ORIGINAL SUBMISSION

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Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
Telephone: 772-299-0746
Facsimile: 772-299-5381
E-mail: msoni@soniassociates.net

June 25, 2015

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Subject: GRAS Notification for Zeaxanthin concentrate (OmniXan™)

Dear Sir/Madam:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), OmniActive Health Technologies Ltd., through Soni & Associates Inc. as its agent, hereby provides notice of a claim that the food ingredient Zeaxanthin concentrate (OmniXan™) described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be Generally Recognized As Safe (GRAS), based on scientific procedures.

As required, please find enclosed three copies of the notification. If you have any questions or require additional information, please feel free to contact me by phone at 772-299-0746 or by email at msoni@soniassociates.net or sonim@bellsouth.net.

Sincerely,

(b) (6)

Madhu G. Soni, Ph.D.

Soni & Associates Inc.

749 46th Square
Vero Beach, FL 32968, USA
Telephone: 772-299-0746
Facsimile: 772-299-5381
E-mail: msoni@soniassociates.net

GRAS NOTIFICATION

I. Claim of GRAS Status

A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

OmniActive Health Technologies Ltd. (the notifier) has determined that zeaxanthin concentrate (OmniXan™) derived from paprika (*Capsicum annum* fruits) oleoresin is Generally Recognized As Safe, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use as a food ingredient. Therefore, the use of zeaxanthin concentrate (OmniXan™) is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Date June 29, 2015

Madhu G. Soni, Ph.D., FACN

Agent for:

OmniActive Health Technologies Ltd.
Cybertech House, Ground Floor,
J B Sawant Marg, Wagle Industrial Estate,
Thane (West) 400 604,
India

B. Name and Address of Notifier

Dr. Vijaya Juturu
OmniActive Health Technologies Ltd
Cybertech House, Ground Floor
J B Sawant Marg, Wagle Industrial Estate,
Thane (West) 400 604
India

Tel: +91-22-25830446/7
Fax: +91-22-25833771
Email: v.juturu@omniactives.com

C. Common or Usual Name of the Notified Substance

The common name of the substance of this notification is zeaxanthin concentrate. The ingredient is a mixture of carotenoid xanthophylls, including zeaxanthin, lutein, β-carotene and β-cryptoxanthin. The trade name of the substance is OminXan™.

D. Conditions of Intended Use in Food

OmniXan™, a zeaxanthin concentrate, is intended for use as a food ingredient in the following food categories: baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, and soft candy] at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). A summary of use levels and food categories for zeaxanthin use is presented in Table I-D. OmniXan™ will not be added to food categories that come under USDA jurisdiction. The intended use of zeaxanthin concentrate is in the same food products and at levels proportional to those mentioned in the GRN 000140 (FDA, 2004), GRN 000110 (FDA, 2003) and GRN 000291 (FDA, 2009). The substance mentioned in GRN 000140 has been reported to contain $\geq$ 74% trans-lutein and $\geq$ 2 and $\leq$ 9% zeaxanthin, while the subject of present GRAS determination (OmniXan™) contains $\geq$ 65% zeaxanthin. On the basis of lutein content, zeaxanthin can be added at a level of 114% that of the substance mentioned in the GRN 000140. Based on these assumptions, the zeaxanthin can be added at level of up to 114% (1.14 times) of the levels of lutein mentioned in GRN 000140. The intended uses of zeaxanthin concentrate in the above mentioned food categories will result in the mean and 90th percentile intake of 7.3 and 13.4 mg zeaxanthin/person/day, respectively.

Table I-D. Summary of Food Categories and Intended Use Levels of Zeaxanthin in the US

Food Category	Proposed Food	Use levels mg/RACC[1]
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.28
	Crackers and Crisp-breads	2.28
Beverages and Beverage Bases	Bottled Water	0.57
	Carbonated Beverages	2.28
	Meal Replacements	2.28
	Tea, Ready-to-Drink	0.68
Breakfast Cereals	Instant and Regular Hot Cereals	2.28
	Ready-to-Eat Cereals	2.28
Chewing Gum	Chewing Gum	1.14
Dairy Product Analogs	Imitation Milks	2.28
	Soy Milks	
Egg Products**	Liquid, Frozen or Dried Egg Substitutes	2.28
Fats and Oils	Margarine-like Spreads	1.71
	Salad Dressings	1.71
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.14
Gravies and Sauces	Tomato Based Sauces	0.34
Hard Candy	Hard Candy	1.14
Infant and Toddler Foods*; **	Junior, Strained and Toddler-Type Baby foods	1.14
Milk Products	Dry Milk	3.41
	Fermented Milk Beverages	0.68
	Flavored Milk and Milk Drinks	3.41
	Milk-Based Meal Replacements	3.41
	Yogurt	3.41
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	2.28
	Fruit-Flavored Drinks	2.28
	Fruit Juice	2.28
	Nectars	2.28
	Vegetable Juice	2.28
Soft Candy	Chewy and Nougat Candy	1.14
	Fruit Snacks	1.14
Soups and Soup Mixes**	Canned Soups	0.68

[1]RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). Uses listed and level proportional as in GRN 000140. *Does not include infant formula. Adapted from GRN 000140 and GRN 000291. **Unlike GRN 000140, Infant and Toddler Foods, Egg products and Soup and Soup mixes are not food categories for the present GRAS determination.

E. Basis for GRAS Determination

In accordance with 21 CFR 170.30, the intended use of zeaxanthin concentrate (OmniXan™) has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. The determination is supported by the opinion of the Expert Panel. A comprehensive search of the scientific literature was also utilized for this determination. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for zeaxanthin concentrate (OmniXan™). Zeaxanthin, the active constituent of OmniXan™, has a safe history of consumption as a dietary component. The source material of zeaxanthin concentrate (OmniXan™), paprika is commonly consumed around the world. Zeaxanthin and its esters are found in a variety of commonly consumed foods such as yellow corn, red pepper, orange pepper, orange juice, honeydew, mango, and chicken egg yolk. Lutein and zeaxanthin are two of the most abundant carotenoids found in the diet. The macula of the eye is a repository for lutein and zeaxanthin. Lutein and zeaxanthin in combination or alone are marketed as dietary supplements.

Since 2003, the FDA has received six separate GRAS notifications on lutein that also contains small amounts of zeaxanthin. In these submissions to the FDA, extensive data from published literature on lutein and zeaxanthin was presented by the notifiers. Lutein and zeaxanthin have identical chemical formulas and are isomers. The FDA did not object the acceptability and suitability of the available evidence to support the use of lutein that also contains zeaxanthin. Additionally, EFSA and JECFA have extensively reviewed the safety data on zeaxanthin. The safety determination of OmniXan™ is further supported by toxicological studies in rats and mutagenicity study conducted according to Ames assay. The safety of zeaxanthin concentrate is corroborated by multiple animal and human studies that have been performed with lutein, lutein-rich foods, lutein supplements, and meso-zeaxanthin.

On the basis of scientific procedures[1], OmniActive considers the consumption of lutein and zeaxanthin preparation (Lutemax 2020™), as a food ingredient to be safe at levels up to 13.4 mg lutein/person/day and 2.4 mg zeaxanthin/person/day.

F. Availability of Information

The data and information that forms the basis for this GRAS determination will be provided to Food and Drug Administration upon request or will be available for FDA review and copying at reasonable times at the above mentioned offices of the notifier (Section I, B) or at the offices of:

Madhu G. Soni, PhD, FACN, FATS
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32068

Telephone: +1-772-299-0746; Email: msoni@soniassociates.net or sonim@bellsouth.net

[1] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

II. Detailed Information About the Identity of the Notified Substance

OminXan™ is a zeaxanthin standardized product obtained from paprika (*Capsicum annum* fruits) oleoresin. It is a reddish-orange color powder with characteristic aroma of paprika. The zeaxanthin concentrate is diluted and standardized into forms such as beadlets, powders, granules or oil suspension that are useful for food and beverage applications. For dilution and standardization common dietary oils (corn oil, sunflower oil, or safflower oil), or food grade carbohydrates are employed. The zeaxanthin content of the concentrate may range from 55 to 75%, with averaging to approximately 65%.

A. Chemical name

Zeaxanthin: β, β -carotene-3,3'-diol; 3R,3'R-Zeaxanthin; 3,3'-dihydroxy-β,β-carotene

B. Trade Name:

The subject of this notification will be marketed as OmniXan™

C. Chemical Abstract Registry Number:

Zeaxanthin: 144-68-3

D. Chemical Formula:

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

E. Structure:

The structural formula of zeaxanthin is presented in Figure II-E.

Figure II-E. Chemical Structure of Zeaxanthin

F. Molecular Weight

The molecular weight of zeaxanthin is 568.88

G. Physical Characteristics

OmniXan™ is a reddish-orange color powder with a characteristic odor of paprika.

H. Typical Composition and Specifications

Food grade specifications of Zeaxanthin are presented in Tables II-H.1. Analytical data from three manufacturing lots is presented in Appendix I. Compositional analysis of zeaxanthin concentrate used in the production of final product (OmniXan™) is summarized in Table II-H.2.

Table II-H.1. Specifications of Food Grade 3R,3'R Zeaxanthin Concentrate*

Parameter	Specification	Method
Appearance	A reddish orange colour powder	Visual inspection
Total 3R,3R'-Zeaxanthin (trans)	≥55%	HPLC
3R,3R'-Zeaxanthin (cis)	NMT 0-2%	HPLC by area percent
Nutritional Characters		
Fats	15-25%	Extraction by Soxhlet
Proteins	NIL	Kjeldahl
Residual Solvents		
Acetone	NMT 30 ppm	GC / FID
Ethanol	NMT 25 ppm	GC/FID
Heavy Metals		
Lead (Pb)	NMT 1 ppm	ICP-MS
Arsenic (As)	NMT 1 ppm	ICP-MS
Cadmium (Cd)	NMT 0.1 ppm	ICP-MS
Mercury (Hg)	NMT 0.05 ppm	ICP-MS
Pesticide content		
Organochlorine pesticides	NMT 0.1 ppm	GC / FID
Organophosphorus pesticides	NMT 0.1 ppm	GC / FID
Dithiocarbamates	NMT 0.1 ppm	GC / FID
Microbiological Contaminants		
Total aerobic plate count	NMT 1,000 CFU	USP <61>
Total coliforms	NMT 10 CFU	AOAC
Yeast and mould	NMT 100 CFU	USP <61>
Escherichia coli	Negative	USP <61>
Salmonella	Negative in 10 g	USP <61>
Staphylococcus aureus	Negative in 10 g	USP <61>

Table II-H-2. Typical Compositional Analysis of Zeaxanthin Concentrate

Parameter	Percent
3R, 3R'-Zeaxanthin (trans)	> 55%
3R,3R'-Zeaxanthin (cis)	NMT 0-2%
Other Carotenoids	
Lutein	0 - 1%
β-Carotene	5 - 16%
β-Cryptoxanthin	5-8%
Moisture	0-1%
Ash	0-1%

I. Manufacturing Process

OmniActive's Zeaxanthin (OmniXan™) is manufactured according to current good manufacturing practices (cGMP), as outlined in Figure 1, at Kalsec Inc., Kalamazoo, USA and also at OmniActive's facility located at Pune, India. Both manufacturing facilities are ISO certified: ISO 9001 2008 (2003/08) and ISO 22000 HACCP (2005/08). Additionally, both facilities have extensive experience in food ingredient production and various international quality management systems, including QS Production, HALAL, Kosher, and SA 8000 certification.

The production process of zeaxanthin concentrate starts with the collection of fresh paprika pods from the *Capsicum annum* plant. The dried fruits are sealed on arrival, pending laboratory inspection and approval. This is followed by cleaning, grinding and extraction with acetone. The acetone used as the extraction solvent is removed. The desolventization process results in the formation of oleoresin. Prior to saponification, a portion of the xanthophyll esters may be removed for the manufacturing of different products. The zeaxanthin content of the oleoresin used for saponification stage is 1-5%. After saponification, the material is slowly cooled to 35°C and dropped into holding tanks in preparation for the next step (concentration). The material is concentrated by filtration or centrifugation. The Wet cake is washed with a water/isopropyl alcohol (IPA) mix. The wet cake obtained is filtered and dried under vacuum. The dried concentrate consists of zeaxanthin. Small quantities of food grade antioxidant (tocopherols; 1-2%) are added to the product in accordance with current good manufacturing practices.

At different stages of the production process, pesticide residues, chemical contaminants and residual solvent levels are checked to make sure the product meets the specifications. The preparations thus obtained are formulated to the desired levels of zeaxanthin with a food grade antioxidant and carrier to prepare oil suspension or beadlets. Processing aids, such as solvents (which are removed by vacuum evaporation) and buffer salts used in the manufacturing process are all of food-grade quality as specified in the 8th Edition of Food Chemicals Codex. The oleoresin, source for the preparation of OmniXan™, was also checked for pesticide and related potential contaminants; none were detected at detection limits of < 0.01 mg/kg (Appendix I). The residual solvent, heavy metal and pesticide levels from three lots are presented in Appendix I.

J. Manufacturing Process Flowchart

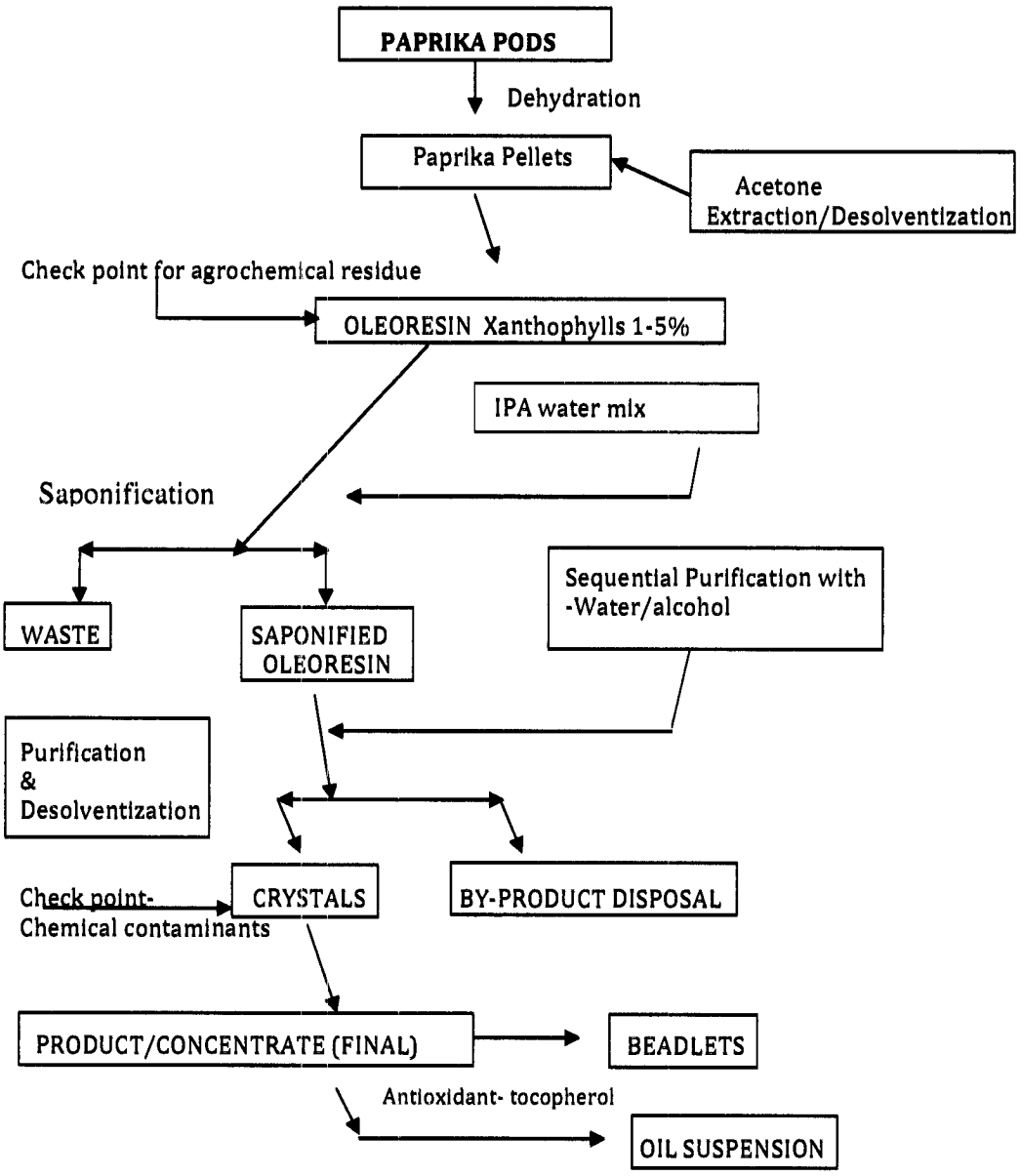

Figure II-I. Manufacturing Process Flow Chart for Zeaxanthin Concentrate

K. Intended Technical Effects

Zeaxanthin preparation is intended for addition to selected foods as a nutritional ingredient to provide consumers with a supplementary source of zeaxanthin in their diets. The use of zeaxanthin preparation is intended for the general population at the levels identified in this document for addition to the following food categories: Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Chewing Gum; Dairy Product Analogs; Egg Products; Fats and Oils; Frozen Dairy Desserts and Mixes; Gravies and Sauces; Hard Candy; Infant and Toddler Foods; Milk Products; Processed Fruits and Fruit Juices; Soft Candy; Soups and Soup Mixes. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, OmniActive does not intend to refer them by the commonly recognized names such as milk, or yogurt.

Use of zeaxanthin preparation in the above described food categories may also incidentally contribute its own color to the product. Its intended nutritional use would thus fall outside the definition of "color additive," in accordance with 21 CFR 70.3(f), "Food ingredients ...which contribute their own natural color when mixed with other foods are not regarded as *color additives*...."

The above exemption from the color additive definition will cover the intended uses of zeaxanthin preparation under the GRAS notification.

III. Summary of the Basis for the Notifier's Determination that zeaxanthin is GRAS

The determination that zeaxanthin preparation (OmniXan™) is GRAS is based on scientific procedures. An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by OmniActive to determine the Generally Recognized As Safe (GRAS) status of zeaxanthin. A comprehensive search of the scientific literature for safety and toxicity information on zeaxanthin and other related carotenoids was conducted through April 2015[2] and was also utilized for this assessment. The updated searches of regulatory databases revealed FDA acceptance of two GRAS notices on meso-zeaxanthin (GRN 550; GRN481). Both these GRAS notices also corroborate the safety of zeaxanthin. Based on a critical evaluation of the pertinent data and information summarized here and employing scientific procedures, it is determined that the addition of lutein and zeaxanthin preparation to the selected foods products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits

[2] The updated database searches performed subsequent to the Expert Panel review of the zeaxanthin (OmniXan™) GRAS assessment in May 2014 did not reveal any significant findings that will affect the panel conclusion.

and fruit juices, and soft candy] at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) meeting the specification cited above and manufactured according to current Good Manufacturing Practice, is GRAS under the conditions of intended use.

In arriving at the decision that zeaxanthin preparation is GRAS, the Expert Panelists relied upon the conclusions that neither zeaxanthin nor any of its degradation products pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the product. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

IV. Basis for a Conclusion that Zeaxanthin is GRAS for its Intended Use.

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to determine the safety of OmniXan™ used as a food ingredient to provide consumers with a supplementary source of lutein and zeaxanthin in their diets. Based on a critical evaluation of the pertinent data and information summarized herein, the Expert Panel members have individually and collectively determined by scientific procedures that the addition of zeaxanthin preparation (OmniXan™) in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes at levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) when not otherwise precluded by a Standard of Identity as described here and resulting in the 90th percentile all-user estimated intake of 13.4 mg zeaxanthin/person/day is GRAS. It is also the opinion of Expert Panel that other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion (see attached Expert Panel Statement).

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ZEAXANTHIN (OMNIXAN™) AS A FOOD INGREDIENT

Prepared for:
OmniActive Health Technologies Ltd.
Cybertech House, First Floor
J B Sawant Marg, Wagle Industrial Estate,
Thane (West) 400 604,
INDIA

Prepared by:
Soni & Associates Inc.
749 46th Square
Vero Beach, FL 32968,
USA

Panel Members

Stanley T. Omaye, Ph.D., F.A.T.S.
Robert L. Martin., Ph.D.
Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

May, 2014

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ZEAXANTHIN (OMNIXAN™™) AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF ZEAXANTHIN (OMNIXAN™) AS A FOOD INGREDIENT

1. INTRODUCTION

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[1], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Soni & Associates, Inc., at the request of OmniActive Health Technologies Ltd. (OmniActive), to determine the Generally Recognized As Safe (GRAS) status of zeaxanthin concentrate (marketed under the trade name OmniXan™) as a nutrient [21 CFR 170.3(o)(20)][2] in selected food products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, and soft candy] at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on zeaxanthin was conducted through January 2014 and made available to the Expert Panel members. The Expert Panel members independently and critically evaluated materials submitted by OmniActive and other information deemed appropriate or necessary. OmniActive assures that all unpublished information in its possession and relevant to the subject of this determination has been provided to Soni & Associates Inc. and has been summarized in this GRAS monograph. Following an independent, critical evaluation, the Expert Panel conferred on May 28, 2014 and unanimously agreed to the decision described herein.

1.1. Background

Over 700 naturally occurring carotenoids have been extracted and identified from a wide variety of biological sources (Nolan et al., 2013). Carotenoids are natural dietary components of higher animals, including humans, which are unable to synthesize these compounds. In recent years, some of these carotenoids are commonly used as dietary supplements, as colorants in cosmetics and foods, as animal feed additives, and in pharmaceuticals. Some carotenoids are known to play an important role in human nutrition and health. Because of their powerful antioxidant properties, carotenoids have received considerable attention in the scientific community. Among the carotenoids, lutein and zeaxanthin are two of the most abundant carotenoids found in the diet (IOM, 2000). Lutein and zeaxanthin have identical chemical formulas and are isomers. These two carotenoids are found in high amounts in green leafy vegetables such as spinach and kale (Khachik et al., 1995; Omaye et al., 1997), and in chicken egg yolk (Handelman et al., 1999). The macula of the eye is a repository for the carotenoids, lutein and zeaxanthin. A higher dietary intake of lutein and zeaxanthin has been shown to reduce the risk of cataracts and age-related macular degeneration (AMD), two eye conditions for which there is minimal options for effective prevention (Moeller et al., 2000). Zeaxanthin is the major carotenoid in the macula and low serum zeaxanthin concentrations is inversely correlated with

[1] Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

[2] "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.

the risk of AMD. Because of the unique nutritional characteristics of zeaxanthin, OmniActive intends to use zeaxanthin (OmniXan™) in a limited number of conventional foods as a dietary ingredient.

1.2. Description

Zeaxanthin concentrate, the subject of this GRAS assessment, is obtained from paprika (*Capsicum annum* fruits) oleoresin, prepared using acetone as a solvent. It is a reddish-orange color product with a characteristic aroma of paprika. The zeaxanthin concentrate is further standardized and diluted into forms useful for food and beverage applications as dry delivery form (beadlets), powders, granules or oil suspension form. The diluted product from the concentrate is marketed under the trade name OmniXan™ either as a standardized oil suspension with commonly used dietary oils (corn oil, sunflower oil, or safflower oil), or as beadlets standardized with food grade carbohydrates. Given the structural similarity between zeaxanthin and lutein, a comparison of general descriptive characteristics and properties between zeaxanthin and lutein is presented in Table 1. As discussed below (section 1.5), chemically zeaxanthin can occur in four different forms, in this document the name zeaxanthin primarily refers to 3R, 3'R-zeaxanthin.

Table 1. General Descriptive Characteristics of Zeaxanthin and Comparison with Lutein

Property/Parameter	Zeaxanthin	Lutein
Chemical names	β, β -carotene-3,3'-diol	Xanthophyll; β, ε-carotene-3,3' –diol
Empirical formula	$C_{40}H_{56}O_2$	$C_{40}H_{56}O_2$
CAS Registry No.	144-68-3	127-40-2
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Solubility- water at 5°C	Insoluble	Insoluble
Melting point	207-215.5°C	177-178°C
Bulk density	0.38-0.41 g/mL	0.35-0.40 g/mL
Stability	Room temperature	Room temperature

*Based on information provided by OmniActive (2014) as well other publicly available sources

1.3. Specifications and Identity

Typical food grade specifications of zeaxanthin concentrate have been established by OmniActive and are presented in Table 2. The zeaxanthin levels may range from 55 to 75%, with averaging to approximately 65%. Analytical results from three non-consecutive lots (Appendix I) demonstrate that zeaxanthin concentrate is consistently manufactured to meet the standard specifications. Typical compositional analysis of zeaxanthin concentrate used in the production of final product (OmniXan™) is summarized in Table 3.

Table 2 Specifications for 3R,3'R Zeaxanthin Concentrate*

Parameter	Specification	Method
Appearance	A reddish orange colour powder	Visual inspection
Total 3R,3R'-Zeaxanthin (trans)	≥55%	HPLC
3R,3R'-Zeaxanthin (cis)	NMT 0-2%	HPLC by area percent
Nutritional Characters		
Fats	15-25%	Extraction by Soxhlet
Proteins	NIL	Kjeldahl
Residual Solvents		
Acetone	NMT 30 ppm	GC / FID
Ethanol	NMT 25 ppm	GC/FID
Heavy Metals		
Lead (Pb)	NMT 1 ppm	ICP-MS
Arsenic (As)	NMT 1 ppm	ICP-MS
Cadmium (Cd)	NMT 0.1 ppm	ICP-MS
Mercury (Hg)	NMT 0.05 ppm	ICP-MS
Pesticide content		
Organochlorine pesticides	NMT 0.1 ppm	GC / FID
Organophosphorus pesticides	NMT 0.1 ppm	GC / FID
Dithiocarbamates	NMT 0.1 ppm	GC / FID
Microbiological Contaminants		
Total aerobic plate count	NMT 1,000 CFU	USP <61>
Total coliforms	NMT 10 CFU	AOAC
Yeast and mould	NMT 100 CFU	USP <61>
Escherichia coli	Negative	USP <61>
Salmonella	Negative in 10 g	USP <61>
Staphylococcus aureus	Negative in 10 g	USP <61>

*Based on information provided by OmniActive (2014)

Table 3. Typical Compositional Analysis of Zeaxanthin Concentrate (OmniActive, 2014)

Parameter	Percent
RR-Zeaxanthin (trans)	> 55%
3R,3R'-Zeaxanthin (cis)	NMT 0-2%
Other Carotenoids	
Lutein	0 - 1%
β-Carotene	5 - 16%
β-Cryptoxanthin	5-8%
Moisture	0-1%
Ash	0-1%

*Based on information provided by OmniActive (2014)

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1.4. Manufacturing Process

Zeaxanthin (OmniXan™) is manufactured according to current good manufacturing practices (cGMP), as outlined in Figure 1, at Kalsec Inc., Kalamazoo, US and also at OmniActive's facility located at Pune, India. Both manufacturing facilities are ISO certified: ISO 9001 2008 (2003/08) and ISO 22000 HACCP (2005/08). Additionally, both facilities have extensive experience in food ingredient production and various international quality management systems, including QS Production, HALAL, Star K Kosher, and SA 8000 certification.

The manufacturing process for the preparation of zeaxanthin concentrate starts with the collection of fresh paprika pods from the *Capsicum annum* plant. The dried fruits are sealed on arrival, pending laboratory inspection and approval. This is followed by cleaning, grinding and extraction with acetone. The acetone used as the extraction solvent is removed. The desolventization process results in the formation of oleoresin. Prior to saponification, a portion of the xanthophyll esters is removed for the manufacturing of different products. The zeaxanthin content of the oleoresin is 1-5%. After saponification, the material is slowly cooled to 35°C and dropped into holding tanks in preparation for the next step (concentration). The material is concentrated by filtration or centrifugation. The Wet cake is washed with a water/isopropyl alcohol (IPA) mix. The wet cake obtained is filtered and dried under vacuum. The dried concentrate consists of zeaxanthin. Small quantities of food grade antioxidant (tocopherols; 1-2%) are added to the product in accordance with current good manufacturing practices.

During the production process, pesticide residues, chemical contaminants and residual solvent levels are checked at different stages to make sure the product meets the specifications. The preparations thus obtained are formulated to the desired levels of zeaxanthin with a food grade antioxidant and carrier to prepare oil suspension or beadlets. Processing aids, such as solvents (which are removed by vacuum evaporation) and buffer salts used in the manufacturing process are all of food-grade quality as specified in the 5th Edition of Food Chemicals Codex. The residual solvent levels in the final product from multiple batches were below NMT. The oleoresin, source for the preparation of OmniXan™, was also checked for pesticide and related potential contaminants; none were detected at detection limits of < 0.01 mg/kg (Appendix I). The residual solvent, heavy metal and pesticide levels from three lots are presented in Appendix I.

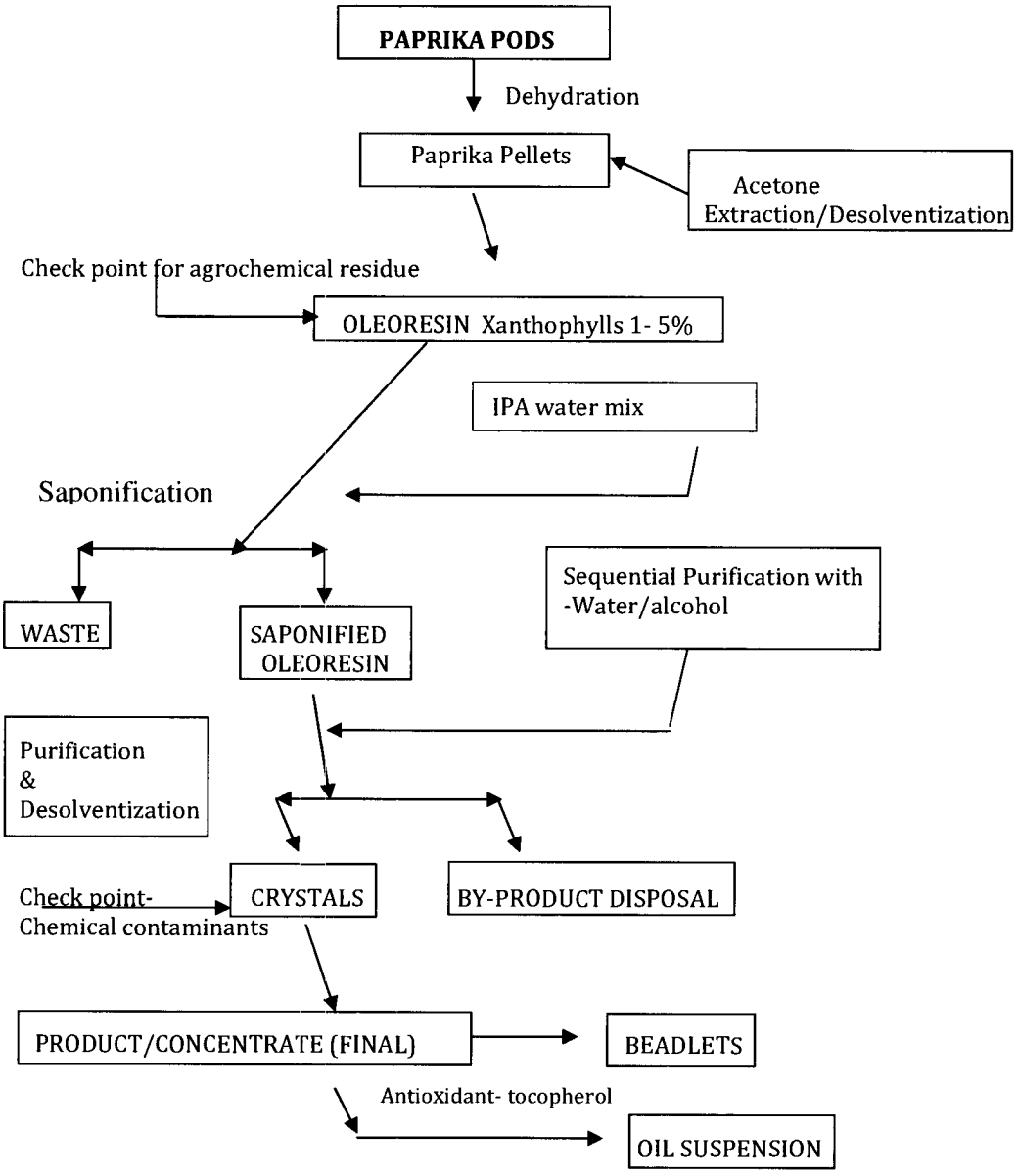

Figure 1. Manufacturing Process Flow Chart for Zeaxanthin Concentrate

1.5. Chemistry

Both zeaxanthin and lutein are naturally occurring xanthophylls and oxycarotenoids. Carotenoids are primarily synthesized by photosynthetic plants and microorganisms and both zeaxanthin and lutein are abundant carotenoids. Lutein occurs with the isomeric xanthophyll, zeaxanthin in many foods, particularly vegetables and fruits. The structural formulas of zeaxanthin and lutein are presented in Figure 2. Chemically, lutein and zeaxanthin contain two

cyclic end groups (a β- and an α-ionone ring) and the basic C40 isoprenoid structure common to all carotenoids. The polyene chain double bonds present in lutein could exist in a *cis* or *trans* configuration and thus can be in a large number of possible mono-*cis* and poly-*cis* isomers. However, the majority of naturally occurring carotenoids are in the all-*trans* configurations (Rice-Evans et al., 1997; IOM, 2000). Structurally, zeaxanthin and lutein have identical chemical formulas and are isomers, but they are not stereoisomers. The main difference between them is in the location of a double bond in one of the end rings. As lutein can absorb blue light, it appears as yellow color; while zeaxanthin appears yellow-orange color (Khoo et al., 2011).

Zeaxanthin occurs primarily as a mixture of three isomers (3R,3'R)-β, β-carotene-3,3'-diol and (3R,3'S)-β, β-carotene-3,3'-diol, with a minor amount of (3S,3'S)-β, β-carotene-3,3'-diol (Sajilata et al., 2008). In the literature, the first two predominant isomers of zeaxanthin are referred as zeaxanthin and meso-zeaxanthin, respectively (Bone et al., 2007). Zeaxanthin and meso-zeaxanthin are classed as diastereomers, and differ only in the spatial orientation of the hydroxyl group on the C3' chiral position. This chiral position within zeaxanthin has an R spatial orientation, whereas meso-zeaxanthin has an S spatial orientation. This difference in spatial arrangement results in zeaxanthin being described as 3R, 3'R zeaxanthin and meso-zeaxanthin being described as 3R,3'S zeaxanthin. 3R,3'R-zeaxanthin is the most commonly found isomer in the diet and is commonly referred as zeaxanthin.

In the lutein and meso-zeaxanthin molecules, the hydroxyl groups located on the 3 and 3' carbon atoms of the carotenoid end-groups is identical. However, in the conversion of lutein into meso-zeaxanthin a shift of one carbon-carbon double bond in the ε-ring of lutein, and change in optical activity will occur thereby resulting in achirality also increasing the conjugation. Alternatively, meso-zeaxanthin may be formed from the metabolite, dehydrolutein via an enzymatic reduction pathway (Bone et al., 2007). Available evidence indicates that the keto-carotenoid canthaxanthin does undergo reduction in the human and primate retina lending credence to this possibility. However, in the plasma, dehydrolutein is formed from both lutein (Thurmann et al., 2005) and zeaxanthin (Heartmann et al., 2004). Unlike β-carotene, α-carotene and β-cryptoxanthin, lutein and zeaxanthin are not considered as provitamin A compounds, as in the human body they are not converted into retinol, an active form of vitamin A.

Figure 1. Chemical Structure of Zeaxanthin

1.6. Natural Occurrence

Carotenoids are some of the most vital colored phytochemicals occurring as all-trans and cis-isomers, and accounting for the brilliant colors of a variety of fruits and vegetables (Khoo et al., 2011). Carotenoids are fat soluble pigments found in some plants, algae, and photosynthetic bacteria. Of the 700 carotenoids identified in nature, over 40 are reported to be present in fruits

and vegetables (Nolan et al., 2013). Despite this, only 14 of these dietary carotenoids may be absorbed, modified, and/or used by the human body, and yet only zeaxanthin, meso-zeaxanthin, and lutein are found at the macula (the innermost part of the eye), reflecting an exquisite degree of biological selectivity. The available information suggest that the concentration of the macular pigment approaches 1 mM in the central regions of the macula more than a 10,000 times more concentrated than that in the blood. The profile of the macular pigment concentration across the retina varies dramatically, > 100 times, from the peripheral retina to the central retina (Landrum and Bone, 2004). Both lutein and two isomeric forms of zeaxanthin are found in the macula of the retina as well as in the crystalline lens (Hendler and Rorvik, 2001). Lutein is the dominant carotenoid in the peripheral macula, zeaxanthin in the mid-peripheral macula, and meso-zeaxanthin at the epicentre of the macula (Nolan et al., 2013).

Humans are not capable of synthesizing zeaxanthin, and thus, the zeaxanthin content of the body is entirely dependent upon dietary intake. Lutein and zeaxanthin are found in green leafy vegetables like spinach, kale, and broccoli and as esters (fatty acid esters) in mango, orange, papaya, red paprika, algae, and yellow corn (van het Hof et al., 1999). Dietary sources of zeaxanthin include yellow corn, red pepper, orange juice, honeydew, mango, and chicken egg yolk (Sajilata et al., 2008). Zeaxanthin has also been identified in extracts from apricots, peaches, cantaloupe, and a variety of pink grapefruit (Ruby seedless). The presence of meso-zeaxanthin has also been reported in shrimp carapace, fish skin, and turtle fat, where all three isomers of zeaxanthin were found (Maoka et al., 1986). Although meso-zeaxanthin is considered a rare isomer, it is present in significant quantities in commercially produced chickens and eggs in Mexico where it is commonly added to the feed to achieve desirable coloration in these products (Bone et al., 2007). The levels of zeaxanthin in several food products, including fruits and vegetables are provided in Table 4.

Table 4. Zeaxanthin in Fruits and Vegetables*

Food	Levels (µmoles)	Food	Levels (µmoles)
Eggs	35	Green grapes	7
Maize (Corn)	25	Brussels sprout	2
Red seedless grapes	10	Scallions	3
Zucchini squash	5	Green beans	3
Orange pepper	37	Orange	15
Cucumber	4	Apple (red delicious)	1
Green pepper	3	Mango	16
Red grape	4	Tomato juice	2
Orange juice	20	Peach	8
Honeydew	18	Nectarine	6
Celery stalk leaves	2		

*Adapted from Sommerberg et al. (1998)

1.7. Current Uses

Lutein and zeaxanthin in free (non-esterified) as well as esterified (with fatty acids) forms are found in numerous dietary supplements commonly marketed around the world. As compared to lutein, the concentration of zeaxanthin in these products is considerably low. Additionally in

recent years, supplements containing meso-zeaxanthin have been marketed. In 2001, FDA accepted a New Dietary Ingredient Notification on synthetically produced zeaxanthin. ConsumerLabs (2007) analyzed over 15 products for its contents of lutein/zeaxanthin and reported that these products contain up to 20 mg lutein and up to 2 mg zeaxanthin in a daily serving. The recommended dose of zeaxanthin in this notice is 1 mg/day (FDA, 2001). The synthetically produced zeaxanthin containing products are marketed under the name ICaps®, MacuShield®, Macusan® Viteyes® Advanced, Eye Essentials and the recommended dose of zeaxanthin ranges from 1.2 mg to as high as 8 mg/day.

In addition to its use as a dietary supplement, lutein containing small amount of zeaxanthin is also used as a food ingredient in some selected foods (Table 5). In response to six separate GRAS notices on lutein, the FDA responded that they had no questions regarding the conclusions that the use of lutein (also contains small amounts of zeaxanthin) is GRAS under the conditions described in those notices. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has reviewed use of zeaxanthin as a food additive and allocated a group acceptable daily intake (ADI) of 0 to 2 mg/kg body weight/day for lutein from *T. erecta* and zeaxanthin (JECFA, 2004).

The source material of zeaxanthin concentrate (subject of this GRAS assessment), *Capsicum annum* fruit pod or paprika, is a commonly used spice. As per 21 CFR 73.340 and 73.345, paprika [ground dried pod of capsicum (*Capsicum annum* L.)] and paprika oleoresin [ground dried pod of mild capsicum (*Capsicum annum* L.)] are listed as color additives. Additionally, both paprika (21 CFR 182.10) and paprika oleoresin (21 CFR 182.10) are GRAS. Thus the source material used in the preparation of zeaxanthin concentrate is a safe food commonly consumed by humans.

1.8. Technological Effects

Zeaxanthin (OmniXan™) is intended for addition to selected foods as a nutrient to provide consumers of all ages with a supplementary source of zeaxanthin in their diets. The use of zeaxanthin is intended for the general population at the levels identified in this document for addition to the following food categories: Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Chewing Gum; Dairy Product Analogs; Fats and Oils; Frozen Dairy Desserts and Mixes; Gravies and Sauces; Hard Candy; Processed Fruits and Fruit Juices; Soft Candy. Foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, and meat and poultry products are excluded from the list of intended food uses of the subject zeaxanthin concentrate. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, OmniActive does not intend to refer to them by the commonly recognized names such as milk or yogurt.

The use of zeaxanthin in the above described food categories may also impart a color to the product. However, the intended use of zeaxanthin would fall outside the definition of "color additive" for the following reasons: (1) The "non-apparent color" Exemption [21 CFR 70.3(f)] - The intended use levels are low enough that it dose not impart a significant color to the food products. Although zeaxanthin has a natural reddish-orange color, in many cases it will be added to food at levels so low that it will not affect the color of the food. Hence, for such types of uses, zeaxanthin may not be regulated as "color additive." (2) "Unimportant color" Exemption [21

CFR 70.3(g)] - For some of the intended uses, when added solely to provide nutritive value, zeaxanthin would contribute a color in a manner that would conform to this exemption. (3) "Food Ingredient" Exemption [21 CFR 70.3(f)] - It is a food use and does not relate to any use of the ingredient as a color additive. The intended use of zeaxanthin in certain specified foods is to provide consumers with a supplementary source of zeaxanthin in their diet and does not relate to any use of the ingredient as a color additive [21 CFR 70.3(f)].

1.9. Intended Uses

Zeaxanthin (OmniXan™) is intended for use in the same foods and at levels proportional to those for lutein mentioned in the GRN 000140 (FDA, 2004) and GRN 000110 (FDA, 2003). As both these notices were reviewed by the FDA and GRN 000140 appeared subsequent to GRN 000110, it is likely that the FDA considered cumulative intake from both notices. There are no new food uses proposed for zeaxanthin (OmniXan™). Unlike GRN 000140, infant and toddler foods, egg products, and soup and soup mixes are not food categories for the present GRAS determination. The substance mentioned in GRN 000140 has been reported to contain $\geq$ 74% trans-lutein and $\geq$ 2 and $\leq$ 9% zeaxanthin, while the subject of present GRAS determination (OmniXan™) contains $\geq$ 65% zeaxanthin. On the basis of lutein content, zeaxanthin can be added at a level of 114% that of the substance mentioned in the GRN 000140. Based on these assumptions, the zeaxanthin can be added at level of up to 114% (1.14 times) of the levels of lutein mentioned in GRN 000140.

The intended uses of zeaxanthin are as a food ingredient in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, and soft candy. The intended food uses and use levels are summarized in Table 5. In the GRN 000140 (FDA, 2004) that received "No Question Asked" letter from the FDA on June 14, 2004, the intake estimates were based on the analysis using United States Department of Agriculture's (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII 1994-1996) and the 1998 Supplemental Children's Survey (CSFII 1998) (USDA, 2000). The compositional analysis of the notified substance was reported to contain 76% lutein and 7% zeaxanthin. Based on this information, the corresponding mean all-user intakes of lutein and zeaxanthin from the consumption of proposed food-uses were 7.3 mg/person/day (0.14 mg/kg body weight/day) and 0.7 mg/person/day (0.01 mg/kg body weight/day), respectively. The 90th percentile all-user intakes of lutein and zeaxanthin were 13.4 mg/person/day (0.28 mg/kg body weight/day) and 1.2 mg/ person/day (0.03 mg/kg body weight/day), respectively.

As the intended use of zeaxanthin (OmniXan™) is in the same food products and at proportional use levels to those described in GRN 000140 (Kemin Foods, 2003), the mean and 90th percentile zeaxanthin intake from its uses will be similar (7.3 and 13.4 mg zeaxanthin/person/day, respectively). For safety assessment purposes the 90th percentile zeaxanthin intake from the proposed uses is considered as 13.4 mg/person/day. A summary of use levels and food categories for zeaxanthin is presented in Table 5. Although the list includes infant and toddler foods, egg products, and soups and soup mixes, these products are excluded from the intended uses of OmniXan™.

Table 5. Summary of the Individual Proposed Food Uses for Zeaxanthin in the US

Food Category	Proposed Food	Use levels mg/RACC[1]
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.28
	Crackers and Crisp-breads	2.28
Beverages and Beverage Bases	Bottled Water	0.57
	Carbonated Beverages	2.28
	Meal Replacements	2.28
	Tea, Ready-to-Drink	0.68
Breakfast Cereals	Instant and Regular Hot Cereals	2.28
	Ready-to-Eat Cereals	2.28
Chewing Gum	Chewing Gum	1.14
Dairy Product Analogs	Imitation Milks	2.28
	Soy Milks	
Egg Products**	Liquid, Frozen or Dried Egg Substitutes	2.28
Fats and Oils	Margarine-like Spreads	1.71
	Salad Dressings	1.71
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.14
Gravies and Sauces	Tomato Based Sauces	0.34
Hard Candy	Hard Candy	1.14
Infant and Toddler Foods*; **	Junior, Strained and Toddler-Type Baby foods	1.14
Milk Products	Dry Milk	3.41
	Fermented Milk Beverages	0.68
	Flavored Milk and Milk Drinks	3.41
	Milk-Based Meal Replacements	3.41
	Yogurt	3.41
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	2.28
	Fruit-Flavored Drinks	2.28
	Fruit Juice	2.28
	Nectars	2.28
	Vegetable Juice	2.28
Soft Candy	Chewy and Nougat Candy	1.14
	Fruit Snacks	1.14
Soups and Soup Mixes**	Canned Soups	0.68

[1]RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). Uses listed and level proportional as in GRN 000140. *Does not include infant formula. Adapted from GRN 000140 and GRN 000291. **Unlike GRN 000140, Infant and Toddler Foods, Egg products and Soup and Soup mixes are not food categories for the present GRAS determination.

2. SAFETY RELATED DATA

In recent years, both zeaxanthin and lutein has become the subject of intense investigations for their potential health benefits. There has been a significant effort to elucidate the biological role and safety of zeaxanthin. A simple search of PubMed scientific database revealed over 2300 published articles on zeaxanthin since 1979. Additionally, the national and international regulatory agencies, such as the FDA, EFSA and JECFA, have extensively reviewed the safety of lutein and zeaxanthin in combination or alone. Since 2003, the FDA has received six separate GRAS notifications on lutein that also contains small amounts of zeaxanthin. In these submissions to the FDA, extensive data from published literature on lutein and zeaxanthin was presented by the notifier. The FDA did not object the acceptability and suitability of the available evidence to support the use of lutein that also contains zeaxanthin. Additionally, EFSA and JECFA have extensively reviewed the safety data on zeaxanthin. The findings from the FDA, EFSA and JECFA reviews and recent publications on this subject, as described below, were utilized for the present safety assessment. Given all this, the following section briefly describes the safety of lutein, while the safety data of zeaxanthin is discussed at length to support its intake from the intended uses.

2.1. Regulatory Agency Excerpt

A comparison between regulatory agencies assessment of lutein and zeaxanthin, in combination or alone, with current GRAS assessment of zeaxanthin is summarized in Table 6. As the subject of this present GRAS assessment contains zeaxanthin, studies described in the FDA GRAS notifications, as well as in JECFA and EFSA assessments are applicable to the proposed use of OmniActive's OmniXan™ that contains zeaxanthin as a food ingredient. Studies published subsequent to the FDA GRAS notices or JECFA and EFSA assessments continue to support the safety of lutein as well as zeaxanthin as a food ingredient. Although, at present, a GRAS notice on the use of meso-zeaxanthin in different foods is under review with the FDA (2013), as discussed below, other international agencies such as JECFA and EFSA have already evaluated the safety-in-use of zeaxanthin as a food ingredient.

2.1.1. FDA Assessment

As of January 6, 2014, the FDA has received seven GRAS notices on the use of lutein and zeaxanthin [lutein ester- GRN 110 (FDA, 2003); crystalline lutein- GRN 140 (FDA, 2004); suspended lutein- GRN 221 (FDA, 2007); crystalline lutein- GRN 291 (FDA, 2009); lutein GRN 385 (FDA, 2011); suspended lutein- GRN 390 (FDA, 2012a); lutein diacetate GRN 432 (FDA, 2012b); and meso-zeaxanthin- GRN 481 (FDA, 2013)] as a food ingredient. Of these seven GRAS notices, FDA has accepted six notices and one on meso-zeaxanthin is currently under review at the agency. Of the seven GRAS notices, GRAS notification 385 was submitted by OmniActive. The subject of GRAS notice 385 contains 13.5% zeaxanthin (isomers 3R,3'R- and meso-, at a ratio of 50:50). In this notice, the resulting 90th percentile zeaxanthin (both isomers) intake was reported as 2.4 mg/person/day (FDA, 2011). This notice received a "no questions" letter from FDA. All these notices also suggest that FDA is comfortable with the conclusion that the consumption of lutein with daily intake of zeaxanthin as proposed in these notices is safe. The information related to the safety and uses of zeaxanthin in the above cited GRAS Notices is hereby incorporated by reference into this GRAS document.

Table 6. Comparison of Regulatory Assessment of Lutein and Zeaxanthin Products

Information Source	Subject	Levels of Lutein and Zeaxanthin in the Product	Estimated Daily Intake (90^{th} percentile)	Acceptable Daily Intake	Safety Assessment Basis
GRN 110	Lutein ester	Lutein esters ≥ 74% Zeaxanthin esters ≥ 7%	Lutein/zeaxanthin ester = 22 mg/p/day Lutein ester = 20.46 mg/p/day Zeaxanthin ester = 1.5 mg/p/day	40 mg/person /day (lutein ester equivalent)	Based on ADI
GRN 140	Crystalline lutein	Lutein ≥ 74% Zeaxanthin 2-9%	Lutein = 13.4 mg/ person/day Zeaxanthin 1.2 mg/ person/day	Not reported	Totality of evidence supports safety
GRN 221	Suspended lutein	Lutein ≥ 74% Zeaxanthin 4-9%	0.20-0.82 and 0.46-1.10 (for 3-6 and 7-11 month old child, respectively)	Not reported	Totality of evidence supports safety
GRN 291	Crystalline lutein	Lutein ≥ 74% Zeaxanthin ≤ 8%	Lutein = 13.4 mg/p/day Zeaxanthin 1.2 mg/p/day	Not reported	Totality of evidence supports safety
GRN 385	Lutein (free and ester form)	Lutein ≥ 67% Zeaxanthin ≥ 13.5% R,R-zeaxanthin ≥ 6.75% Meso-zeaxanthin ≥ 6.75%	Lutein = 13.4 mg/ p/day Zeaxanthin 2.4 mg/ p/day R,R-Zeaxanthin 1.2 mg/p/day Meso-zeaxanthin 1.2 mg/p/day	Not reported	Totality of evidence supports safety
GRN 390	Suspended lutein	Lutein ≥ 74% Zeaxanthin 4-9%	37 µg/kg bw/day (for infants)	Not reported	Totality of evidence supports safety for infants
GRN 481 (under review with FDA)	Meso-zeaxanthin	Lutein = 15% Zeaxanthin = 65% R,R-Zeaxanthin = 5% Meso-zeaxanthin = 60%	Lutein = 2.57 Zeaxanthin = 11.46 R,R-Zeaxanthin = 0.88 Meso-zeaxanthin = 10.75	Not determined	Totality of evidence
JECFA	Lutein	Not reported	Up to 120 mg/day	0–2 mg/kg bw (group ADI for lutein and zeaxanthin)*	Based on ADI
JECFA	Zeaxanthin	Synthetic	Not reported	0-2 mg/kg bw (120 mg/person/day for 60 kg individual). Group ADI for lutein and synthetic zeaxanthin	Based on ADI
EFSA	Zeaxanthin	Synthetic (96%)	2 mg/person/day	53 mg/person (70 kg person)	Based on ADI
Present GRAS	**R,R Zeaxanthin**	**Zeaxanthin 65%**	**13.4 mg/person/day**	**Not determined**	Totality of evidence

*This group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications.

000026

In the GRAS notices (six) that are accepted by the FDA, biological data on the natural occurrence, metabolism, and safety of lutein containing zeaxanthin has been extensively presented and discussed. In these notices, the safety of lutein containing small amounts of zeaxanthin has been established through toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. Additionally, the safety is corroborated by additional studies conducted with other sources of lutein, lutein-rich foods, and lutein supplements. Furthermore, historical consumption of eggs and fruits and vegetables where these carotenoids predominate (*e.g.*, green leafy vegetables, such as spinach and kale) confirm the safety of lutein and zeaxanthin. Based on the data submitted in these Notices, and supported by FDA's determination that it had no questions regarding the determinations in these Notices, it can be concluded that consumption of lutein and zeaxanthin from conventional foods that results in daily intake of up to 20 mg/person/day is safe. The most recent review and response from the FDA was to GRN 432 (lutein diacetate) during 2012. The FDA did not question the acceptability and suitability of the available evidence to support the use of lutein containing small amounts of zeaxanthin.

2.1.2. JECFA Assessment

In separate safety evaluations, JECFA assessed the safety of lutein containing small amount of zeaxanthin, and zeaxanthin. In 2006, JECFA evaluated safety-in-use of lutein containing small amounts of zeaxanthin as a food additive (JECFA, 2006). The committee noted that there were no adverse effects documented in any of the toxicity studies in animals, including mice, rats, and monkeys, or in humans. JECFA assigned a group ADI of 0 to 2 mg lutein and zeaxanthin/kg bw. The ADI determination was based on no observed adverse effect level (NOAEL) of 200 mg lutein/kg bw/day (the highest dose tested) reported in a 90-day rat study (Pfannkuch et al., 2000, 2001; Kruger et al., 2002), to which a safety factor of 100 was applied. Given the lack of adverse effects reported at much higher doses than 200 mg/kg body weight/day (up to 1,000 mg/kg bw/day in a study of developmental toxicity), the safety factor was considered appropriate by JECFA. The JECFA determined ADI of 2 mg/kg bw/day will be equivalent to a dose of 120 mg/person/day for an individual weighing 60 kg.

JECFA also evaluated the safety of synthetic zeaxanthin and summarized its findings from some clinical, toxicological, and mutagenicity tests performed in animals, in a report (WHO, 2006). In this report, findings from a 13-week study on mice and rats receiving oral doses of zeaxanthin at levels of 250, 500, 1000 mg/kg bw/day were described. No treatment-related adverse effects were observed throughout the study. In addition, hematology, blood chemistry, and urine analysis measurements showed no evidence of toxicity. The NOAEL of zeaxanthin from this study was determined to be 1000 mg/kg bw/day, the highest dose tested (Ettlin et al., 1980a; 1980b). In this report, additional ocular toxicity studies performed on monkeys were also described. These studies also did not reveal any evidence of treatment-related changes (Pfannkuch et al., 2000a, 2000b; Pfannkuch, 2001).

2.1.3. EFSA Assessment

Initially in 2008, the EFSA Panel evaluated information on synthetic zeaxanthin and concluded, based on the available data, that the safety of zeaxanthin as an ingredient in food supplements at the proposed use level of up to 20 mg/person/day has not been established (EFSA, 2008). However, in a recent scientific opinion, the EFSA (2012) Panel reevaluated the safety of synthetic zeaxanthin as a novel food ingredient in food supplements at a dose of 2 mg/person/day.

For the reevaluation, the Panel reviewed additional toxicological information, in particular a two-generation reproduction toxicity study. Based on the two-generation study, the Panel identified a NOAEL at 150 mg/kg bw/day. Given the absence of a chronic toxicity/carcinogenicity study, the Panel applied an uncertainty factor of 200. The Panel concluded that, based on the available data, intakes of 0.75 mg/kg bw per day for synthetic zeaxanthin, corresponding to a daily intake of 53 mg for a person with a body weight of 70 kg do not raise safety concerns. The Panel also noted that human intervention studies, in which zeaxanthin has been supplemented at doses of up to 20 mg/day for up to 6 months, or 8 mg/day for a year, were without evidence of adverse effects. Available epidemiological studies do not indicate that dietary zeaxanthin is linked to an increased risk of lung cancer (EFSA, 2012).

2.1.4. Specific Toxicity Studies of OmniXan™

In a series of studies, OmniActive Health Technologies investigated acute and subchronic toxicity, and mutagenicity of zeaxanthin concentrate (the subject of present GRAS determination). The results of these studies are in the process of publication. The reports of these studies and the manuscript under publication were provided for this GRAS assessment.

2.1.4.1. Acute (LD_{50}) Study

The acute toxicity study was performed according to OECD Guidelines for Testing of Chemicals (No. 423, Section 4, Health Effects). For these investigations, in house bred nulliparous/non-pregnant female Wistar rats were used. Based on findings from sighting study (step I and step II), 2000 mg/kg bw dose was gavage administered to rats (n=4). The oral LD_{50} of the zeaxanthin concentrate in rats was found to be greater than 2000 mg/kg bw. The 14-day observation period and body weight measurements did not reveal any toxic effects. Necropsy at the end of study did not reveal any gross pathological abnormalities. The results of the oral acute toxicity study suggest that zeaxanthin concentrate is unlikely to be toxic. The results of this study suggest that LD_{50} of zeaxanthin concentrate is greater than 2 g/kg bw (Ravi et al., 2014).

2.1.4.2. Subchronic (90-day) Study

In the repeat-dose 90-day toxicity study, conducted according to OECD and Redbook 2000 guidelines for such studies, the potential adverse effects of zeaxanthin concentrate (OmniXan™) were investigated (Ravi and Vinay Babu, 2014; Ravi et al., 2014). In this study, zeaxanthin concentrate dissolved in refined safflower oil was administered orally (gavage) once daily at dose levels of 0, 4, 40 and 400 mg/kg bw/day (control, low, mid, and high dose group, respectively) for at least 90 days to Wistar rats (10/sex/group). Additional, ten male and ten female rats were allocated to control and high dose recovery groups. The highest dose (400 mg/kg/day) selected for this study is approximately 100X of the human dose based on body weight and body surface area. Additionally, a preliminary 14-day repeat-dose range (100, 200, 400 and 1000 mg/kg bw/day) finding study was conducted (OECD Guidelines 425) to support the dose selection for the subchronic study. Clinical signs (daily), body weights, and feed consumption (once weekly) were recorded during the course of study, while organ weights, hematology, clinical biochemistry, and urine analysis were recorded at the end of treatment (after 13 weeks) and recovery (after 17 weeks) was performed. At the end of the treatment period, macroscopic and microscopic observations were performed. Histopathology of the preserved tissues of all the animals in the control and high dose groups as well as any gross lesions observed in other group animals were performed.

No mortalities or treatment related clinical signs were observed at any of the doses tested in either sex throughout the experimental period, except for stained (light brown colored) feces in the high dose group that disappeared with the discontinuation of treatment (recovery group). The feces coloration was attributed to the direct contact of the test substance with the contents of the gastrointestinal tract. There were no treatment related changes observed during the ophthalmological examination carried out at the end of treatment period for the treatment groups and at the end of the recovery period for the recovery groups. Similarly, no treatment related changes were observed in neurological/functional examinations carried out at the end of the treatment period for the treatment groups and at the end of the recovery period for the recovery groups. There were no treatment related changes noted in mean body weight or in feed consumption in either sex at any of the doses tested. Some statistically significant changes noted in body weights and feed consumption were considered incidental as there was no dose dependency and the change was sporadic in nature (Ravi and Vinay Babu, 2014; Ravi et al., 2014).

No toxicologically relevant treatment-related findings were noted in hematology or clinical biochemistry parameters at the end of the treatment and recovery period. However, some clinical chemistry parameters did show some statistically significant changes. For example a small but significant ($p<0.05$) increase in plasma levels of sodium was noted in all treated males. However, there was no clear dose response in these changes and the changes were within the historical control data of the performing laboratory. Additionally, other related parameters such as plasma potassium and calcium, as well as urinary parameters (such as urine volume, pH and specific gravity) did not show any dependent changes. Furthermore, at termination there were no histological changes noted in kidneys at dose level of 400 mg/kg bw/day. In the absence of changes in other blood or urine parameters, or histopathological observations of kidney, the mild variation in plasma sodium is not considered a cause of concern and without any toxicological and biological consequence. Besides an increase in plasma sodium in male rats, a slight but statistically significant ($p<0.05$) increase in sodium, total protein, urea and globulin were observed in female rats treated with high dose (400 mg/kg/day) of zeaxanthin. These changes were considered toxicologically and biologically insignificant as there were no microscopic changes observed in any of the dependent organ/tissues (Ravi and Vinay Babu, 2014; Ravi et al., 2014).

There were no treatment related variations in the urinalysis parameters at any of the doses tested on both the sex. As compared to control group rats, no treatment-related changes in absolute organ weights were noted in male and female rats following administration of the zeaxanthin concentrate. The gross macroscopic examinations did not reveal any treatment-related changes in any of the groups. The histopathological changes observed were considered spontaneous and incidental to Wistar rats of this particular species, strain and age. The results of this study suggest that the NOAEL of zeaxanthin concentrate (OmniXan^{TM}) was 400 mg/kg bw/day, the highest dose tested (Ravi and Vinay Babu, 2014; Ravi et al., 2014).

2.1.4.3. Mutagenicity Studies

In an *in vitro* study (Ames assay), potential mutagenic effects of zeaxanthin (OmniXan™) were investigated according to the plate incorporation test (Trial I) and the pre-incubation test (Trial II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 in the presence and absence of rat liver microsomal S9 fraction (Ravi et al., 2014). The study was performed in compliance with OECD principles of GLP. Zeaxanthin

concentrate was tested at the following concentrations: 62.5, 125, 250, 500 and 1000 µg/plate. No substantial increase in revertant colony numbers of any of the tester strains were observed following treatment with zeaxanthin concentrate at any selected dose level in both the confirmatory trials, neither in the presence nor in the absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. The results of this study revealed that zeaxanthin concentrate at a selected dose levels used in this study did not induce gene mutations by base pair changes or frameshifts in the genome of the *Salmonella* strains used (Ravi et al., 2014).

2.1.5. Other Safety Studies of Zeaxanthin

In a long-term study in monkeys, Khachik et al. (2006b) investigated the effects of lutein, zeaxanthin, or a combination of the two, on changes in plasma levels of these carotenoids as well as ocular (fundus photography and retina histopathology) and renal (biomarkers) toxicity. In this study, eighteen female rhesus macaques were divided into control (n=3), lutein-treated (n=5; 9.34 mg lutein/kg bw and 0.66 mg zeaxanthin/kg bw), zeaxanthin-treated (n=5; 10 mg zeaxanthin/kg bw), and lutein/zeaxanthin-treated groups (n=5; lutein and zeaxanthin, each 0.5 mg/kg). The animals were supplemented with these levels daily for a period of 12 months. Plasma and ocular tissue concentrations of lutein or zeaxanthin and their metabolites determined at baseline and at the end of 6 and 12 months revealed a significant increase in the supplemented groups. Lutein and zeaxanthin supplementation did not cause ocular toxicity and had no effect on biomarkers and indicators of renal toxicity such as urinary creatinine and protein. The results of this study demonstrate that administration of either lutein or zeaxanthin to monkeys for one year at a dose of approximately 10 mg/kg bw/day did not cause ocular or renal toxicity. For a 60 kg human, this dose is equivalent to 600 mg/day. The investigators suggested that future long-term human supplementation studies with these carotenoids at a much lower dose (e.g., 0.5 mg/kg body weight or lower), should not present any problems associated with toxicity.

In the EFSA (2012) assessment, several recent published and unpublished safety-related studies of zeaxanthin are summarized. In a two-generation reproduction toxicity study, carried out in accordance with OECD Guideline 416, Zeaxanthin was administered with the diet to groups of 24 male and 24 female rats resulting in overall combined intake of 52, 155 and 508 mg zeaxanthin/kg bw/day, low, mid and high dose group, respectively. Additionally, one placebo group and another standard rodent diet (conventional control) group were also included. The results of this study revealed that compared with the placebo group, administration of zeaxanthin at a dose of approximately 500 mg/kg bw/day to rats for two successive generations induced a slightly lower post-implantation survival index in the high dose group in the P generation and a slightly lower body weight gain during the gestation period of the F1 generation. There was an adverse effect on fertility of the F1 generation (statistically significantly reduced mating index), slightly fewer pups were born and the post-implantation survival index was also slightly lower. Based on these observations, the NOAEL was considered to be the nominal dosage of 150 mg zeaxanthin/kg bw/day.

2.1.6. Toxicity Studies of Meso-Zeaxanthin

As mentioned earlier, zeaxanthin and meso-zeaxanthin are diastereomers, and differ only in the spatial orientation of the hydroxyl group on the C3' chiral position. As both these isomers occur in macular pigment, there has been considerable scientific interest to explore the

mechanism of action and safety of meso-zeaxanthin, as well. In the published literature several studies, including toxicity studies, of meso-zeaxanthin have appeared. Given the similarity between zeaxanthin and meso-zeaxanthin (structural isomers), safety studies of meso-zeaxanthin are discussed below.

Thurnham and Howard (2013) examined the potential genotoxicity (Ames test) and subchronic toxicity of meso-zeaxanthin. For the genotoxicity investigations, the ability of meso-zeaxanthin to induce reverse mutations (in the presence and absence of microsomal enzymes) at 2 genomic loci; the histidine locus of 4 strains of *S. typhimurium* and the tryptophan locus of *Escherichia coli* strain WP2uvrA was studied. Six doses of meso-zeaxanthin ranging from 10 to 5000 μg/plate were tested twice with vehicle and positive controls using 3 plates/dose. Meso-zeaxanthin did not cause any increase in the mean number of revertants/plate with any bacterial strain, with or without microsomal enzymes, and was therefore unlikely to be mutagenic.

For the subchronic toxicity study conducted according to OECD and FDA guidance for such studies, meso-zeaxanthin was administered daily to rats for 13 weeks followed by a 4-week recovery period (Thurnham and Howard, 2013). Rats were randomly assigned to four groups (10/sex/group) to receive corn oil (control) or meso-zeaxanthin at dose levels of 2, 20 and 200 mg/kg bw/day by oral gavage. Additional rats (five of each sex) in the control and the 200 mg/kg/day groups were retained for the recovery period. No compound-related clinical, biochemical or pathological signs or symptoms were noted. Based on these results, the investigators determined the NOAEL of meso-zeaxanthin as >200 mg/kg bw/day, the highest dose tested.

In a comprehensive safety profile, Xu et al. (2013) investigated the effects of meso-zeaxanthin in a series of toxicity tests that included acute toxicity, genetic toxicity (Ames test, mice bone marrow erythrocyte micronucleus and mice sperm abnormality) and 90-day sub-chronic toxicity. In the acute oral toxicity tests, maximum tolerable dose was more than 10.0 g/kg bw in Sprague Dawley rats and ICR mice, and showed no toxicological signs during the period of the study. These observations suggest that meso-zeaxanthin has no acute toxicity (practically non-toxic). In the genotoxicity studies, as evaluated by *in vitro* Ames test (*S. typhimurium* TA97, TA98, TA100, and TA102) and in the *in vivo* mice experiments as per bone marrow erythrocyte micronucleus assay and as per sperm abnormality test in male mice (treated at dose levels of 1.25, 2.5 and 5.0 g/kg bw), meso-zeaxanthin did not reveal any genotoxic or mutagenic potentials.

In the subchronic toxicity study by Xu et al. (2013), Sprague Dawley rats were randomly divided into four groups (10/sex/group) and were orally treated with meso-zeaxanthin at dose levels of 0, 300, 600 and 1200 mg/kg bw/day for 13 consecutive weeks. During the course of the study and at termination all standard parameters for such type of studies were measured. At termination, necropsy and pathological examination revealed histological changes in liver cells with vacuolar degeneration increased significantly in 1200 and 600 mg/kg dose group, as well as those with inflammatory cell infiltration within liver lobule and spotted liver cell necrosis with inflammatory cell infiltration increased significantly in 1200 mg/kg dose group compared with the negative control group. At the dosage of 300 mg/kg/day in both male and female SD rats, no noticeable toxicological effects were observed. The investigators concluded that meso-zeaxanthin has no acute toxicity and no genotoxicity and the use of meso-zeaxanthin is safe at dose of 300 mg/kg bw/day in rats from a 90-day feeding study. After the application of a 100-fold safety factor, the investigators determined the ADI (acceptable daily intake) value of 3

mg/kg bw/day (Xu et al., 2013). The results of this study further support findings from the above study that meso-zeaxanthin is unlikely to cause any adverse effects at the dose level of 200 mg/kg be/day, the highest dose tested (Thurnham and Howard, 2013).

In two separate studies, Firdous et al. (2011) investigated anti-mutagenic *(in vitro)* and anti-carcinogenic *(in vivo)* potentials of meso-zeaxanthin. For the anti-mutagenic activity, Ames test (*Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 1535) with direct acting mutagens as well as mutagen needing microsomal activation were employed. Meso-zeaxanthin was found to inhibit the mutagenicity induced by direct acting carcinogens in a concentration dependent manner, as well as with indirect acting carcinogens. The *in vivo* anti-carcinogenicity activity of meso-zeaxanthin was evaluated using nitroso diethyl amine (NDEA) induced hepatocellular carcinoma in rats. For these studies, groups of male Wistar rats were treated (oral gavage) 5 days a week with NDEA alone or in combination with meso-zeaxanthin at two dose levels (50 and 250 mg/kg/day) for 20 weeks. As compared to positive control group, treatment with meso-zeaxanthin reduced the tumor incidence. The results of these experiments suggest anti-mutagenic and anti-carcinogenic potentials of meso-zeaxanthin.

2.2. Bioavailability

Oral ingestions of zeaxanthin and lutein are likely to follow the same digestion and intestinal absorption pathways as dietary fat. Upon absorption, both zeaxanthin and lutein are incorporated into chylomicrons, and are approximately evenly distributed between high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in circulation (Olson, 1996; Furr and Clark, 1997; Goulinet and Chapman, 1997). Lutein appears to be distributed to tissue following the interaction of lipoprotein particles with receptors, and degradation of lipoproteins by extra-hepatic enzymes such as lipoprotein lipase (Boileau et al., 1999). A specific xanthophyll-binding protein from human macula that mediates the uptake of lutein and zeaxanthin from the bloodstream has been isolated (Yemelyanov et al., 2001). Leo et al. (1995) suggested that lutein is likely to be excreted primarily through the bile into the feces. Part of lutein may be excreted by subcutaneous glands and sweat but not urine (Bendich, 1988). Given the similarity between lutein and zeaxanthin, it is likely that zeaxanthin will follow similar metabolic path.

Perez-Galvez et al. (2003) assessed the availability of carotenoids from paprika oleoresin, including zeaxanthin in human subjects. In this study, after overnight fasting, nine volunteers (four female and five male, 23 - 31 years old, non-smokers) ingested a single dose (1 g) of the paprika oleoresin containing 6.4 mg zeaxanthin, 4.2 mg β-cryptoxanthin, 6.2 mg β-carotene, 35.0 mg capsanthin and 2.0 mg capsorubin. Blood samples (10 ml) were collected at baseline (0 h) and 2, 4, 5, 6, 7, 9 and 12 hours after intake of the paprika oleoresin. Blood samples were centrifuged after clotting to obtain serum and chylomicrons were prepared according to a standard method described in the article. At different time points the carotenoid pattern in the chylomicron fraction was analyzed to evaluate carotenoid absorption. From the major carotenoids present in the paprika oleoresin only zeaxanthin, β-cryptoxanthin and beta-carotene were detectable. Free zeaxanthin and beta-cryptoxanthin were also detected. The investigators concluded that oleoresin is a suitable source for the provitamin A carotenoids β-carotene and β-cryptoxanthin and the macular pigment zeaxanthin.

In another study, Thurnham et al. (2008) measured the blood uptake of zeaxanthin and lutein in human subjects. In this study, human volunteers (ten male and nine female) received

one capsule containing 20 mg of lutein (10.8 mg), (3R,3'R)-zeaxanthin (1.2 mg) and meso-zeaxanthin (8.0 mg) daily for 21 days. Plasma lutein and total zeaxanthin concentrations were quantified at baseline, day 10, and day 22. Plasma concentrations per mg dose at day 22 suggested that (3R,3'R)-zeaxanthin (0·088 mmol/L per mg) was about 50% more actively retained by the body than lutein (0·056 mmol/L per mg) (although the difference was not significant in women) and 2.5 to 3.0 times more than meso-zeaxanthin (0·026 mmol/L per mg). Concentrations of meso-zeaxanthin at day 22 were 2.5 times higher in women than men. The results of this study also indicate that the plasma responses from lutein and (3R,3'R)-zeaxanthin were lower than those of the pure substances from the same laboratory. The uptake of these carotenoids appeared to be slightly depressed by the presence of meso-zeaxanthin. Plasma concentrations of β-carotene were depressed by about 50% at day 10 and about 35% at day 22. The investigators concluded that the lower plasma response to meso-zeaxanthin compared with (3R,3'R)-zeaxanthin probably indicates that meso-zeaxanthin is less well absorbed than (3R,3'R)-zeaxanthin.

Given its rare presence in human diet, meso-zeaxanthin has not been found to naturally occur in human blood. The available evidence suggests that the presence of high levels of meso-zeaxanthin in the eye despite being a minor component of the diet is likely to be its formation from lutein. Initially this view was proposed on the basis of chemical reactions, however recent studies in monkeys support this hypothesis (Johnson et al., 2005). Monkeys raised on a carotenoid-free diet, and then supplemented with lutein, subsequently exhibited both lutein and meso-zeaxanthin in the macular pigment. Those supplemented with zeaxanthin only, exhibited no presence of meso-zeaxanthin in the macular pigment. These observations support the hypothesis of conversion of lutein to meso-zeaxanthin. The exact mechanism of the conversion remains to be elucidated.

Bone et al. (2006) measured the serum concentrations of R,R-zeaxanthin and meso-zeaxanthin in two human volunteers who consumed a mixed suspension of lutein (5.5 mg), zeaxanthin (1.4 mg) and meso-zeaxanthin (14.9 mg) daily with a meal for 6 weeks. In one subject, the serum concentrations of zeaxanthin and meso-zeaxanthin increased by 0.043 and 0.044 μmol/l, respectively, while in the other subject it increased by 0.133 and 0.145 μmol/l. In both the subjects, the increase in the serum concentrations was approximately equimolar for both the isomers of zeaxanthin. The concentration of the meso-isomer was ten times the concentration of the R,R-isomer of zeaxanthin in the supplement. These observations suggests that the plasma uptake of R,R-zeaxanthin (commonly present in diet) in human subjects is much higher (90%) as compared with meso-zeaxanthin (10%).

Connolly et al. (2011) compared the differing serum carotenoid and macular pigment responses from several published studies. These investigators suggested that the differences noted in the serum levels may be due to several factors, such as daily dose of carotenoids consumed, type of carotenoids in the supplement (free versus ester), matrix in which carotenoids are consumed (oil versus microencapsulated), whether the supplement was consumed alone or in the presence of other antioxidants, and noncompliance with the study supplement regimen. In another review article, Abdel-Aal et al. (2013) reported that absorption of carotenoid released from food involves dispersion in the gastric emulsion to be incorporated into lipid droplets, followed by transfer to mixed micelles involving bile salts, biliary phospholipids, dietary lipids and others. Following solubilization, carotenoids are absorbed by the intestinal cell for transportation into blood system. These steps may include simple diffusion, uptake by micelles

and receptor mediated and other transporter (Abdel-Aal et al., 2013). It should be noted that because of the hydroxyl groups, lutein and zeaxanthin are polar compounds compared with the hydrocarbon carotenoids (α-, β-carotene, and lycopene).

In summary, pharmacokinetic studies indicate that orally administered zeaxanthin or lutein (free or ester form) is bioavailable. Upon absorption, zeaxanthin is likely to be incorporated into chylomicrons, and distributed in HDL and LDL in circulation. Extra-hepatic tissue uptake appears to be a receptor medicated enzyme (lipase) reaction. The available evidence suggests that lutein is converted to meso-zeaxanthin (limited to macular area). There is no evidence to suggest that lutein or meso-zeaxanthin is converted to zeaxanthin.

2.3. Human Studies

In the recent opinion on the safety of synthetic zeaxanthin as an ingredient in food supplements, the EFSA (2012) Panel mentioned several human intervention studies related to visual function and eye research in which zeaxanthin, alone or in combination with lutein. In these studies, human subjects has been supplemented at doses of up to 20 mg/day for up to 6 months (Huang et al., 2008; Stringham and Hammond, 2008; van de Kraats et al., 2008; Forma et al., 2011; Carboni et al., 2011) or 8 mg/day for a year (Richer et al., 2011) and no evidence of adverse effects was noted.

In a 4 month study in 19 volunteers, Bone et al. (2007) examined macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. In this study, 10 participants were given supplement containing 14.9 mg of meso-zeaxanthin, 5.5 mg of lutein and 1.4 mg of R,R-zeaxanthin, while 9 subjects received placebo for 4 months. At initiation of study and during the supplementation period, blood serum samples were analyzed for carotenoid content. Similarly, macular pigment optical density was measured. Supplementation with the carotenoids revealed the presence of all three carotenoids in the blood. As compared to placebo group, the macular pigment optical density significantly increased in the supplemented subjects. No adverse effects were reported by the investigators.

Connolly et al. (2010b; 2011) investigated changes in macular pigment optical density (MPOD), and serum concentrations of the macular carotenoids in response to supplemental meso-zeaxanthin, lutein and R,R-zeaxanthin, in healthy volunteers. In this double-blind, randomized, placebo controlled trial, 44 subjects were recruited of which 22 subjects (male/female = 8/14; age 43±13 years; BMI = 27.2±6.1) were randomized to consume a formulation containing 10.6 mg of meso-zeaxanthin, 5.9 mg of lutein and 1.2 mg of R,R-zeaxanthin (Intervention group), and 22 subjects (male/female = 9/13; age 45±12 years; BMI = 26.8±5) consumed a placebo everyday over a six month period (Placebo group). At baseline, 3 months, and 6 months changes in macular pigment and serum levels of lutein and zeaxanthin was measured. A statistically significant increase in serum concentrations of lutein and zeaxanthin and macular pigment density in the intervention group at 3 and 6 month visit was noted. The safety of consumption was assessed by analyzing blood samples for changes in renal and liver function, as well as lipid profile, hematologic profile, and markers of inflammation after 6 months of supplementation.

Among the clinical pathology parameters analyzed, a statistically significant variation from baseline to 6 months in 8 of the 25 variables measured in the intervention group and 9 of the 25 biochemical parameters analyzed in the placebo group was noted (Connolly et al., 2011). However, all parameters were within the normal reference range, with the exception of total

cholesterol and LDL, which had a baseline value outside the accepted normal reference range in both the groups before supplementation. There were no adverse events recorded or reported by any subject participating in this study. The results of this clinical trial suggest that consumption of a supplement containing meso-zeaxanthin, lutein and R,R-zeaxanthin is safe (Connolly et al., 2011).

In a randomized, double-blind trial, in human volunteers, Khachik et al. (2006a) investigated the effect of lutein (with 6% zeaxanthin) on serum carotenoids. In this study, 45 elderly subjects (> 60 years of age), with and without AMD were supplemented with lutein at doses of 2.5, 5.0, or 10 mg/day for six months. Lutein supplementation (10 mg/day) resulted in a significant increase in serum levels of lutein and its metabolites. The increase in the serum levels of lutein/zeaxanthin correlated with increases in the serum levels of their metabolites. The investigators also reported that based on the results of the liver function tests and visual-function examinations, no toxicity or adverse effects were associated with lutein supplementation at dose levels up to 10 mg/day. Based on the results of this study, the investigators concluded that consumption of lutein at doses up to 10 mg/day for six months by elderly subjects with and without AMD is safe.

3. SUMMARY AND DISCUSSION

OmniActive Health Technologies Ltd. intends to use zeaxanthin concentrate (marketed under trade name OmniXan™) obtained from paprika (*C. annum* L) as a food ingredient. OmniXan™ is a reddish-orange color product with a characteristic aroma of paprika. The compositional analysis of zeaxanthin concentrate demonstrated that it primarily contains zeaxanthin with a small amount of other carotenoids, such as lutein (0-1%), β-carotene (5-15%) and β-cryptoxanthin (4-6%). The marketed product (OmniXan™) is formulated to desired levels of zeaxanthin as a standardized oil suspension with commonly used dietary oils (corn oil, sunflower oil, or safflower oil), or as beadlets standardized with food grade carbohydrates. OmniActive intends to use zeaxanthin at concentrations up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, and soft candy. The intended use of OmniXan™ in the above mentioned food categories will result in a mean and 90th percentile estimated daily zeaxanthin intake of 7.3 and 13.4 mg/person/day.

The active constituent of OmniXan™, zeaxanthin has a safe history of consumption as a dietary component. Zeaxanthin concentrate (OmniXan™) is derived from paprika that is commonly consumed around the world. Zeaxanthin and its esters are found in a variety of commonly consumed foods such as yellow corn, red pepper, orange pepper, orange juice, honeydew, mango, and chicken egg yolk. Lutein and zeaxanthin are two of the most abundant carotenoids found in the diet. The macula of the eye is a repository for lutein and zeaxanthin. Lutein and zeaxanthin in combination or alone are marketed as dietary supplements. Lutein and zeaxanthin have identical chemical formulas and are isomers. The recommended dose of zeaxanthin as a dietary supplement ranges from 1.2 to 8.0 mg/day. Zeaxanthin has been the subject of a New Dietary Ingredient Notification that was accepted by FDA for filling. There is sufficient qualitative and quantitative scientific as well as history of use evidence to determine the safety-in-use of OmniXan™ and its constituent in the above mentioned food applications.

The FDA has reviewed six separate GRAS notices on lutein that also contains small amount of zeaxanthin. In response to these notices the agency did not question the safety of lutein or zeaxanthin. The subject of the present GRAS determination is similar to these GRAS notices particularly as regards the presence of zeaxanthin. In addition to these regulatory reviews by FDA, JECFA has completed separate safety evaluations of lutein as well as zeaxanthin and assigned a group ADI of 0 to 2 mg lutein and zeaxanthin/kg bw (combined). Furthermore, recently EFSA has evaluated the safety of synthetic zeaxanthin and concluded that intake of synthetic zeaxanthin at levels of 0.75 mg/kg bw/day, corresponding to a daily intake of 53 mg for a person with a body weight of 70 kg, do not raise safety concerns (EFSA, 2012).

The available evidence from animal and human studies suggests that oral administration of zeaxanthin ester or its free form results in zeaxanthin being bioavailable. In a study, supplementation of zeaxanthin (10 mg/kg/day) to rhesus monkeys for one year did not reveal ocular toxicity and had no effect on biomarkers of kidney toxicity. In a genotoxicity study, zeaxanthin did not cause mutagenic effects. The available evidence suggests that zeaxanthin is unlikely to be mutagenic or cause DNA damage. The available studies of meso-zeaxanthin also provide support for the safety-in-use of zeaxanthin.

In a specifically designed subchronic toxicity study, the safety of zeaxanthin concentrate (OmniXan™) was investigated. In this study, conducted as per OECD guidelines, zeaxanthin was administered (gavage) daily to rats at doses of 0, 4, 40, or 400 mg/kg bw/day for 90 days. No treatment-related adverse effects on any of the series of parameters investigated were noted. Based on the results of this study, the NOAEL for zeaxanthin (OmniXan™) is determined as 400 mg/kg bw/day, the highest dose tested. The estimated daily intake of zeaxanthin (13.4 mg) from its intended food use is approximately 1800 fold lower compared to the NOAEL determined from the subchronic toxicity study in rats.

In summary, considering the totality of the evidence, on the basis of scientific procedures[3], history of exposure and use, the consumption of zeaxanthin (OmniXan™) derived from paprika (*Capsicum annum* L) as a food ingredient at use levels of 0.3 to 3 mg/serving in certain specified foods resulting in a 90th percentile intake of 13.4 mg zeaxanthin is considered safe. The proposed uses are compatible with current regulations, *i.e.*, zeaxanthin concentrate (OmniXan™) is used as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes, when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

[3] 21 CFR 170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

4. CONCLUSION

Based on a critical review of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that consumption of zeaxanthin (OmniXan™) as a nutrient [21 CFR 170.3(o)(20)] in selected food products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, milk products, processed fruits and fruit juices, and soft candy] at levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12) when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile all-user estimated intake of 13.4 mg zeaxanthin/person/day is Generally Recognized As Safe (GRAS).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that zeaxanthin (OmniXan™ when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

Stanley T. Omaye, Ph.D., F.A.T.S. — 5/28/2014 Date

(b) (6)

Robert L. Martin., Ph.D. — June 1, 2014 Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — June 4, 2014 Date

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6. APPENDIX I

Batch Specifications

Analytical data from three manufacturing lots
(RR-Zeaxanthin Concentrate- OmniXan™)

Parameters		Batch Nos.		
		ZNPE55	**ZNPE56**	**ZNPE57**
Assay	RR Zeaxanthin (Trans)	66.17%	68.13%	69.37%
	TXC (total xanthophyll concentration)	84.18%	80.26%	87.09%
	Total Mixed Carotene	18.01%	12.13%	17.72%
Nutritional Characters	Fats	20.92 g/100 g	21.59 g/100 g	19.82 g/100 g
	Proteins	0.0 g/100 g	0.0 g/100 g	0.0 g/100 g
Heavy Metals (ICP-MS)	Cadmium	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg
	Lead	<0.1 mg/kg	<0.1 mg/kg	0.25 mg/kg
	Mercury	<0.025 mg/kg	<0.025 mg/kg	<0.025 mg/kg
	Arsenic	<0.1 mg/kg	0.84 mg/kg	<0.1 mg/kg
Pesticides	Organochlorine pesticides	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
	Organophosphorus pesticides	<0.05 mg/kg	<0.05 mg/kg	<0.05 mg/kg
	Dithiocarbamates	<0.1 mg/kg	<0.1 mg/kg	<0.05 mg/kg
Residual Solvent	Acetone	18 ppm	26 ppm	15 ppm
	Ethanol	0 ppm	3 ppm	4 ppm
Microbial Analysis	Total Plate Count	<10 cfu/g	<10 cfu/g	<10 cfu/g
	Coliform count	<10 cfu/g	<10 cfu/g	<10 cfu/g
	Escherichia coli	<3 MPN/g	<3 MPN/g	<3 MPN/g
	Staphylococcus aureus	<10 cfu/g	<10 cfu/g	<10 cfu/g
	Salmonella spp.	Absent/25 gm	Absent/25 gm	Absent/25 gm
	Yeast & Mould Count	<10 cfu/g	<10 cfu/g	<10 cfu/g

SUBMISSION END