the ingestion of zeaxanthin.

### 5.11.1 Genotoxicity

Gocke (1987) and Strobel (1986) (both as cited in FDA 2001 and FAO/WHO 2006) have investigated the mutagenicity of zeaxanthin. In the study by Gocke (1987; as cited in FDA 2001 and FAO/WHO 2006), a beadlet formulation of zeaxanthin was tested in seven *S. typhimurium* strains (TA1535, TA1537, TA1538, TA97, TA98, TA100 and TA102) using an Ames assay. Both the plate incorporation and the preincubation methods were utilized. As a positive control, a set of tester strains were also mixed with an exogenous microsomal fraction (S9) derived from male albino rats treated with phenobarbital/β-naphthoflavone. Concentrations of 2.4 to 1500 µg/plate and 5 to 500 µg/plate were tested in the plate incorporation and pre-incubation methods, respectively. No increase in the number of mutants in any of the tester strains was observed. The positive controls verified the sensitivity of the strains and the activity of the S9 mix. Strobel (1986; as cited in FDA 2001 and FAO/WHO 2006) tested zeaxanthin for its ability to induce gene mutations at the Hypxanthine Guanine Phosphoribosyl Transferase (HGPRT) locus in cultured mammalian cells (V79) derived from Chinese hamster lung cells. One to 16 µg/mL did not induce mutations to 6-Thioguanine resistance in V79 cells *in vitro*, neither in the absence nor presence of a rat liver activation system.

Roche's synthetic zeaxanthin has also been tested for its ability to induce DNA damage (Strobel 1987; as cited in FDA 2001 and FAO/WHO 2006). One to 16 µg/mL zeaxanthin was added to an Unscheduled DNA Synthesis assay (UDS test) on radiolabeled nucleotides in non-replicated DNA of male FU-albino rat hepatocytes for 20 hours. DNA repair synthesis was not induced in the primary cultures of the rat hepatocytes.

Strobel and Bonhoff (1987; as cited in FDA 2001 and FAO/WHO 2006) evaluated the potential clastogenic activities of zeaxanthin on human peripheral blood lymphocytes. The test was conducted in both the absence and presence of a rat liver activating enzyme system. There were three exposure periods of 1, 2 and 24 hours to evaluate the following doses: 6, 30, 60 and 120 µg/mL, 60 and 120 µg/mL and 40, 50, 60, 70 and 80 µg/mL, respectively. Investigators reported that neither zeaxanthin nor its metabolites induce chromosomal aberrations.

An *in vivo* micronucleus test of bone-marrow cells was conducted in mice administered oral doses of zeaxanthin (Gallandre 1980; as cited in FDA 2001 and FAO/WHO 2006). Mice were administered powdered beadlets of synthetic zeaxanthin at doses of 44.5, 89, or 178 mg/kg-bw either 30 or 6 hours prior to sacrifice. Investigators observed no increase in micronuclei and concluded that under study conditions, zeaxanthin did not induce chromosomal breaks or mitotic non-disjunctions in the mouse bone marrow cells.

## 5.11.2 Acute Studies

In a series of two studies, the acute toxicity of zeaxanthin and its precursors were studied in mice and rats (Baechtold 1977a, 1977b; as cited in FDA 2001 and FAO/WHO 2006). Mice were administered a single dose of 8000 mg/kg-bw zeaxanthin while rats received 4000 mg/kg-bw zeaxanthin by gavage. All mice and rats survived for the 10 days prior to scheduled sacrifice. The author established an $LD_{50}$ of >8000 mg/kg-bw and >4000 mg/kg-bw for mice and rats, respectively.

## 5.11.3 Subchronic Studies

Roche[®] conducted subchronic oral studies with mice, rats and beagle dogs treated with synthetic zeaxanthin in their diet for 13 weeks (Ettlin et al. 1980a; Buser 1985; Ettlin 1985; all as cited in FDA 2001 and FAO/WHO 2006).

In a study with albino-SPF mice, 15 mice were administered 250, 500, or 1000 mg/kg-bw/day all-*trans*-3R,3'R-zeaxanthin in gelatin coated beadlets with 97.6% purity for 13 weeks in their diet (Ettlin et al. 1980a; as cited in FDA 2001 and FAO/WHO 2006). Investigators found no treatment-related abnormalities in ophthalmoscopic examinations or in the hematological and biochemical investigations in the blood. No discoloration of adipose tissue was reported. No changes were observed at necropsy or during histopathological examination. No toxic effects were attributed to zeaxanthin and a No Observable Effects Level (NOEL) of 1000 mg/kg-bw/day zeaxanthin (the highest dose tested) was established for mice.

In an oral toxicity study in rats, 18 rats were administered 250, 500 or 1000 mg/kg-bw/day synthetic zeaxanthin for 13 weeks (Buser 1985; as cited in FDA 2001 and FAO/WHO 2006). No effects on food intake or body weights were observed throughout the study. Decreases in leukocyte numbers, bilirubin concentrations, total protein serum and α-1-globulin were observed in the higher dose groups as well as increases in $Na^+$ concentrations. These changes were not considered to be biologically significant. Yellow-orange discoloration was observed in the feces and adipose tissue of all treated rats. No treatment-related changes were observed in organ weights or during microscopic examination of the tissues. The investigator established a NOEL for rats of 1000 mg/kg-bw/day zeaxanthin; the highest dose tested.

The oral toxicity of zeaxanthin was also evaluated in 10-month old beagle dogs over a 13 week period in a study which reportedly complied with GLP (Ettlin 1985; as cited in FDA 2001 and FAO/WHO 2006). Three males and 3 females were included in one of three groups administered diets containing different concentrations of zeaxanthin. Male beagles ingested 123, 204, or 422 mg zeaxanthin/kg-bw/day; females received 104, 238, or 442 mg zeaxanthin/kg-bw/day. No treatment-related toxicity was observed throughout the study. Furthermore, no treatment-related findings were reported in the ophthalmologic examination, urinalysis, hematological, or serum clinical chemistry investigations. A NOEL of 430 mg zeaxanthin/kg-bw/day was established for dogs.

## 5.11.4 Developmental and Reproductive Toxicity

A developmental toxicology study was conducted with synthetic zeaxanthin administered in the diets of mated female FU-albino outbred rats (Kistler 1984; as cited in FDA 2001 and FAO/WHO 2006). 108 rats were included in the study, receiving 250, 500 or 1000 mg/kg-bw/day zeaxanthin in a beadlet formulation in their diets. The zeaxanthin was administered for 10 days during days 7 through 16 of gestation. The rats were necropsied on day 21 of gestation and fetuses from 15 litters per group underwent skeletal and soft tissue examination. The uteri of the female rats revealed no increase in numbers or locations of implantations or resorptions. No developmental effects were found in the fetuses examined. Litters from each group were raised until weaning and no abnormalities were reported. A NOEL of 1000 mg zeaxanthin/kg-bw/day was established for rats.

The same investigator conducted a similar developmental study in mated female FU-albino rabbits (Kistler 1984; as cited in FDA 2001 and FAO/WHO 2006). Rabbits received 100, 200 or 400 mg/kg-bw/day zeaxanthin in their diets for 13 days during days 7 through 19 of gestation. No treatment-related deaths or signs of maternal toxicity were observed. Dams were necropsied on day 30 of gestation and fetal viability was tested in incubators. No treatment-related abnormalities were observed in uteri or during fetal gross and skeletal examinations. Some malformed fetuses were found in both test and control groups. A NOEL of 400 mg/kg-bw/day, the highest dose tested, was established for rabbits.

## 5.11.5 Chronic Studies

The long-term effects of zeaxanthin administration by oral gavage were evaluated in cynomolgus monkeys in a 52-week study which reportedly complied with GLP (Pfannkuch et al. 2000a, 2000b; Pfannkuch 2001; all as cited in FDA 2001 and FAO/WHO 2006). Two male and two female monkeys were assigned to one of two treatment groups and were administered vehicle, 0.2 mg zeaxanthin/kg-bw/day, or 20 mg zeaxanthin/kg-bw/day. Two additional monkeys were sacrificed after 6 months of treatment in the high-dose group. A control group was also included in the study. All animals survived until scheduled sacrifice. No effect on overall mean body weight gain or mean food intake was observed. No changes in hematology, blood chemistry, urine analyses, EKG or blood pressure were observed. No clinical signs of toxicity or abnormalities at necropsy or histopathological examinations were observed. Eye examinations revealed no crystalline deposits in the retina of the treated monkeys. Electroretinograms revealed no treatment-related changes throughout the study. A dose-dependent increase in zeaxanthin concentration in the retina was observed.

## 5.11.6 Skin Sensitization Studies

Csato and Arcelin (2000a; as cited in FDA 2001 and FAO/WHO 2006) determined the allergenicity of zeaxanthin via intradermal administration of a 3% solution of 98.3% pure zeaxanthin in either polyethylene glycol 300 or a 1:1 mixture of Freund's complete adjuvant and physiological saline in the nuchal region of female Himalayan spotted guinea pigs. Epidermal induction of sensitization was conducted for 48 hours under an occlusive dressing with 25% zeaxanthin. At 1 and 2 weeks after administration, no signs of irritation were observed.

### 5.11.7 Conclusion

The publicly available information on studies conducted Roche Vitamins®, Inc.'s proprietary formulation of synthetic zeaxanthin do not present any findings that would bring the safety of the supplemental use of zeaxanthin into question. These data provide additional supportive evidence for the safety of the ingestion of zeaxanthin.

## 5.12 Conclusions of the Safety Data (2007)

Results from studies indicate that the pharmacokinetics of the xanthophylls lutein and zeaxanthin are similar and studies in which the plasma and tissue responses to zeaxanthin intake have been monitored in humans and animals demonstrate that ingestion of zeaxanthin or lutein increases plasma levels of the ingested carotenoid and its metabolites. In addition, the intake of zeaxanthin does not appear to affect plasma concentrations of other dietary carotenoids.

The bioavailability of xanthophylls is affected by several factors, including the food matrix and processing, structure of the carotenoid molecule, interactions with other nutrients and nutritional status. Results from *in vitro* studies suggest that micellarization and absorption of lutein and zeaxanthin are comparable. Some *in vivo* studies indicate that lutein provided in a supplement is more bioavailable as compared to lutein in a food matrix; other studies, however, have shown no difference between these two forms in lutein bioavailability.

Increased ingestion of zeaxanthin supplements and foods rich in lutein+zeaxanthin has been reported to increase macular pigment density. No adverse effects on tissue concentrations of carotenoids, including ocular damage, have been observed in human or animal supplementation studies. Individuals consumed as much as 30 mg zeaxanthin per day for periods up to 120 days.

The available genotoxicity and *in vivo* toxicological studies that have been conducted provide compelling evidence for the safety of the ingestion of Kemin ZeaONE™. These findings corroborate the safety of ingestion of the whole product as determined by evaluating the source of the product, production process, nature and quantity of impurities, and product specifications, as well as safety of ingestion of the major constituents, zeaxanthin and lutein. Toxicological studies provide evidence that Kemin ZeaONE™ is not mutagenic and a 28-day dietary rat study with Kemin ZeaONE™ reported NOAELs of 548 mg zeaxanthin/kg-bw/day and 549 mg/kg-bw/day for male and female rats, respectively. These were the highest doses tested and they correspond to a NOAEL of 2068 mg Kemin ZeaONE™/kg-bw for males and 2072 mg Kemin ZeaONE™/kg-bw for females. Further, results from toxicological studies conducted with synthetic zeaxanthin in mice, rats, dogs, and monkeys by Roche® Vitamin Inc. and submitted as a New Dietary Ingredient (NDI) Notification to the US FDA provide corroborative evidence of the safety of ingestion of zeaxanthin from Kemin ZeaONE™.

Kemin intends to use Kemin ZeaONE™ in a variety of foods and beverages as a source of zeaxanthin. The estimated mean and 90th percentile 2-day average intakes of zeaxanthin by the U.S. population ages 1 year and older from the proposed uses are 840 and 1477 µg, respectively. As previously noted, the resulting total intakes of lutein+zeaxanthin based on the proposed uses of Kemin ZeaONE™ are therefore at most approximately twice the current

intakes of these carotenoids from naturally occurring sources, and lower than potential intakes resulting from consumption of foods containing other marigold-derived sources of lutein+zeaxanthin.

Additionally, the estimated intakes of antheraxanthin, violaxanthin, mutatoxanthin and waxes present in Kemin ZeaONE™ resulting from the proposed uses are below intakes from foods commonly consumed in the diet. Canthaxanthin is a carotenoid that is present in many plants and has been reported to cause a crystalline retinopathy in animals and humans that consume this compound as a dietary supplement. Based on the detected levels of canthaxanthin in Kemin ZeaONE™, individuals consuming the concentrate at 90th percentile daily intake levels ingest 9 µg of canthaxanthin, or approximately 0.16 µg/kg-bw for a 60 kg person. This level of intake is considerably lower than the current canthaxanthin ADI.

The estimated mean and 90th percentile intakes of Kemin ZeaONE™ correspond to intakes of 14 and 25 µg zeaxanthin/kg bw/day for a 60 kg adult, respectively. These intakes are significantly lower (approximately 150000 and 83000 times lower) than the reported NOAELs (average of 2070 mg zeaxanthin/kg bw/day for male and female rats) for zeaxanthin from the ingestion of Kemin ZeaONE™.

## 5.13 Conclusions of the Updated Safety Data (2015)

In 2007 the Expert Panel found that Chrysantis® Purified Zeaxanthin Extract Concentrate is safe, and Chrysantis® Purified Zeaxanthin Extract Concentrate is GRAS at the originally proposed levels of addition to foods and based on the safety data presented above.

Since the 2007 determination Kemin has revised the manufacturing process to create ZeaONE™, a zeaxanthin product with higher purity. The resulting product consists of > 50% zeaxanthin and > 3% lutein with a corresponding decrease in residual components.

Recent toxicological studies with Kemin ZeaONE™ support the safety of zeaxanthin generally but also demonstrate the safety of the ZeaONE™ product specifically. The studies with Kemin ZeaONE™ reported NOAELs of 550 mg ZeaONE/kg-bw/day (77 mg zeaxanthin/kg bw/day) in rats; these were the highest doses tested. Kemin ZeaONE™ is neither mutagenic nor genotoxic. Kemin ZeaONE™ does not contain residual components from the manufacturing process with allergenic potential and there is no evidence to suggest that it may cause adverse effects in sensitive populations because the revised manufacturing process reduces residual components from the 2007 ZEC product and does not introduce allergenic substances.

The estimated mean (4.9 mg/day) and 90th percentile (9.4 mg/day) intakes of Kemin ZeaONE™ correspond to intakes of 82 and 157 µg Kemin ZeaONE™/kg bw/day for a 60 kg adult, respectively, or 67 µg Kemin ZeaONE™/kg bw/day for a 60 kg pregnant woman taking the prenatal supplement. These intakes are significantly lower (approximately 940, 490, and 1150 times lower, respectively) than the reported NOAEL of (550 mg Kemin ZeaONE™/kg bw/day for male and female rats) for zeaxanthin from the ingestion of Kemin ZeaONE™.

For an individual with average intakes of lutein+zeaxanthin from current dietary sources, combined lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 3.4 to 5.2 mg/d. For an individual with lutein+zeaxanthin intakes

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from current dietary sources at the 90th percentile of intake, total lutein+zeaxanthin intakes from the current diet and Kemin ZeaONE™ sources are estimated to be in the range of 15.4 to 17.2 mg/d. These estimates are well below the ADI of 60 mg/d established by EFSA for marigold-derived lutein.

A critical evaluation of the available evidence indicates that foods containing up to 600 µg Kemin ZeaONE™/serving are safe and suitable for the general population, daily supplementation of up to 10 mg Kemin ZeaONE™ is safe and suitable for the adult general population, and that prenatal supplements containing up to 4.0 mg Kemin ZeaONE™ are safe and suitable for pregnant women.

ENVIRON concludes that the suitability and safety of the proposed uses of Kemin ZeaONE™ are supported by the appropriate, publicly available, scientific data.

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