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GRAS Notice (GRN) No. 385

<http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/default.htm>

ORIGINAL SUBMISSION

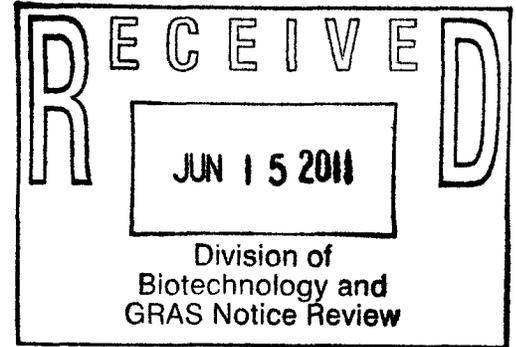
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Soni & Associates Inc.

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June 7, 2011

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 lutein and zeaxanthin preparation (Lutemax 2020™)

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 lutein and zeaxanthin preparation (Lutemax 2020™) 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.

Given the compositional differences (particularly isomers of zeaxanthin) and manufacturing process, we believe that Lutemax 2020™ is somewhat different compared to the lutein products that have already been reviewed by FDA under other GRAS notices.

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.

Sincerely,
(b) (6)

Madhu G. Soni, Ph.D.

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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 lutein and zeaxanthin preparation (Lutemax 2020™) derived from Marigold flowers (*Tagetes erecta* L) 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 lutein and zeaxanthin preparation (Lutemax 2020™) is exempt from the requirement of premarket approval.

Signed
(b) (6)



Date June 8, 2011

Madhu G. Soni, Ph.D., FACN

Agent for:

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India

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C. Common or Usual Name of the Notified Substance:

The common name of the substance of this notification is lutein and zeaxanthin preparation. The ingredient is a mixture of carotenoid xanthophylls, including lutein and zeaxanthin. Generally, the term lutein is used as the name of the principal component of the mixture of carotenoids xanthophylls and often denotes a mixture of the carotenoids lutein and zeaxanthin. The trade name of the substance is Lutemax 2020™.

D. Conditions of Intended Use in Food

Lutemax 2020™, a lutein (> 67%) and zeaxanthin (>13.5%) preparation, 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, 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 use levels of 0.3 to 3 mg lutein/serving (reference amounts customarily consumed, 21CFR 101.12). A summary of use levels and food categories for lutein is presented in Table I-D. Lutemax 2020™ will not be added to food categories that come under USDA jurisdiction. The intended use of lutein and zeaxanthin preparation 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 intended uses of lutein and zeaxanthin in the above mentioned food categories will result in the mean and 90th percentile intake of 7.3 and 13.4 mg/person/day, respectively. Lutemax 2020™ contains 13.5% zeaxanthin and the resulting 90th percentile zeaxanthin intake will be approximately 2.4 mg/person/day. As zeaxanthin in Lutemax 2020™ exists as 3R,3'R- and 3R,3'S-isomer at a 50:50 ratio, the 90th percentile consumption of each isomer will be 1.2 mg/person/day. These isomers are called RR-zeaxanthin and meso-zeaxanthin, respectively. In this document, unless otherwise specified, Lutemax 2020™ represents the concentrated form with >67% lutein and >13.5% zeaxanthin.

Table I-D. Food Categories and Intended Use Levels of Lutein and Corresponding Use Levels of Lutemax 2020™ in the US.

Food Category	Proposed Food	Use levels	
		Lutein mg/RACC ¹	Lutemax 2020™ mg/RACC
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.0	2.40
	Crackers and Crisp-breads	2.0	2.40
Beverages and Beverage Bases	Bottled Water	0.5	0.60
	Carbonated Beverages	2.0	2.40
	Meal Replacements	2.0	2.40
	Tea, Ready-to-Drink	0.6	0.72
Breakfast Cereals	Instant and Regular Hot Cereals	2.0	2.40
	Ready-to-Eat Cereals	2.0	2.40
Chewing Gum	Chewing Gum	1.0	1.20
Dairy Product Analogs	Imitation Milks	2.0	2.40
	Soy Milks	1.5	1.94
Egg Products	Liquid, Frozen or Dried Egg Substitutes	2.0	2.40
Fats and Oils	Margarine-like Spreads	1.5	1.80
	Salad Dressings	1.5	1.80
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.0	1.20
Gravies and Sauces	Tomato Based Sauces	0.3	0.36
Hard Candy	Hard Candy	1.0	1.20
Infant and Toddler Foods*	Junior, Strained and Toddler-Type Baby foods	1.0	1.20
Milk Products	Dry Milk	3.0	3.60
	Fermented Milk Beverages	0.6	0.72
	Flavored Milk and Milk Drinks	3.0	3.60
	Milk-Based Meal Replacements	3.0	3.60
	Yogurt	3.0	3.60
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	2.0	2.40
	Fruit-Flavored Drinks	2.0	2.40
	Fruit Juice	2.0	2.40
	Nectars	2.0	2.40
	Vegetable Juice	2.0	2.40
Soft Candy	Chewy and Nougat Candy	1.0	1.20
	Fruit Snacks	1.0	1.20
Soups and Soup Mixes	Canned Soups	0.6	0.72

¹RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC. Uses listed and level same as in GRN 000140. *Does not include infant formula. Adapted from GRN 000140 and GRN 000291.

As Lutemax 2020™ represents the concentrated form with >67% lutein and >13.5% zeaxanthin, this table represents the quantity of Lutemax 2020 when used as such, or the quantity present in a standardized diluted form using food grade/GRAS ingredients.

E. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of lutein and zeaxanthin preparation (Lutemax 2020™) 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 lutein and zeaxanthin (Lutemax 2020™). Lutein has been the subject of four separate GRAS notifications. For these GRAS notifications (GRN 000140- 2004; GRN 000110- 2003; GRN 000221- 2007; and GRN 000291- 2009), FDA responded that the agency had no questions regarding the conclusions that the use of lutein is GRAS under the conditions described in the notices. The safety determination of Lutemax 2020™ is further supported by toxicological studies in rats and mutagenicity study conducted according to Ames assay. The safety of Lutemax 2020™ is corroborated by multiple animal and human studies that have been performed with similar sources of lutein, lutein-rich foods, lutein supplements, and meso-zeaxanthin. Additionally, the safety of lutein is well established in the literature based on the dietary consumption of foods such as fruits and vegetables, and eggs that are known to contain this carotenoid.

Both isomers of zeaxanthin in Lutemax 2020™ are found naturally in food that has long been consumed. Recently, in a scientific opinion on the substantiation of health claims related to meso-zeaxanthin, European Food Safety Authority (EFSA) Panel clearly stated, “Meso-zeaxanthin is a dietary carotenoid and is measurable in foods by established methods.”¹ Meso-zeaxanthin is found in food sources such as shrimp carapace, fish skin, and turtle fat.² While these portions of marine animals are not eaten often, the carotenoids in these portions are fat-soluble and may dissolve in fat during cooking and thus be consumed along with the shrimp, fish, or turtle meat. Additionally, meso-zeaxanthin (as part of a natural lutein and zeaxanthin mixture) is used in Mexico in chicken feed for the purpose of affecting the color of chicken flesh and egg yolks.³ Meso-Zeaxanthin is then found in significant quantities in the resultant poultry products, which are used for human food. RR-Zeaxanthin is present in a range of fruits and vegetables. Furthermore, traces of meso-zeaxanthin are known to be

¹ EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to meso-zeaxanthin and maintenance of vision (ID 2096) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010; 8(2):1483. [11 pp.]. doi:10.2903/j.efsa.2010.1483. Available online: www.efsa.europa.eu.

² Maoka, T., Arai, A., Sinuzu, M., Matsuno, T., 1986. The first isolation of enantiomeric and *meso*-zeaxanthin in nature. *Comparative Biochemistry and Physiology B*, 83:121-123. Meso-Zeaxanthin was found in the skin of commonly consumed fish such as tilapia, catfish, halibut, and Alaska pollock. *Id. See also* Connolly, E.E., Beatty, S., Thurnham, D.I., Loughman, J., Howard, A.N., Stack, J., Nolan, J.M., 2010. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study, *Curr Eye Res.* 35(4):335-51.

³ Thurman, D.I., 2007. Macular zeaxanthins and lutein — a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. *Nutrition Research Reviews* 20:163-179; Bone R.A., Landrum, J.T., et al. 2007. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutrition & Metabolism* 4:12 doi:10.1186/1743-7075-4-12. Related marigold meal and extracts are authorized for this use in the United States; see 21 C.F.R. § 73.295.

formed when lutein esters are commercially saponified at standard elevated temperatures to form free lutein, and these traces are clearly seen in a number of market samples of lutein containing products evaluated by OmniActive Health Technologies Ltd.

Additionally, constituents of Lutemax 2020™, lutein and both principal isomers of zeaxanthin, are found in the macular region of the retina in the eye. The importance of meso-zeaxanthin has been discovered relatively recently.⁴ At least two recently published studies have documented that supplementation with all three carotenoids may help support eye health, and that meso-zeaxanthin may play a particularly special role.⁵

On the basis of scientific procedures⁶, 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:

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Vero Beach, FL 32068

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

II. Detailed Information About the Identity of the Notified Substance:

Lutemax 2020™ is a lutein and zeaxanthin standardized product obtained from Marigold flowers (*Tagetes erecta* L). It is a reddish-orange color crystalline powder that will be marketed either as a standardized powder (water dispersible), as an oil suspension with commonly used dietary (food-grade) oils (such as corn oil, sunflower oil, safflower oil or soybean oil), or as beadlets standardized with food-grade carbohydrates. Lutemax 2020™ contains a minimum of 67% lutein and 13.5% zeaxanthin isomers. As regards the zeaxanthin isomer distribution, the product contains about 50:50 of (3R,3'R)-β, β-carotene-3,3'-diol and (3R,3'S)-β, β-carotene-3,3'-diol.

⁴ Notably, some of this research has been done by a researcher with the USDA Human Nutrition Research Center on Aging at Tufts University has studied these carotenoids, and the USDA reports that her "research has led to new findings about the source of an important form of zeaxanthin, called meso zeaxanthin" which, in the macula, "may be better than lutein at reducing damage from light entering the eye." USDA/ARS, "Scientists Link Nutrition & Eye Health," available at <http://www.ars.usda.gov/is/AR/archive/aug03/eye0803.htm?pf=1>.

⁵ See Bone, R.A., Landrum JT, et al., *supra* note 3; Connolly EE, Beatty S, et al., *supra* note 2.

⁶ 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.

A. Chemical name

Lutein: β , ϵ -carotene- 3,3'-diol; Zeaxanthin: β , β -carotene-3,3'-diol

B. Trade Name:

The subject of this notification will be marketed as Lutemax 2020™

C. Chemical Abstract Registry Number:

Lutein: 127-40-2; Zeaxanthin: 144-68-3

D. Chemical Formula:

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

E. Structure:

The structural formulae of lutein and zeaxanthin isomers are presented in Figure II-E.

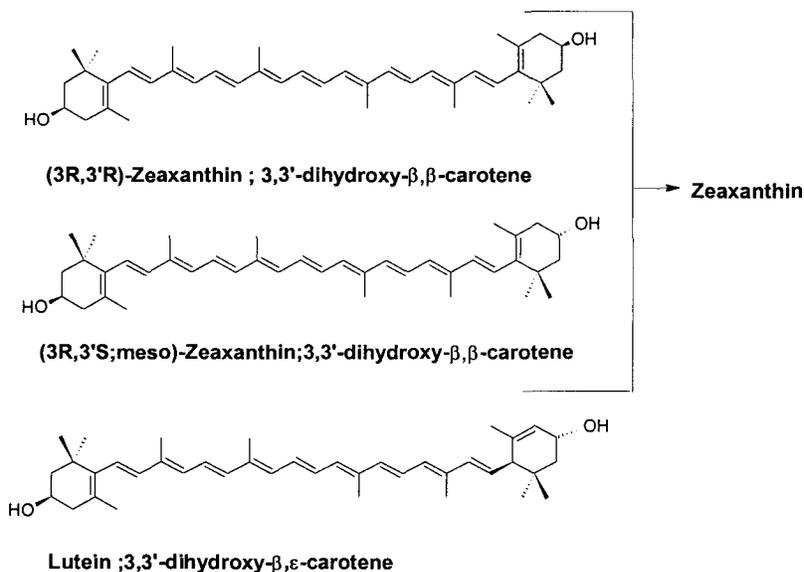


Figure II-E. Chemical Structure of Lutein and Zeaxanthin

F. Molecular Weight

The molecular weight of lutein as well zeaxanthin is 568.88

G. Physical Characteristics

Lutemax 2020™ is a reddish-orange color crystalline powder with a characteristic odor of Marigold flowers.

H. Typical Composition and Specifications

Typical food grade specifications of lutein are presented in Tables II-H.1. Analytical data from five manufacturing lots is presented in Appendix I-A.

Table II-H.1. Specifications of Lutemax 2020™ (Lutein & zeaxanthin preparation)

Parameters	Specifications	Methods
Appearance	Reddish orange colored crystal	Sensory observation
Odor	Characteristic of Marigold flowers	Sensory observation
Identity	The retention time for the major peaks in chromatogram of the test solution corresponds to that in the chromatogram of the standard solution	ANA/TM-001
Moisture	Max 1%	USP
Total Carotenoids	Min 80%	AOAC 17th Ed
Lutein	Min 67%	AOAC 17th Ed
Zeaxanthin	Min 13.5%	AOAC 17th Ed
Heavy metals		
Lead	< 1.0 ppm	AOAC 17th Ed
Arsenic	< 1.0 ppm	AOAC 17th Ed
Mercury	< 1.0 ppm	AOAC 17th Ed
Cadmium	< 1.0 ppm	AOAC 17th Ed
Microbiological		
Total plate count	<1000 cfu/g	US FDA (BAM)
Yeast and molds	<100 cfu/g	US FDA (BAM)
<i>E. coli</i> and Salmonella	Negative	US FDA (BAM)
Salmonella	Negative	US FDA (BAM)
<i>Pseudomonas aeruginosa</i>	Negative	US FDA (BAM)
Staphylococcus	Negative	US FDA (BAM)

Typical compositional analysis of lutein is compared with other FDA reviewed GRAS notices in Table II-H.2. As mentioned earlier, the zeaxanthin isomers in Lutemax 2020™ are present as 3R, 3'R-zeaxanthin and 3R, 3'S-zeaxanthin at a ratio of 50:50.

Table II-H-2. Typical Compositional Analysis of Lutein and Comparison with other GRAS Notices

Constituent	Lutemax 2020™ (OmniActive)	Kemin (GRN 140)	IOSA (GRN 291)
Total carotenoid	> 80%	> 80%	> 90%
Lutein	≥ 67%	≥ 74%	≥ 74%
Zeaxanthin	~13.5%	≥ 2 - ≤ 9%	≤ 8%
Waxes	14 to 16%	≤ 14%	≤ 7%
Moisture	≤ 1%	≤ 1%	≤ 1%
Ash	≤ 1%	≤ 1%	≤ 1%

I. Manufacturing process

OmniActive's Lutemax 2020™ manufacturing process starts with the collection of fresh Marigold flowers (Figure II-I). The fresh flowers are dried to prepare meal that is extracted with solvent (hexane) to obtain oleoresin. The oleoresin is then subjected to saponification⁷ to obtain lutein crystals of defined quality. The process consist of admixing marigold oleoresin with excess alcoholic alkali (potassium hydroxide and 1-propanol) under controlled heating and stirring at a specific range of temperature and period, diluting with water and extracting the oily layer with ethyl acetate to get lutein extract, purified by washing with polar and non-polar solvents resulting in purified free lutein and zeaxanthin crystals. 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 lutein and zeaxanthin preparations thus obtained are formulated with a food grade antioxidant and carrier to prepare lutein oil suspension or lutein 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 for hexane in the final product from multiple batches were below 50 ppm. Ethyl acetate and ethanol were detected at levels below 25 ppm (Appendix II). The marigold oleoresin, source for the preparation of Lutemax 2020™ was also checked for pesticide and related potential contaminants and none were detected at detection limits of < 0.01 mg/kg (Appendix III).

⁷ Saponification of the esterified xanthophyll is a standard step commonly employed in the manufacturing of non-esterified and free forms of xanthophylls such as lutein.

J. Manufacturing process diagram

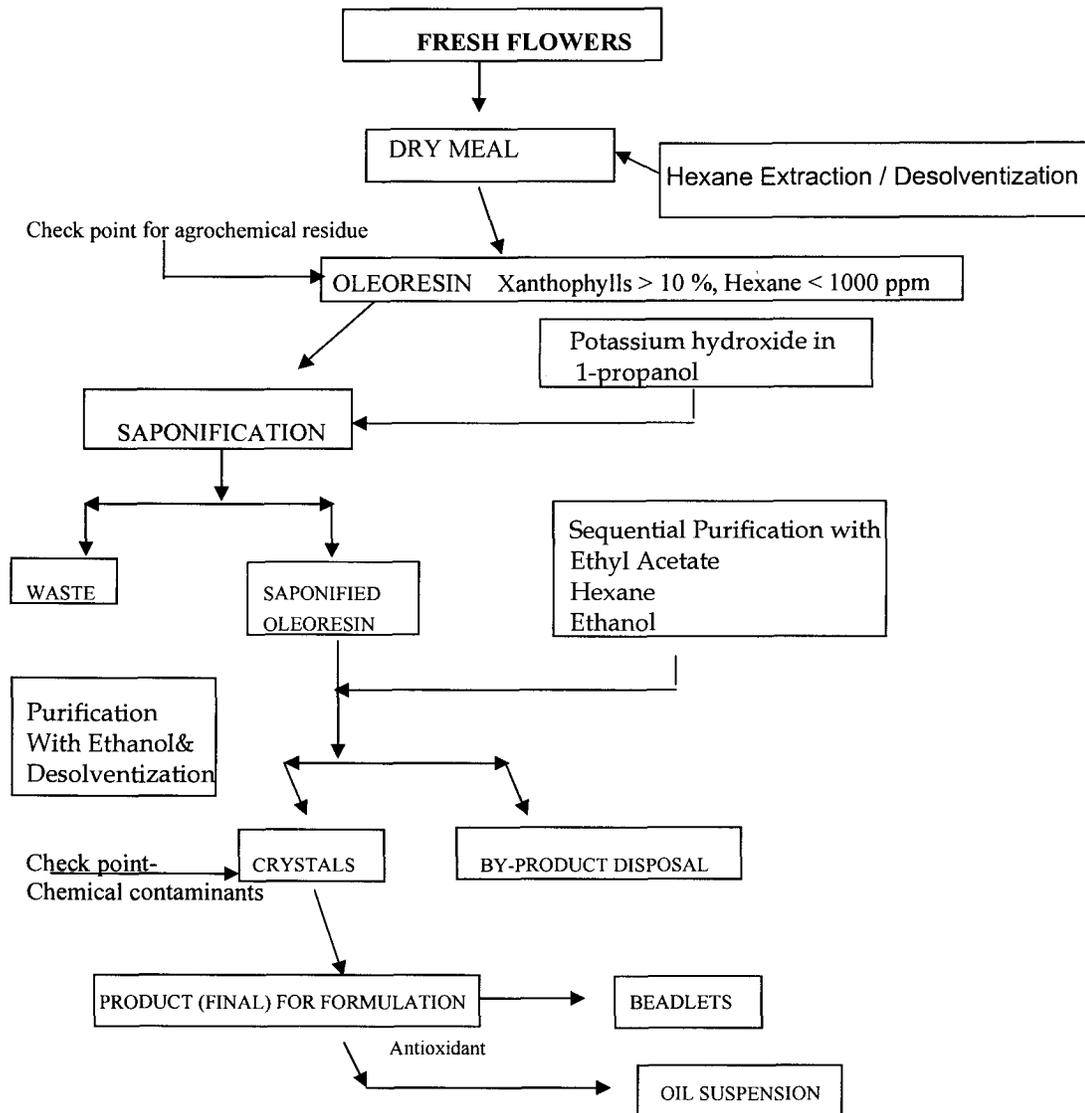


Figure II-I. Manufacturing process flow chart for free lutein (OmniActive, 2009)

K. Intended Technical Effects

Lutein and zeaxanthin preparation is intended for addition to selected foods as a nutritional ingredient to provide consumers with a supplementary source of lutein and zeaxanthin in their diets. The use of lutein and 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 lutein and 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 lutein and zeaxanthin preparation under the GRAS notification.

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

The determination that Lutemax 2020™ is GRAS is based on scientific procedures. A comprehensive search of the scientific literature for safety and toxicity information on lutein and zeaxanthin in its free and ester form was conducted through April 2011⁸ and was also utilized for this assessment. 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 described in this notice and at use levels of 0.3 to 3 mg/serving (in accordance with established 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 as specified herein.

In coming to this decision that lutein and zeaxanthin preparation is GRAS, OmniActive relied upon the conclusions that neither lutein nor zeaxanthin nor any of their 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.

⁸ The updated database searches performed subsequent to the Expert Panel review of the Lutemax 2020™ GRAS assessment in October 2010 did not reveal any significant findings that will affect the panel conclusion.

IV. Basis for a Conclusion that Lutein and 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 Lutemax 2020™ 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 lutein and zeaxanthin preparation (Lutemax 2020™) 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 lutein/person/day and 2.4 mg zeaxanthin/person/day is GRAS. It is also their opinion 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 LUTEIN (LUTEMAX 2020™) AS A FOOD
INGREDIENT**

Prepared for:
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Madhusudan G. Soni, Ph.D., F.A.C.N.

October 15, 2010

EXPERT PANEL STATEMENT
DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE
(GRAS) STATUS OF LUTEIN (LUTEMAX 2020™) AS A FOOD
INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LUTEIN (LUTEMAX 2020™) 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 lutein (Lutemax 2020™) as a nutrient [21CFR 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, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, and soft candy] at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on lutein was conducted through September 2010 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 and unanimously agreed to the decision described herein.

1.1. Background

More than 600 naturally occurring carotenoids have been identified. Some of these carotenoids are known to play an important role in human nutrition and health. These carotenoids are used as dietary supplements, as colorants in cosmetics and foods, as animal feed additives, and in pharmaceuticals. Among the carotenoids, lutein and zeaxanthin are two of the most abundant carotenoids found in the diet (IOM, 2000). 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, two eye conditions for which there is minimal options for effective prevention (Moeller *et al.*, 2000). Because of the unique nutritional characteristics of lutein and zeaxanthin, OmniActive intends to use lutein (Lutemax 2020™) in a limited number of conventional foods as a dietary ingredient.

1.2. Chemistry

Lutein and zeaxanthin are naturally occurring xanthophylls and oxycarotenoids. Carotenoids are primarily synthesized by photosynthetic plants and microorganisms and lutein is

¹Modeled 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.

one of the most abundant carotenoids. Lutein occurs with the isomeric xanthophyll, and zeaxanthin in many foods, particularly vegetables and fruits. The structural formulas of lutein and zeaxanthin isomers are presented in Figure 1. 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 carotenoids are in the all-*trans* configurations (Rice-Evans *et al.*, 1997; IOM, 2000). Structurally, lutein and zeaxanthin 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.

While lutein is present as a single stereoisomer [(3R,3'R,6'R)- β , ϵ -carotene-3,3'-diol], zeaxanthin occurs primarily as a mixture of (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). The first two predominant zeaxanthin isomers are referred to as zeaxanthin and *meso*-zeaxanthin, respectively (Bone *et al.*, 2007). The hydroxyl groups located on the 3 and 3' carbon atoms of the carotenoid end-groups is identical in the lutein and *meso*-zeaxanthin molecules. 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 (Figure 1). 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). In order to be consistent with the observation that the proportion of *meso*-zeaxanthin within the retina is dependent upon the location, an enzymatic reaction is the most likely way. 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.

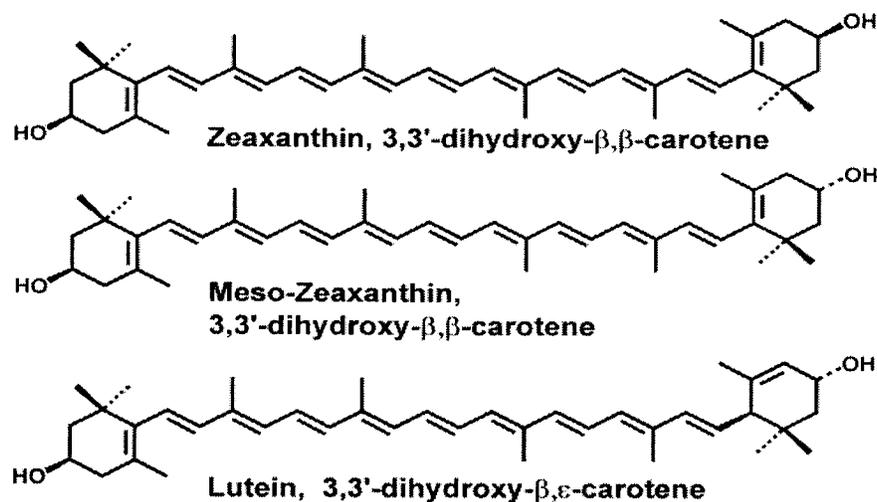


Figure 1. Chemical structure of lutein, zeaxanthin and *meso*-zeaxanthin
 Zeaxanthin, 3,3'-dihydroxy- β , β -carotene is synonymously referred to as 3R,3'R Zeaxanthin, while
meso-Zeaxanthin, 3,3'-dihydroxy- β , β -carotene is referred as 3R,3'S *meso*-Zeaxanthin

1.3. Description

Lutemax 2020™ is a lutein and zeaxanthin enriched standardized product obtained from Marigold flowers (*Tagetes erecta* L). The lutein and zeaxanthin concentrate is standardized and diluted into forms useful for food and beverage applications as dry delivery forms or oil suspension forms. Lutemax 2020™ is a reddish-orange color crystalline powder with a characteristic odor of Marigold flowers. Lutemax 2020™ for food uses will be marketed either as a standardized powder (water dispersible), as an oil suspension with commonly used dietary oils (corn oil, sunflower oil, or safflower oil), or as beadlets standardized with food grade carbohydrates. The product contains a minimum of 67% lutein and 13.5% zeaxanthin isomers. The subject of this GRAS determination, lutein (Lutemax 2020™) is substantially equivalent to lutein that was the subject of GRAS notified substances reviewed by the FDA without any questions [lutein ester- GRN 000110 (FDA, 2003); crystalline lutein- GRN 000140 (FDA, 2004); suspended lutein- GRN 000221 (FDA, 2007); crystalline lutein- GRN 000291 (FDA, 2009)]. General descriptive parameters and properties of lutein and zeaxanthin are described in Table 1.

Table 1. General descriptive characteristics of lutein and zeaxanthin free forms

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical names	Xanthophyll; β , ϵ -carotene-3,3'-diol	β , β -carotene-3,3'-diol
Empirical formula	C ₄₀ H ₅₆ O ₂	C ₄₀ H ₅₆ O ₂
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting point	177-178°C	207-215.5°C
Bulk density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility- water at 5°C	Insoluble	Insoluble
Stability	1 year at room temperature	1 year at room temperature

1.4. Specifications and Identity

Typical food grade specifications of lutein (Lutemax 2020™) have been established by OmniActive and are presented in Table 2. Analytical results from five non-consecutive lots (Appendix I) demonstrate that lutein (Lutemax 2020™) is consistently manufactured to meet these specifications. Typical compositional analysis of Lutemax 2020™ is summarized in Table 3. In addition to the carotenoids, the product contains waxes that range from 14 to 16%. The final product is prepared from the concentrate along with food grade antioxidant and carrier. The product contains 13.5% zeaxanthin with about a 50:50 distribution as a mixture of (3R,3'R)- β , β -carotene-3,3'-diol and (3R,3'S)- β , β -carotene-3,3'-diol, commonly referred to as zeaxanthin and *meso*-zeaxanthin, respectively. The analytical results from multiple lots suggest that the ratio of these two isomers varies between 40 and 60%.

Table 2. Specifications of Lutemax 2020 / Lutein & zeaxanthin concentrate

Parameters	Specifications	Methods
Appearance	Reddish orange colored crystal	Sensory observation
Odor	Characteristic of Marigold flowers	Sensory observation
Identity	The retention time for the major peaks in chromatogram of the test solution corresponds to that in the chromatogram of the standard solution	ANA/TM-001
Moisture	Max 1%	USP
Total Carotenoids	Min 80%	AOAC 17th Ed
Lutein	Min 67%	AOAC 17th Ed
Zeaxanthin	Min 13.5%	AOAC 17th Ed
Heavy metals		
Lead	< 1.0 ppm	AOAC 17th Ed
Arsenic	< 1.0 ppm	AOAC 17th Ed
Mercury	< 1.0 ppm	AOAC 17th Ed
Cadmium	< 1.0 ppm	AOAC 17th Ed
Microbiological		
Total plate count	<1000 cfu/g	US FDA (BAM)
Yeast and molds	<100 cfu/g	US FDA (BAM)
<i>E. coli</i> and Salmonella	Negative	US FDA (BAM)
Salmonella	Negative	US FDA (BAM)
<i>Pseudomonas aeruginosa</i>	Negative	US FDA (BAM)
Staphylococcus	Negative	US FDA (BAM)

Table 3. Typical compositional analysis of Lutemax 2020 / Lutein and zeaxanthin concentrate (OmniActive, 2009)

Parameter	Percent
Lutein	> 67
Zeaxanthin	> 13.5
Waxes	14 – 16
Moisture	Max 1
Ash	Max 1

1.5. Manufacturing Process

Lutein (Lutemax 2020™) is manufactured according to current good manufacturing practices (cGMP), as outlined in Figure 3, at OmniActive's facilities located in India at Pune and Cochin. The manufacturing process starts with the collection of fresh Marigold (*Tagetes erecta*) flowers. The oleoresin material is obtained by solvent extraction of dried Marigold flowers. The solvents used in manufacturing include hexane, ethanol, ethyl acetate, and acetone. The flowers are subjected to silaging and drying to obtain marigold meal. The oleoresin is obtained by hexane solvent extraction. The oleoresin is then subjected to saponification with potassium hydroxide and 1-propanol. Following saponification, the contents are washed with ethyl acetate, extracted with hexane and then filtered. The wet cake obtained is washed with ethanol, filtered and is dried under vacuum. The dried concentrate consisting of lutein and zeaxanthin (Lutemax 2020™) is then converted into an oil suspension or vegetarian beadlets by using food grade materials. Small

quantities of food grade antioxidant (tocopherols) are added to the product in accordance with good manufacturing practices. Processing aids, such as potassium hydroxide and solvents used in the manufacturing process are all of food-grade quality as specified in the Food Chemicals Codex (5th Edition). The residual solvent levels from multiple lots are presented in Appendix II. The product was also checked for pesticide and related potential contaminants and none were detected at detection limits of < 0.01 mg/kg for oleoresin from which Lutemax 2020™ is prepared (Appendix III).

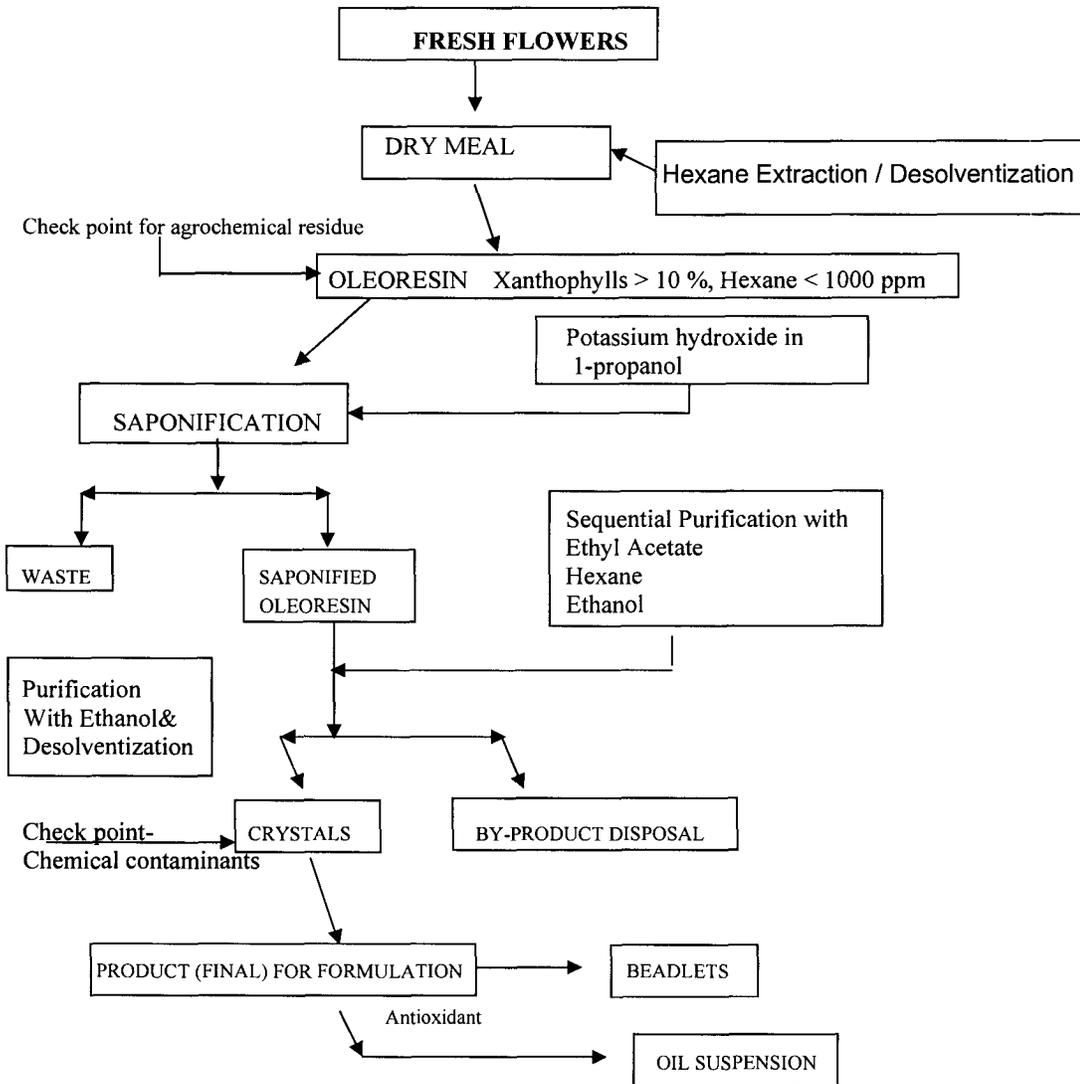


Figure 3. Manufacturing process flow chart for free lutein (OmniActive, 2009)

1.6. Natural Occurrence

Of the over 600 naturally occurring carotenoids identified (Moeller *et al.*, 2000; Rice-Evans *et al.*, 1997), less than 20 have been found in the human body (Roberts *et al.*, 2009). Carotenoids are fat soluble pigments found in some plants, algae, and photosynthetic bacteria. Lutein in free as well as the esterified form is found primarily in green leafy vegetables, yellow-orange fruits, yellow-orange vegetables, and egg yolks (Sies and Stahl, 2003). Lutein and zeaxanthin are present in the free form 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). The levels of lutein in some fruits and vegetables are presented in Table 4. Additionally, in a review article, Calvo (2005) summarized the lutein content of over 70 vegetables and fruits fresh or submitted to different treatments. In humans, lutein is exclusively provided by the diet and may be considered as a marker of vegetable intake (Thurnham *et al.*, 1997). Both lutein and zeaxanthin are found in the macula (the innermost part of the eye) of the retina as well as in the crystalline lens (Hendler and Rorvik, 2001). The two isomeric forms of zeaxanthin predominate in the macula while lutein is found principally in the more peripheral areas of the retina. The presence of lutein, primarily in the free form has been reported in human breast milk (~3 to 232 µg/L) (Canfield *et al.*, 2003). Human beings are unable to synthesize lutein and hence depend entirely on dietary sources such as vegetables, eggs, or dietary supplement in the form of lutein pills.

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).

Table 4. Lutein and lutein ester content of some commonly consumed fruits and vegetables

Fruit/vegetable	Lutein* (mg/100 g)	Lutein ester** (mg/100 g)
Broccoli	1.770	
Cabbage	0.280	
Spinach	14.400	
Brussels sprouts	1.340	
Kale	34.200	
Blood orange		0.902
Mango		1.012
Papaya		2.436
Peach		1.489
Pepper (yellow)		2.067
Potato		0.087
Pumpkin		0.738
Tangerine (Spain)		1.454

Adapted from- *Khachik *et al.*, 1986; **Breithaupt and Bamedi, 2001

1.7. Current Uses

In recent years, lutein and zeaxanthin in free (non-esterified) and esterified (with fatty acids) forms are found in numerous dietary supplements appearing on the international market. Compared to lutein, the amount of zeaxanthin in these products is considerably low. In recent years, supplements containing *meso*-zeaxanthin have been marketed. Nutritional supplements containing lutein are reported to deliver from 250 µg to 20 mg daily dosage of this nutrient (Hendler and Rorvik, 2001). Commonly marketed multivitamins such as Natural Brand™ from the retailer General Nutrition Centers, Inc. (GNC) contains a recommended daily dose of 12 mg lutein ester. Another dietary supplement, Centrum® (American Home Products, Madison, NJ) contains a recommended daily dose of 250 µg unesterified lutein (~500 µg lutein ester equivalents). ConsumerLabs (2007) analyzed over 15 products for its contents of lutein/zeaxanthin and reported its findings. These products were reported to contain up to 20 mg lutein and up to 2 mg zeaxanthin in a daily serving. Based on the available information and the levels recommended in labeling for the use of lutein as a dietary supplement, theoretical lutein ester consumption from dietary supplements may range from 500 µg to 20 mg/day.

In addition to its use as a dietary supplement, lutein is also used as a food ingredient in some selected foods. In response to four separate GRAS notices on lutein [lutein ester- GRN 000110 (FDA, 2003); crystalline lutein- GRN 000140 (FDA, 2004); suspended lutein- GRN 000221 (FDA, 2007); crystalline lutein- GRN 000291 (FDA, 2009)], the FDA responded that they had no questions regarding the conclusions that the use of lutein is GRAS under the conditions described in these notices. The use of lutein in specified food categories in these notices was estimated to result in levels of up to 13.4 mg/day and was considered as safe. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has reviewed lutein 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 JECFA reported uses of lutein as a food coloring agent and nutrient supplement (food additive) include 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, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes at levels ranging from 2 to 330 ppm. Additionally, the European Food Safety Authority panel (EFSA) also reviewed the use of lutein by infants and young children and concluded that there is no concern of safety for recommended use of lutein at the use level of 250 µg lutein/L of infant formula (EFSA, 2008).

1.8. Technological Effects

Lutein (Lutemax 2020™) is intended for addition to selected foods as a nutrient to provide consumers of all ages with a supplementary source of lutein in their diets. The use of lutein 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; Infant and Toddler Foods; Milk Products; Processed Fruits and Fruit Juices; Soft Candy. 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 lutein in the above described food categories may also impart a color to the product, the intended use 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 does not impart a significant color to the food products. Although lutein has a natural reddish-orange or light-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, lutein 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, lutein 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 lutein in certain specified foods is to provide consumers with a supplementary source of lutein 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

Lutein (Lutemax 2020™) is intended for use in the same foods and at levels proportional to those mentioned in the GRN 000140 (FDA, 2004) and GRN 000110 (FDA, 2003). As both 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 Lutemax 2020™. Unlike GRN 000140, 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 ≥ 2 and $\leq 9\%$ zeaxanthin, while the subject of present GRAS determination (lutein) contains $\geq 67.5\%$ lutein and $\geq 13.5\%$ zeaxanthin. On the basis of lutein content, lutein can be added at a level of 110% (1.1-fold) that of the substance mentioned in the GRN 000140. Thus, in order to deliver equivalent levels of lutein to that mentioned in GRN 000140, the addition of lutein will be approximately 1.1 times the overall bulk food addition compared to that of GRN 000140.

The intended uses 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, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, and soft candy. The intended food uses and use levels are summarized in Table 5. The application of lutein to the same foods and at the same levels as those in GRN 000140 (FDA, 2004) is not expected to notably affect the intake of lutein in the diet of the public from introduction into the market by another supplier who will have to compete in essentially the same market and foods.

In the GRN 000140 (FDA, 2004) that received "No Question Asked" letter from the FDA on June 14, 2004, the intake of lutein and zeaxanthin from all sources described in the notification for mean and 90th percentile all-users was estimated as 9.6 mg/person/day (0.18 mg/kg body weight/day) and 17.6 mg/person/day (0.37 mg/kg body weight/day), respectively. These 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 lutein (Lutemax 2020™) is in the same food products, the mean and 90th percentile lutein intake from its uses will be similar (7.3 and 13.4 mg lutein/person/day, respectively). As lutein (Lutemax 2020™) contains a relatively higher concentration of zeaxanthin compared to that in GRN 000140, the resulting 90th percentile zeaxanthin intake will be about 2.4 mg/person/day. As the two isomers of zeaxanthin are present at a ratio of 50:50, the intake of each isomer of zeaxanthin will be 1.2 mg/person/day. In other words, the intake of *meso*-zeaxanthin will be 1.2 mg/person/day. Given that the equivalent amounts of the product on the basis of lutein is intended for use in the same foods at levels of addition as notified in GRN 000140, the estimates of intake for lutein are considered to be the same and would not be additive. A summary of use levels and food categories for lutein is presented in Table 5. Although the list includes egg products, and soups and soup mixes, these products are excluded from the intended uses of Lutemax 2020™.

Table 5. Summary of the individual proposed food uses for lutein in the US

Food Category	Proposed Food	Use levels	
		mg/RACC ¹	mg/kg
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.0	50
	Crackers and Crisp-breads	2.0	67
Beverages and Beverage Bases	Bottled Water	0.5	2.1
	Carbonated Beverages	2.0	8.3
	Meal Replacements	2.0	8.3
	Tea, Ready-to-Drink	0.6	2.6
Breakfast Cereals	Instant and Regular Hot Cereals	2.0	8.3
	Ready-to-Eat Cereals	2.0	36 - 130
Chewing Gum	Chewing Gum	1.0	330
Dairy Product Analogs	Imitation Milks	2.0	8.3
	Soy Milks	1.5	6.3
Egg Products**	Liquid, Frozen or Dried Egg Substitutes	2.0	40
Fats and Oils	Margarine-like Spreads	1.5	36 - 130
	Salad Dressings	1.5	330
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.0	8.3
Gravies and Sauces	Tomato Based Sauces	0.3	6.3
Hard Candy	Hard Candy	1.0	40
Infant and Toddler Foods*	Junior, Strained and Toddler-Type Baby foods	1.0	36 - 130
Milk Products	Dry Milk	3.0	36 - 130
	Fermented Milk Beverages	0.6	330
	Flavored Milk and Milk Drinks	3.0	8.3
	Milk-Based Meal Replacements	3.0	6.3
	Yogurt	3.0	40
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	2.0	36 - 130
	Fruit-Flavored Drinks	2.0	8.3
	Fruit Juice	2.0	8.3
	Nectars	2.0	8.3
	Vegetable Juice	2.0	8.3
Soft Candy	Chewy and Nougat Candy	1.0	25

Food Category	Proposed Food	Use levels	
		mg/RACC ¹	mg/kg
	Fruit Snacks	1.0	25
Soups and Soup Mixes**	Canned Soups	0.6	2.6

¹RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC. Uses listed and level same as in GRN 000140. *Does not include infant formula. Adapted from GRN 000140 and GRN 000291. **Unlike GRN 000140, egg products and soup and soup mixes are not food categories for the present GRAS determination.

2. BIOLOGICAL DATA

In recent years lutein has become the subject of intense investigations for its potential health benefits. There has been a significant effort by researchers to elucidate the biological role and safety of lutein. The literature is full of information on lutein. Additionally, the national and international regulatory agencies such as the FDA and JECFA have extensively reviewed the safety of lutein. Since 2003, the FDA has received four separate GRAS notifications on lutein [lutein ester- GRN 000110 (FDA, 2003); crystalline lutein- GRN 000140 (FDA, 2004); suspended lutein- GRN 000221 (FDA, 2007); GRN 000291 (FDA, 2009)]. In these submissions to the FDA, extensive data from published literature on lutein esters was presented by the notifier. The FDA did not object the acceptability and suitability of the available evidence to support the use of lutein ester, crystalline lutein, and suspended lutein. As the subject of this GRAS determination is substantially equivalent to the products of the FDA notification, studies described in these notifications can be utilized for the present GRAS determination of lutein. Additionally, JECFA has also extensively reviewed the biological data on lutein. The findings from the FDA and JECFA reviews and recent publications on this subject as described below were utilized for the present safety assessment.

2.1. Regulatory Agency Reviews

In the four GRAS notices to the FDA, biological data on the natural occurrence, metabolism, and safety of lutein has been extensively presented and discussed. The recent review and response from the FDA was during 2009. Given the substantial equivalence of lutein products between FDA notices and the present, it is instructive to review the FDA's responses to these notices and the information presented in these on lutein before considering the recent publications on this subject. The discussion presented below suggests that the agency is comfortable with the GRAS determination of lutein at its proposed use levels in selected foods. In addition to the FDA, JECFA also reviewed published and other information on lutein to determine acceptable daily intake (ADI). These regulatory agency reviews are fully applicable to the present assessment of OmniActive's lutein. Based on the review of GRAS notices that are available on the FDA website and as described below, the consumption of lutein or its ester as an ingredient from conventional foods resulting in daily intake of up to 40 mg/person/day of lutein ester (or lutein ester equivalent; 20 mg free lutein) is safe. Additionally, JECFA evaluation of lutein further supports the safety of lutein and its esters. A summary of the findings from the FDA GRAS notices and JECFA evaluation is briefly presented below. A comparison between regulatory agencies finding with current GRAS assessment is presented in Table 6.

GRN 000110- Lutein esters: This notice (FDA, 2003) discusses published and unpublished studies conducted in humans and various animal species regarding the absorption, distribution, metabolism, excretion, bioavailability, and toxicity of lutein esters. The notifier concluded that

the results of these studies show no toxic or adverse effects from the consumption of lutein esters and that long-term consumption of lutein esters is well tolerated. In one study, carotenoderma was noted in subjects consuming supplements containing 30 mg/person/day of a mixed lutein esters product for 112 days. In contrast, in a study conducted by the notifier, carotenoderma was not noted in subjects consuming supplements containing up to 40 mg/person/day of lutein esters product for more than 90 days. In this notice, the acceptable daily intake (ADI) for lutein esters (or lutein ester equivalents) was determined as 40 mg/person/day; a dose that has been administered without evidence of carotenoderma.

GRN 000140- Crystalline lutein: In this notice (FDA, 2004), the notifier discussed published studies conducted in humans and various animal species regarding the metabolism, bioavailability, toxicity, and mutagenicity of crystalline lutein and zeaxanthin. The notifier concluded that results from animal, human, and mutagenicity studies did not reveal any toxic or adverse effects (including ocular endpoints) from the consumption of crystalline lutein or lutein and zeaxanthin from other sources. Carotenoderma was reported in a study in which healthy subjects consumed 15 mg/person/day of lutein (from mixed ester forms extracted from Marigold flowers) for 4 months. However, carotenoderma is considered a harmless and reversible biological effect of high carotenoid intake, and that no signs of its occurrence have been reported in other populations (e.g., patients with cataracts or patients with age-related macular degeneration) following exposure of approximately 25 mg/person/day of lutein (3 times/week) for 13 months. The mean and 90th percentile daily intake of crystalline lutein in this notice from its proposed uses was estimated to be approximately 10 and 18 mg/person/day, respectively. Based on the carotenoid composition of crystalline lutein, it was estimated that the intake of lutein and zeaxanthin would be approximately 7 mg/person/day (lutein) and 0.7 mg/person/day (zeaxanthin) at the mean and approximately 13 mg/person/day (lutein) and 1.2 mg/person/day (zeaxanthin) at the 90th percentile, respectively. The average dietary intake of lutein and zeaxanthin from plant sources was estimated to range from 2 to 4 mg/person/day.

GRN 000221- Suspended lutein: In this notice (FDA, 2007), the use of suspended lutein in infant formula is described. The notifier estimates that the mean and 90th percentile intake of dietary carotenoids for children ages 2 through 6 months to be 0.2 and 0.82 mg/day, respectively, and for children between 7 to 11 months of age to be 0.46 and 1.10 mg/day, respectively. The source of lutein described in this notice was the subject of a previous notice (GRN 000140). This notice discusses published and unpublished studies described in GRN 000140, as well as several additional published animal studies that became available after the agency review of GRN 000140, and two unpublished growth studies conducted in healthy term infants. The notice described that the safety of lutein has been established in toxicological studies in rats, mutagenicity studies from *Salmonella typhimurium*, and is further supported by intervention studies conducted with healthy subjects designed to measure metabolic endpoints. The safety of lutein for use in infant formulas was further supported by a 13-week toxicity study in Wistar rats at doses up to 260 mg lutein/kg body weight/day (corresponding to 210 mg lutein+zeaxanthin/kg body weight/day) and a developmental toxicity study conducted in Sprague-Dawley rats at doses up to 1100 mg lutein/kg body weight/day. Additionally, the results of an infant growth and safety study conducted with lutein at a targeted concentration of 0.2 mg lutein/L in infant formula, for a period of 16 weeks further supports the safety of lutein.

GRN 000291- Crystalline lutein: The subject of this notice is a mixture of carotenoids, lutein, and zeaxanthin referred to by the notifier as crystalline lutein (FDA, 2009). As part of its notice,

the notifier included the report of two panels of individuals (GRAS panel 2006 and GRAS panel 2008) who evaluated the data and information that formed the basis for GRAS determination of crystalline lutein. For the estimated daily intake (EDI), this notice used the calculation from GRN 000140. In the GRN 000140 an estimate of the current intake of crystalline lutein from the diet and the intended intake of crystalline lutein from the foods was provided. The notifier concluded that, due to the substantial equivalency in composition with the crystalline lutein from GRN 000140 and the fact that the intended use would be substitutional and not an additive, the EDI calculations would be considered the same for the uses of crystalline lutein in both GRN 000140 and its notice. The intake of lutein and zeaxanthin from specified foods was estimated to be 7.3 and 0.7 mg/person/day, respectively, at the mean, and 13.4 and 1.2 mg/person/day, respectively, at the 90th percentile. The notice discussed published and unpublished studies that were described in GRN 000140, as well as several published animal and human studies that became available after the agency review of GRN 000140. Based upon the totality of the safety studies, the notifier concluded that crystalline lutein in the diet at the estimated levels is not considered to pose any safety concerns.

JECFA Assessment: In addition to the FDA review of GRAS notices, JECFA (2006) also reviewed the scientific literature on lutein and evaluated its safety as a food additive. The committee noted that there were no adverse effects documented in any of the toxicity studies in animals, including mice, rats, monkeys, or in humans. JECFA assigned a group ADI of 0 to 2 mg lutein and zeaxanthin/kg body weight. The ADI determination was based on no observed adverse effect level (NOAEL) of 200 mg lutein/kg body weight/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 body weight/day in a study of developmental toxicity), the safety factor was considered appropriate by JECFA. The JECFA determined ADI of 2 mg/kg/day will be equivalent to a dose of 120 mg/person/day for an individual weighing 60 kg.

Given the differences in metabolism of carotenoids in humans and animals, the JECFA determination of ADI of 0 to 2 mg/kg body weight/day (120 mg/person for an individual weighing 60 kg) based on animal studies may be high. Available evidence indicates that at low levels of exposure (physiological levels) rats do not transport carotenoids (β -carotene) intact but rather metabolizes it to vitamin A in the intestinal cells. In order to raise blood carotenoid levels, rats need to be exposed to very high doses ($> 0.02\%$ of the diet) of β -carotene (White *et al.*, 1993, Lee *et al.*, 1999; Wolf, 2002). Although lutein does not serve as a vitamin A precursor, there is limited comparative data on lutein between rodents and humans. Rats and mice may not be the most appropriate models for studying carotenoid absorption and bioavailability (Krinsky *et al.*, 1990; Lee *et al.*, 1999). Generally, high levels of dietary carotenoid are fed in these studies to achieve adequate tissue levels.

Table 6. Summary of assessments of lutein reported in GRAS notices, JECFA and current GRAS determination

Parameters	GRN 110	GRN 140	GRN 221	GRN 291	JECFA	Current
Subject	Lutein ester	Crystalline lutein	Suspended lutein	Crystalline lutein	Lutein	Lutein and lutein ester
Estimated daily intake (mg/day)	22 (90 th percentile)	13 (90 th percentile)	0.20-0.82 and 0.46-1.10 (for 3-6 and 7-11 month old child, respectively)	13 (90 th percentile)	Not reported	13 (90 th percentile)
Acceptable daily intake (mg/person/day)	40 (lutein ester equivalent)	Not reported.	Not reported.	Not reported	Up to 120 mg/day*	Not reported
Safety determination	Based on ADI	Totality of evidence supports safety	Totality of evidence supports safety	Totality of evidence supports safety	Based on ADI	Totality of evidence supports safety

*For an individual weighing 60 kg

2.2. Bioavailability

The National Health and Nutrition Examination Survey III, conducted in the US during 1988 to 1994, suggest that the daily median intake of lutein and zeaxanthin from dietary sources ranges from 1.35 to 1.97 mg resulting in serum lutein concentrations of ~ 0.4 µmol/L (CDC, 1998). An oral single dose administration of lutein to human subjects showed peak concentrations in the plasma at 16 hours (Kostic *et al.*, 1995). In another study, Khachick *et al.* (1995) reported that daily administration of 10 mg lutein to humans for 18 days resulted in an increase in plasma lutein concentrations over time. Steady state serum lutein levels of 0.26 to 1.76 µmol/L were achieved in humans consuming supplements containing 16 to 62 mg lutein esters/day (equivalents to 8 to 31 mg free lutein/day). Following intestinal hydrolysis of lutein esters, lutein appears to be absorbed intact. Khachik *et al.* (1995) identified at least six lutein metabolites, four of which resulted from oxidation and two from non-enzymatic dehydration.

Oral ingestions of lutein and zeaxanthin are likely to follow the same digestion and intestinal absorption pathways as dietary fat. Upon absorption, both lutein and zeaxanthin 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). In the FDA GRAS notifications and JECFA evaluation, these and other studies on the metabolism of lutein and lutein esters are described in detail.

Thurnham *et al.* (2008) measured the blood uptake of *meso*-zeaxanthin. In this study human volunteers (ten men and nine women) 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. However, to confirm that the lower plasma response was not due to the large amount of lutein, studies with pure *meso*-zeaxanthin will be required.

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.

In summary, pharmacokinetic studies of lutein indicate that orally administered lutein or lutein ester is bioavailable. Following oral administration, lutein esters are hydrolyzed in the gut to the free form of lutein and it is the free form that enters circulation. Upon absorption, lutein is incorporated into chylomicrons, and distributed in HDL and LDL in circulation. Extra-hepatic tissue uptake of lutein appears to be a receptor mediated enzyme (lipase) reaction. Lutein is likely to be excreted primarily through the bile into the feces. Available metabolism-related information suggests that the results from studies that used lutein (free form or ester form) are equally valid for the safety assessment. Given the structural similarity, zeaxanthin is also expected to follow similar metabolic pathway. Available studies suggest that *meso*-zeaxanthin is absorbed from the gastrointestinal track.

2.3. Toxicological Studies

As indicated earlier, the safety of lutein has been extensively described in the FDA GRAS notifications and JECFA evaluation. Since these assessments, several additional articles have appeared in the publicly available databases on lutein. Relevant findings from these recent publications are summarized in the following sections.

2.3.1. Review Articles

In an extensive and critical review, Shao and Hathcock (2006) described the safety-related information on lutein and assessed the risk. For the risk assessment, "Observed Safe

Level"³ (OSL) methodology was utilized. Based on the review of safety data, these investigators identified the OSL for lutein as 20 mg/day. This method provided strong evidence supporting the safety of lutein intake at levels up to 20 mg/day. The authors stated that lutein has been tested at much higher levels without adverse effects, the data for intakes above 20 mg/day are not sufficient for a confident conclusion of long-term safety. In this assessment, of the 30 peer-reviewed human clinical trials, 11 of the most relevant studies related to the safety of lutein were considered. As described in the article, human clinical trials involved lutein doses of 8, 10, 12, 15, 20, 20.5, 30, or 40 mg/day. In the trial with the highest dose, subjects with retinitis pigmentosa were treated with lutein (40 mg/day) for nine weeks followed by an additional 17 weeks with 20 mg/day. The longest duration trial was for 12 months and in this trial, patients with age-related macular degeneration AMD received lutein at a dose of 10 mg/day. No adverse effects were observed in any of the clinical trials. The absence of any pattern of adverse effects in these clinical trials provides support for a high level of confidence for the safety of lutein. Because of the complete absence of adverse effects in human trials using lutein doses above, at, and below the 20 mg level and other considerations, 20 mg/day was designated as the OSL. Additionally, these investigators also considered animal data to determine the NOAEL. A dose of 639 mg/kg/day in rats for four weeks had no adverse effects, and this dose was considered as the NOAEL. The application of a 1000-fold uncertainty factor would result in an acceptable daily intake of 38 mg/kg/day, which is higher than that, determined on the basis of human studies, and supports the safety of lutein at 20 mg/day for human consumption.

2.3.2. Animal Studies

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 body weight and 0.66 mg zeaxanthin/kg), zeaxanthin-treated (n = 5, 10 mg zeaxanthin/kg), 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 body weight/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 a subchronic toxicity study, Kumar *et al.* (2009) evaluated dose-related effects of lutein diacetate L20% (ester form of lutein with acetic acid) in rats. In this study, Sprague-Dawley rats (10/sex/group) were orally administered once daily with 0 (control), 2.1, 22.5, and 210 mg lutein acetate/kg body weight for 90 days. Two additional control and high dose recovery groups were also included. No treatment-related clinical signs were noted in any of the

³ This methodology is defined as "the highest intake with convincing evidence of safety, even if there are no established adverse effects at any level."

groups, except stained (light brown colored) feces in the high dose group. The feces coloration appears to be due to the direct contact of the test substance with the contents of the gastrointestinal tract. No changes in ophthalmologic or neurological examinations were noted. Similarly lutein diacetate administration did not affect mean body weight, net body weight gains, food intake, organ weights, and relative (to body weight) organ weights. Compared to control rats, no changes in hematology or clinical chemistry parameters were noted in any of the treatment groups. Histological examinations did not reveal any treatment-related microscopic findings. Incidental changes noted in some of the parameters were not considered as treatment-related. Based on the results of this study, the investigators determined the no-observed-adverse-effect level (NOAEL) for lutein diacetate as 210 mg/kg body weight/day, the highest dose tested.

In a series of studies, Harikumar *et al.* (2008) investigated acute, short-term, and subchronic toxicity of lutein and lutein esters. For these studies, lutein (0.05, 0.5, and 5% prepared in sunflower oil) and lutein ester (0.05, 0.5, and 5% of molar equivalent of lutein prepared in sunflower oil) were provided by OmniActive. In the acute toxicity study, oral administration of lutein or lutein ester (molar equivalent of lutein) at doses up to 4 g/kg did not produce any mortality. The results of this study suggest that the LD₅₀ of lutein or lutein ester is greater than 4 g/kg body weight. In the short-term study, lutein or lutein ester (molar equivalent of lutein) was administered at a dose level of 4, 40, or 400 mg/kg body weight/day to Wistar rats for four weeks. Compared to the control rats, the administration of lutein or lutein ester did not affect body weight or feed consumption. Necropsy did not reveal any unusual findings and no changes in organ weights were noted compared to the control group. Hematological parameters, such as RBC, platelet, hemoglobin, WBC, and lymphocytes did not reveal any treatment-related changes. No treatment related effects of lutein or lutein ester on serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin/globulin ratio, creatinine, cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol, or blood urea nitrogen (BUN) were noted. A slight increase in serum total bilirubin was noted in animals treated with 400 mg/kg lutein or lutein ester. However, the increase was within the limits of normal range (historical control data). Histological evaluation of brain, spleen, kidney, liver, and eyes did not reveal any treatment-related pathological lesions. The results of this study suggest that oral administration of lutein or lutein esters at levels up to 400 mg/kg body weight/day to rats did not produce any toxic effects (Harikumar *et al.*, 2008).

In the subchronic toxicity study by Harikumar *et al.* (2008), 70 Wistar rats were divided in seven groups (5/sex/group) and were treated (gavage) daily with vehicle (control), lutein (4, 40, or 400 mg/kg body weight/day) or lutein ester (4, 40, or 400 mg/kg body weight/day) for 13 weeks. Animals were monitored for mortality, clinical signs, behavioral symptoms, and any adverse effects. Feed consumption and body weights were recorded every fifth day. At the end of 13 weeks, the animals were euthanized and a necropsy was performed. Blood was collected for hematology (hemoglobin, RBC, platelet, WBC, lymphocytes) and clinical chemistry (ALT, AST, ALP, bilirubin, protein, BUN, creatinine, sodium, potassium, chloride, bicarbonate, cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and VLDL) parameters. Selected organs such as the liver, lung, thymus, spleen, kidney, brain, and eyes were collected and processed for histological observations. The administration of lutein or its ester did not result in any mortality, or changes in body weight gain, feed consumption, and organ weight compared to controls. Administration of lutein or lutein ester did not alter the hepatic and renal function as evaluated by serum chemistry parameters, and did not produce any treatment-related alterations in the

hematological parameters and lipid profile. A slight decrease in AST was noted in male and female rats treated with 400 mg/kg lutein ester and in female rats receiving 40 mg/kg lutein ester. An increase in ALT levels is a known marker of liver injury. In the present study, lutein esters resulted in a slight decrease in ALT but this decrease was not considered an adverse effect. Histological examinations of the organs (brain, spleen, kidney, liver, and eyes) did not reveal any treatment-related pathological lesions. The results of this study suggest that the NOAEL of lutein (free or in ester form) was 400 mg/kg body weight/day, the highest dose tested.

2.3.3. Genotoxicity/Carcinogenicity Studies

Wang *et al.* (2006) investigated the mutagenic and clastogenic potential of lutein in Ames test and chromosome aberration. Mutagenicity was studied at concentrations of 334, 668, and 1335 µg lutein/plate using the standard Ames assay (*S. typhimurium* strains TA97, TA98, TA100, and TA102) in the presence and absence of S9 fraction. Lutein was not mutagenic at any of the tested concentrations. In anti-mutagenic investigations, the effects of lutein in *S. typhimurium* strains TA98 and TA100 following the addition of the known mutagens (2-aminofluorene for the strain TA98 and cyclophosphamide and sodium azide for TA100) were studied. In these assays, a dose-related anti-mutagenic effect of lutein was noted. In a standard chromosome aberration test using Chinese hamster ovary (CHO) cells, lutein (66.8, 133.5, and 267.0 mg/L) did not cause clastogenicity, but did show anti-clastogenic effects. The results of these investigations suggest that lutein is neither mutagenic nor clastogenic.

In an unpublished report, Kuttan and Sabu (2004) investigated the mutagenic and anti-mutagenic potential of free lutein using the standard Ames assay (*S. typhimurium* strains TA98, TA100, and TA1535) in the presence and absence of rat liver microsomal S9 fraction. For the anti-mutagenic activity of lutein, direct acting mutagens (4-nitro-o-phenylenediamine- NPD; methylnitronitrosoguanidine- MNNG) and mutagens requiring activation (2-acetylaminofluorene- AAF) were used. The results of this study revealed that lutein does not possess any mutagenic property. Additionally the results of anti-mutagenic investigation suggest that lutein possesses significant anti-mutagenic activity.

In an *in vivo* rat model of hepatocarcinogenesis, Moreno *et al.* (2007) reported that administration of lutein during the promotion phase of carcinogenesis inhibited the size of hepatic macroscopic nodules and DNA damage. These investigators studied the effects of lutein on hepatic preneoplastic lesions and DNA strand breakage induced in Wistar rats. In this model (initiation with diethylnitrosamine and promotion with 2-acetylaminofluorene coupled with partial hepatectomy), the effects of lutein (70 mg/kg; alternate day) administration via gavage were investigated specifically during the initiation or promotion phase, for 2 and 6 weeks, respectively. Lutein administration during the initiation phase neither inhibited nor induced hepatic preneoplastic lesions and DNA damage. However, lutein administration during the promotion phase inhibited the size of hepatic macroscopic nodules and DNA damage. These observations suggest that lutein acts as an inhibitor during promotion of hepatocarcinogenesis.

2.3.4. Safety Studies with Lutemax 2020™

In a series of studies, OmniActive Health Technologies investigated acute and subchronic toxicity, and mutagenicity of Lutemax 2020™ (the subject of present GRAS determination). The results of these studies are in the process of publication. The reports of these studies and the draft manuscript for publication were provided for this GRAS assessment.

2.3.4.1. Acute (LD₅₀) Study

For the acute toxicity study, two groups (Step I and Step II), each with three female HanRcc:WIST rats were treated with Lutemax 2020™ (using sunflower oil as vehicle) by oral gavage administration at a dosage of 2000 mg/kg bw (Ilamurugan *et al.*, 2010a). Following the treatments, the rats were monitored for 14 days for mortality and clinical signs. At the end of the study all animals were necropsied and examined macroscopically. All animals appeared normal during the study period. The administration of Lutemax 2020™ at doses up to 2 g/kg did not produce any mortality. At terminal euthanasia one animal from the Step I experiment showed hyperemia of the small intestine. No abnormalities were observed in any of the remaining animals from Step I and Step II. The results of this study suggest that LD₅₀ of Lutemax 2020™ is greater than 2 g/kg bw.

2.3.4.2. Subchronic (90-day) Study

In a toxicity study, conducted according to OECD and Redbook 2000 guidelines for such studies, the potential effects of lutein (Lutemax 2020™) were investigated (Ilamurugan *et al.*, 2010b). In this study, lutein dissolved in sunflower oil was administered (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 males and ten females were allocated to control and high dose recovery groups. 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 mortality or clinical signs were observed during the dosing period of 90 days and recovery period of 28 days. An eye examination was performed for all the animals before the start of treatment. In the 13th week, an eye examination was performed for the control and high dose groups. Ophthalmologic examination did not reveal any abnormalities. The quantity of feed consumed by the animals across different dose groups was found to be comparable with that of the control animals. A significant decrease in feed consumption was noted in males during week 8 and 10 in all treatment groups during the treatment phase. In females, a significant decrease in feed consumption was noted during week 8 in all treatment groups and during weeks 9 and 10 in mid and high dose groups as compared to the control. Also a significant decrease in feed consumption was noted in the high dose treated recovery group male rats during weeks 9, 10, 11, and 12 as compared to that of the control group. In the high dose treated recovery group females, a significant decrease was noted during weeks 9 and 10 as compared to that of the control females. All these variations were observed to be within the normal biological and laboratory limits and the effect was not dose-dependent (Ilamurugan *et al.*, 2010b).

The body weight of males and females of all the treatment group rats were comparable with the control group. No significant changes were noted during the dosing period of 90 days and the recovery period of 28 days. Body weight gain (%) was significantly increased on day 8 in all three treatment group male animals as compared to the control group animals. However, at later time in the experimental period of 90 day and up to the end of the recovery period (28 days) no significant changes in the treatment group were noted compared to the control. These changes

were within the normal range with respect to age and sex of the animals (Ilamurugan *et al.*, 2010b).

No toxicologically relevant findings were noted in hematology or clinical biochemistry parameters at the end of the treatment and recovery period. Hematology parameters such as red blood cell counts (RBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet (thrombocyte) count, white blood cell count (WBC), and differential leukocyte count did not show any toxicologically relevant findings in both sexes. However, there was a significant decrease in clotting time in males in all treatment groups as compared to the control group, whereas RBC counts and hemoglobin were significantly decreased in females in the low dose and high dose group when compared with that of the control group. However, these changes observed were not considered to be treatment related as these values were within the normal biological variation (Ilamurugan *et al.*, 2010b).

Clinical biochemistry parameters such as glucose, urea, creatinine, cholesterol, total triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, sodium, potassium, chloride, total protein, albumin, globulin, and A/G ratio did not show any toxicologically relevant findings in both the sexes in treatment and recovery groups with the exception of a decrease in sodium levels in males in the high dose recovery group when compared with that of control recovery. However, the changes observed were not considered to be treatment related as these values were within the normal biological variation. Urine analysis parameters (volume, specific gravity, color, clarity, pH, RBC, WBC, urobilinogen, bilirubin, ketone bodies, proteins and glucose) did not reveal any treatment-related changes in both sexes during the dosing period of 90 days and recovery period of 28 days, with the exception of an increase in the number of erythrocytes in the high dose group treated males compared with the control group. This variation was not considered treatment related since all the values were within the normal biological variation (Ilamurugan *et al.*, 2010b).

There were no significant differences in absolute and relative organ weights between control and treated groups in both males and females. However, there was a significant decrease in relative organ weight of brain in males in the low and mid dose group as compared with that of the control group. There was also a significant decrease in relative organ weight of the liver in males in the high dose recovery group as compared to that of control recovery group. However, these statistically significant variations observed were not considered to be treatment related as these variations were within the normal biological limits. Histological examinations of the tissue samples (brain, spleen, kidney, liver, and eyes) from the high dose group did not reveal any treatment-related pathological lesions as compared with the control group. The results of this study suggest that the NOAEL of lutein (Lutemax 2020™) was 400 mg/kg bw/day, the highest dose tested (Ilamurugan *et al.*, 2010a).

2.3.4.3. Mutagenicity Studies

Rusia *et al.* (2010) investigated the mutagenic potential of lutein (Lutemax 2020™) 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. The study was performed in compliance with OECD principles of GLP. Lutein was tested at the following concentrations: 0.031, 0.063, 0.125,

0.25 and 0.5 mg/plate. At a lutein concentration of 5 mg/plate, precipitation was noted. No substantial increase in revertant colony numbers of any of the tester strains were observed following treatment with lutein (Lutemax 2020™) 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 lutein at a selected dose level did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

2.3.5. Safety Studies of Meso-Zeaxanthin

As mentioned earlier, *meso*-zeaxanthin is also a minor component of Lutemax 2020™ and its intended use in specified food categories will result in estimated daily intake of total zeaxanthin of 2.4 and that of *meso*-zeaxanthin of 1.2 mg/person/day. Although the safety of *meso*-zeaxanthin as a component of Lutemax 2020™ has been supported by the above described specific studies on this ingredient, in a series of unpublished studies publicly available at the Howard Foundation website, the safety of *meso*-zeaxanthin has been investigated. These studies are described below.

Based on a final complete study report, Chang (2006) investigated the potential toxicity of *meso*-zeaxanthin in a subchronic toxicity study. In this study, *meso*-zeaxanthin was administered (gavage) once daily at dose levels of 0, 2, 20 or 200 mg/kg bw/day (control, low, mid, and high dose group, respectively) for at least 90 days to Han Wistar rats (10/sex/group). In addition, five males and five females were allocated to control and high dose recovery groups. The parameters evaluated included mortality, clinical observations, body weights, ophthalmology, clinical pathology, organ weights, gross pathology, and histopathology. No compound-related mortality, clinical signs of toxicity, changes in body weights, ophthalmology, clinical pathology, gross pathology, or histopathology were noted. The results of this study suggest the NOAEL of *meso*-zeaxanthin in rats was >200 mg/kg bw/day when administered orally for 13 consecutive weeks.

Mecchi (2006) evaluated the ability of *meso*-zeaxanthin to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes in *S. typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (strain WP2uvrA). The tester strains used were *S. typhimurium* tester and *Escherichia coli* tester. The doses tested in the mutagenicity assay with all tester strains in both the presence and absence of S9 mix were 10.0, 33.3, 100, 333, 1000, and 5000 µg per plate. The results of the initial mutagenicity assay were confirmed in an independent experiment. The results of these assays indicate that under the conditions of this study, *meso*-zeaxanthin, did not cause a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9).

2.3.6. Human Safety Data

In a prospective, randomized, controlled, and double-blind trial with parallel groups, Capeding *et al.* (2010) fed healthy term infants either control formula or experimental formula (fortified with lutein at 200 µg/L) for 16 weeks. In this study, 232 infants ≤14 days postnatal age were randomized and 220 (94.8%) completed the study. At 4, 8, 12, and 16 weeks, weight, head circumference, and length were measured. The primary endpoint of the study was weight gain from baseline to week 16. Safety was assessed through monitoring of study events throughout

the study and evaluation of selected blood chemistry tests performed at week 16. At 4 and 12 weeks, the lutein fortified formula intake was 964 and 1237 mL/day, respectively, corresponding to daily lutein intake of 193 and 247 µg/day, respectively. Infants in both groups demonstrated appropriate growth. No differences between treatment groups were found in any of the measures of growth at any of the measurement time points. Both formulas were well tolerated. The mean values of all measured blood chemistry parameters fell within the modified normal ranges for infants, and the values for both groups for any measured parameter were similar. The investigators concluded that lutein fortification of infant formula at 200 µg/L is safe.

Rosenthal *et al.* (2006) examined the dose-response relationship between oral lutein supplementation and serum lutein concentrations in subjects aged 60 years and older (n=45), with or without age-related macular degeneration (AMD). In this randomized trial, 45 subjects with no AMD, large drusen, or advanced AMD, received 2.5, 5, or 10 mg lutein daily for six months. Adverse events and additional safety-related parameters such as visual acuity, comprehensive ophthalmic examination, fundus photography, liver function tests, visual field tests, and the "Age-Related Eye Disease Study" (AREDS) side-effect questionnaire were assessed. Lutein ingestion resulted in a dose-related increase in serum levels of lutein. Lutein supplementation did not reveal any toxicity. Similarly no adverse effects were recorded on the AREDS side-effects questionnaire or in visual function. The liver function test results remained unchanged and normal. The results of this study suggest that supplementation of lutein at doses up to 10 mg/day was safe.

In a Letter to Editor, Gaynes (2007) reported that the conclusion drawn by Rosenthal *et al.* (2006) in the above described study is a result of oversimplification of essential concepts in clinical drug testing as related to study power (statistical) and detectable event rate. Gaynes (2007) also suggested that animal studies have identified three target organs for lutein bioaccumulation following intravenous administration, and suggested that lutein may be metabolized resulting in liver toxicity. Furthermore, the author mentioned that a close chemical correlate of lutein, β-carotene, is metabolized to vitamin A, which is known to cause liver toxicity. In responding to Gaynes (2007), one of the coauthors of the Rosenthal study, Chew *et al.* (2007) agreed that the Rosenthal *et al.* (2006) study design has low statistical power to detect adverse side effects. However, Chew *et al.* (2007) claimed that the totality of evidence based on other studies suggests that the dose of lutein of 10 mg/day is safe. In regards to the argument for liver toxicity, Chew *et al.* (2007) suggested that oral lutein supplementation may result in different metabolic patterns than if administered *via* an intravenous route. In another report from the same group but in an animal study (Khachik *et al.*, 2006b; described at length in Section 2.3.2.), oral supplementation of lutein to female monkeys increased concentrations of lutein in both plasma and tissue (liver, lung, colon, kidney, breast, ovaries, spleen, cervix, and other ocular tissue) without detectable toxicity. The doses of lutein and zeaxanthin used in the monkey study were equivalent to human doses of about 600 mg/day for an individual weighing 60 kg. Although lutein is a member of the carotenoid family, it is not considered to be provitamin A or β-carotene type compounds. Lutein does not share the metabolic pathways of β-carotene because it is not a substrate for the 15,15'-monooxygenase enzyme that cleaves β-carotene into vitamin A. Lutein, therefore, does not possess provitamin A activity and is unlikely to cause liver toxicity. The totality of evidence from available animal and clinical studies suggest that lutein supplementation at dose level of 10 mg/day is safe.

In a randomized, double-blind clinical trial, 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.

Vu *et al.* (2006) and Robman *et al.* (2007) reported complex interactions between lutein/zeaxanthin and ω -3 fatty acids intake with the progression of AMD. Vu *et al.* (2006) reported a marked increase in the risk of both early and late AMD among people who consumed greater than the median intake of linoleic acid and higher dietary intakes of lutein/zeaxanthin. These investigators recommended against the supplementation of lutein/zeaxanthin. This report used cross-sectional data based on photographic macular assessments of 71.9% of their sample of 2448 persons, who attended follow-up examinations. Robman *et al.* (2007) re-examined 254 subjects identified with early AMD to determine the progression of AMD over a seven year period. Lutein/zeaxanthin and fatty acid intakes were estimated using food frequency questionnaires. Energy-adjusted lutein/zeaxanthin as well as ω -3 fatty acid intakes was found to be associated with AMD progression. It is possible that multiple factors (described below) may have affected the outcome of these reports. The available evidence does not present a clear picture of the association of lutein/zeaxanthin and lipid intake with AMD.

In contrast to the above described findings (Vu *et al.*, 2006; Robman *et al.*, 2007), Flood *et al.* (2006) did not find any association with energy adjusted lutein/zeaxanthin intake (n = 986) and the incidence of early, late, or any AMD, whether or not this was stratified by linoleic acid intake. In this study, the median linoleic acid was less than the median used by Vu *et al.* (2006) (6.6 g versus 7.2 g). Stratification of the data by the highest tertile of linoleic acid intakes (cut-point 8.5 g) also did not reveal any association between lutein/zeaxanthin and incidence of AMD. These investigators suggested that even though the examination of cross-sectional data can be used to investigate associations with this disease, conclusions drawn from such data need to be made with care, in light of other known literature.

In the first study, in which 2448 subjects were followed, 212 persons who did not have photographic macular assessment (10.8% of those with dietary assessments) were included and this may have affected the outcome (Vu *et al.*, 2006). The dietary assessment method (food frequency questionnaire, FFQ) may have affected the outcome as it was not conducted at baseline, which only allows measurements of association from the follow-up examination (Flood *et al.*, 2006). As the study was cross-sectional, it is plausible that participants with known signs of early macular degeneration or associated visual changes may have increased their dietary antioxidant intakes (indication bias). This may have occurred in particular amongst those consuming higher linoleic acid diets, as higher intakes of linoleic acid have been suggested to increase the risk of AMD.

In a cross-over, double-blind, placebo-controlled trial, Bahrami *et al.* (2006) randomized 34 adult patients with retinitis pigmentosa into two groups. One group (n = 16), received lutein supplementation (10 mg/day for 12 weeks followed by 30 mg/day) for the first 24 weeks and

then placebo for the following 24 weeks, while the other group (n = 18) received placebo treatment (24 weeks) prior to lutein (received placebo during the first half and lutein in the second half of the study). Lutein supplementation did not result in any significant adverse effects. One subject on lutein, and two on placebo had impaired liver function tests at 1 of their 6 week visits. However, in these three subjects the serum liver enzyme levels returned to within normal range when tests were repeated. The investigators concluded that lutein supplementation at 10-30 mg/day for up to six months is safe.

The VITamins And Lifestyle (VITAL) cohort study examined the association of supplemental β -carotene, retinol, vitamin A, lutein, and lycopene with lung cancer risk among participants, aged 50-76 years (Satia *et al.*, 2009). Eligible individuals (n = 77,126) completed a baseline questionnaire, including details of supplement (multivitamins and individual supplements/mixtures) use (duration, frequency, dose) during the previous 10 years. The incidence of lung cancers (n = 521) was identified by linkage to the Surveillance, Epidemiology, and End Results cancer registry. Longer duration of use of individual β -carotene, retinol, and lutein supplements (but not total 10-year average dose) was associated with statistically significantly elevated risk of total lung cancer and histologic cell types. The investigators concluded that long-term use of individual β -carotene, retinol, and lutein supplements should not be recommended for lung cancer prevention, particularly among smokers. Similarly, earlier in the α -Tocopherol, β -Carotene Cancer Prevention (ATBC) trial, it was also noted that a sub-population (alcohol users) seemed to be more associated with the risk of cancers (Mayne *et al.*, 1996; Omaye *et al.*, 1997). It is difficult to interpret the results of epidemiological studies, however, the available evidence indicate that the intake of lutein is safe for healthy individuals.

3. SUMMARY AND DISCUSSION

OmniActive Health Technologies Ltd. intends to use lutein (Lutemax 2020™) obtained from Marigold flowers (*T. erecta* L) as a food ingredient. Lutemax 2020™ is a reddish-orange crystalline powder with a characteristic odor of Marigold flower. The compositional analysis of Lutemax 2020™ demonstrated that it primarily contains lutein with a small amount of zeaxanthin. The product contains either corn oil, or sunflower oil or carbohydrate as carrier. OmniActive intends to use lutein at concentrations up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 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, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, and soft candy. The intended use of Lutemax 2020™ in the above mentioned food categories will result in a mean and 90th percentile estimated daily lutein intake of 7.3 and 13.4 mg lutein/person/day and zeaxanthin intake of 1.4 and 2.4 mg/person/day, respectively. The 90th percentile *meso*-zeaxanthin intake is estimated as 1.2 mg/person/day.

The active constituents of Lutemax 2020™, trans-lutein, and zeaxanthin (except *meso*-zeaxanthin) are commonly found in the human diet and there is a safe history of consumption of these components. Lutein and its esters are found in a variety of commonly consumed green leafy vegetables, yellow-orange fruits, yellow-orange vegetables, and egg yolks. Lutein esters are commonly marketed as dietary supplements and its recommended doses range from 0.5 to 20 mg/day. Additional scientific evidence from animal and human studies also supports the safety

of lutein. Lutein and its ester has been the subject of four GRAS notices to the FDA. Thus, there is sufficient qualitative and quantitative scientific as well as history of use evidence to determine the safety-in-use of Lutemax2020™ and its constituents in the above mentioned food applications.

Lutein esters are hydrolyzed in the gastrointestinal tract to free or unesterified lutein and the free form of lutein is absorbed and enters circulation. Studies conducted in animals and humans suggest that oral administration of lutein ester or its free form results in lutein being bioavailable. Given the metabolism of lutein or its ester form, studies that used lutein in its free or esterified forms are equally important and applicable to the safety assessment of lutein.

The FDA has reviewed four separate GRAS notifications on lutein (lutein ester- GRN 000110; crystalline lutein- GRN 000140; suspended lutein- GRN 000221; crystalline lutein- GRN 000291) and in response to these notices the agency did not question the safety of lutein ester, crystalline lutein, and suspended lutein for their intended food applications. The subjects of the present GRAS determination, lutein in free (non-esterified) and esterified (with fatty acids) forms are substantially equivalent to the subjects of FDA GRAS notified substances (GRN 000110; GRN 000140; GNR 000221). The FDA responses to the GRAS notifications on lutein and its esters indicate that the FDA is comfortable with the safety of the lutein, as long as consumption is below 20 mg/person/day. In addition to the FDA, JECFA also evaluated safety-in-use of lutein and assigned a group ADI of 0 to 2 mg lutein and zeaxanthin/kg body weight. Studies that appeared subsequent to the FDA and JECFA reviews also supports the safety-in-use of lutein (free or ester form). In a recent study, supplementation of lutein (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, lutein did not cause mutagenic or clastogenic effects. The available evidence suggests that lutein is unlikely to be mutagenic or cause DNA damage.

In two human (cross-sectional) studies (Vu *et al.*, 2006; Robman *et al.*, 2007), a positive association between the intake of lutein/zeaxanthin and ω -3 fatty acids with progression of age-related macular degeneration (AMD) was noted. However, no such association was noted in another study. The cross-sectional studies using dietary survey data may have some limitations because it measures the current diet in a group of people with a disease or other condition which may well be altered by the presence of the disease. Another limitation may be related to errors in recall of the exposure and possibly outcome. Furthermore, other clinical studies did not reveal any toxic effects of lutein. In a review article (Shao and Hathcock, 2006), the use of “observed safe level” methodology for risk assessment revealed a strong evidence for the safety of lutein intake at levels up to 20 mg/day for humans. The authors of this study suggested that lutein at levels up to 40 mg/day may be safe, but the data for intakes above 20 mg/day are not sufficient for a confident conclusion.

In acute and subchronic studies, the safety of lutein (Lutemax 2020™) was tested. In the acute study, the LD₅₀ of lutein was greater than 2 g/kg body weight. In the subchronic study, lutein was administered (gavage) daily to rats at doses of 0, 4, 40, or 400 mg/kg body weight/day for 90 days, respectively. 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 lutein (Lutemax 2020™) is determined as 400 mg/kg body weight/day, the highest dose tested. The estimated daily intake of Lutemax 2020™ from its intended food use is about 2000 fold lower compared to the NOAEL determined from the subchronic toxicity study. Similarly, the estimated daily intake of lutein from the intended uses of Lutemax 2020™ is approximately

1000 fold lower compared to the NOAEL of 200 mg/kg body weight/day from the rat study used to establish the JECFA ADI. The safety determination of lutein is based on the totality of available evidence such as human observations, animal studies, *in vitro* studies, and clinical trials. Furthermore specific toxicity studies conducted with Lutemax 2020™ support its safety. Additionally, the safety of *meso*-zeaxanthin, one of the components of Lutemax 2020™ is also supported by the available evidence related to the safety studies of this isomer. The intended uses of Lutemax 2020™ will result in daily *meso*-zeaxanthin intake of 1.2 mg/person/day and this level is over 1000-fold lower than the NOAEL of 200 mg/kg/day for *meso*-zeaxanthin in a rat study.

In summary, on the basis of scientific procedures⁴, history of exposure and use, the consumption of Lutemax 2020™ derived from Marigold flowers (*Tagetes erecta* L) as a nutrient at use levels of 0.3 to 3 mg/serving in certain specified foods resulting in a 90th percentile intake of 13.4 mg lutein/person/day and 0.6 mg zeaxanthin/person/day is considered safe. The intended uses are compatible with current regulations, *i.e.*, Lutemax 2020™ is used as a nutrient [21 CFR 170.3(o)(20)] 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, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, and soft candy, when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

⁴ 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 lutein (Lutemax 2020™) 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, infant and toddler foods (other than infant formula), 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 lutein/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 lutein (Lutemax 2020™), when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

John A. Thomas, Ph.D., F.A.C.T., D.A.T.S.

10/13/10
Date

(b) (6)

Stanley T. Omaye, Ph.D., D.A.T.S.

10/14/10
Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N.

10/14/2010
Date

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6. APPENDIX I

Batch Specifications
Analytical data from five manufacturing lots
(Lutemax 2020 / Lutein and Zeaxanthin Concentrate)

Analytical data from five manufacturing lots (Lutemax 2020 / Lutein and Zeaxanthin Concentrate)

Parameters	Specifications	HPMCZ-0406872	HPMCZ -0406873	HPMCZ-0406874	HPMCZ-0406875	HPMCZ-0406876
Appearance	Reddish orange colored crystals					
Odor:	Characteristic of Marigold flowers					
Identity	HPLC	Complies	Complies	Complies	Complies	Complies
Moisture	Max 1%	0.25%	0.18%	0.27%	0.20%	0.21%
Carotenoids composition						
Total Carotenoids	Min 80%	80.00%	81.28%	82.21%	81.21%	81.17%
Lutein	Min 67%	67.20%	67.50%	68.20%	67.45%	67.30%
Zeaxanthin	Min 13.5%	13.80%	13.78%	13.91%	13.76%	13.87%
3R, 3'S / meso Zeaxanthin	25 to 75% of total zeaxanthin	7.12 %	6.80 %	6.86 %	6.28 %	7.02 %
3R, 3'R Zeaxanthin	25 to 75% of total zeaxanthin	6.68 %	6.98 %	7.05 %	7.48 %	6.85 %
Heavy metals (ppm)						
Lead	<1	Passes	Passes	Passes	Passes	Passes
Arsenic	<1	Passes	Passes	Passes	Passes	Passes
Mercury	<1	Passes	Passes	Passes	Passes	Passes
Cadmium	<1	Passes	Passes	Passes	Passes	Passes
Microbiology assay						
Total plate count (cfu/g)	<1000	Passes	Passes	Passes	Passes	Passes
Yeast and molds (cfu/g)	<100	Passes	Passes	Passes	Passes	Passes
<i>E. coli</i> and <i>Salmonella</i>	Negative	Passes	Passes	Passes	Passes	Passes
<i>Salmonella</i>	Negative	Passes	Passes	Passes	Passes	Passes
<i>Pseudomonas auriginosa</i>	Negative	Passes	Passes	Passes	Passes	Passes
<i>Staphylococcus</i> sp.	Negative	Passes	Passes	Passes	Passes	Passes

7. Appendix II

Residual solvents of five manufacturing lots (Lutemax 2020 / Lutein & Zeaxanthin Concentrate)

Parameters	Specifications	HPMCZ-0406872	HPMCZ-0406873	HPMCZ-0406874	HPMCZ-0406875	HPMCZ-0406876
Total Residual solvent (ppm)	< 100	52	45	38	55	58
Hexane (ppm)	< 50	31	20	21	25	41
Ethyl acetate (ppm)	< 25	10	8	5	9	5
Ethanol (ppm)	< 25	11	17	12	21	12

8. Appendix III

Pesticide residue data from three lots of lutein oleoresin



TEST REPORT

Report No. : CH:GL:9110012744

DATE : 01/04/2009

JOE No. : 911103594

Report Control No.:9115011969

SAMPLE NOT DRAWN BY LABORATORY

SAMPLE SUBMITTED AND IDENTIFIED BY CUSTOMER AS : MARIGOLD OLEORESIN

CUSTOMER NAME OMNIACTIVE HEALTH TECHNOLOGIES LIMITED
ADDRESS PLOT NO.38/39,RAJIV GANDHI INTERNATIONAL BIO-TECH
PARK,PHASE-II,HINJEWADI,
CITY PUNE-411057
COUNTRY A/C SGS INDIA - THANE
INDIA
SAMPLING METHOD N.A.
SAMPLE DESCRIPTION MARIGOLD OLEORESIN
SAMPLE QTY. 25GM
BATCH NO. MRL/108/01
SAMPLE RECD ON 24-03-2009
TEST START DATE 24/03/2009
TEST END DATE 01/04/2009

Table with 3 columns: TESTS, PROTOCOL, RESULT. Rows include Pesticides, Alpha-BHC, Beta-BHC, Gamma-BHC, Delta-BHC, O,P'-DDT, P,P'-DDT, Endrin, Dieldrin, EndoSulfan, Heptachlor, Parathion Methyl, Diazinon, Fenitrothion, Aldrin, Chlorfenvinfos, Chlorothalonil.

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TEST REPORT

Report No. : CH:GL:9110012744 DATE : 01/04/2009

JOE No. : 911103594 Report Control No.:9115011969

TESTS	PROTOCOL	RESULT
Mevinphos		ND[DL:0.01 mg/kg]
Phosalone		ND[DL:0.01 mg/kg]
Heptachlor Epoxide		ND[DL:0.01 mg/kg]
Chlordane		ND[DL:0.01 mg/kg]
OP-DDE		ND[DL:0.01 mg/kg]
PP-DDE		ND[DL:0.01 mg/kg]
OP-DDD		ND[DL:0.01 mg/kg]
PP-DDD		ND[DL:0.01 mg/kg]
Pirimiphos methyl		ND[DL:0.01 mg/kg]
Chlorpyrifos methyl		ND[DL:0.01 mg/kg]
Methoxy Chlor		ND[DL:0.01 mg/kg]
Chlorpyrifos		ND[DL:0.01 mg/kg]
Azinphos methyl		ND[DL:0.01 mg/kg]
Dicofol		ND[DL:0.01 mg/kg]
Ethion		ND[DL:0.01 mg/kg]
Parathion		ND[DL:0.01 mg/kg]
Malathion		ND[DL:0.01 mg/kg]
NOTE: ND - Not Detected, DL - Detection Limit.		

**** End of Report ****

per pro SGS India Private Ltd.

(b) (6)

Authorised Signatory
A.V ABRAHAM
Asst. Manager

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TEST REPORT

Report No. : CH:GL:9110012740 DATE : 01/04/2009

JOE No. : 911103594

Report Control No.:9115011965

SAMPLE NOT DRAWN BY LABORATORY

SAMPLE SUBMITTED AND IDENTIFIED BY CUSTOMER AS : MARIGOLD OLEORESIN

CUSTOMER NAME OMNIACTIVE HEALTH TECHNOLOGIES LIMITED
ADDRESS PLOT NO.38/39,RAJIV GANDHI INTERNATIONAL BIO-TECH
PARK,PHASE-II,HINJEWADI,
CITY PUNE-411057
COUNTRY A/C SGS INDIA - THANE
INDIA
SAMPLING METHOD N.A.
SAMPLE DESCRIPTION MARIGOLD OLEORESIN
SAMPLE QTY. 25GM

BATCH NO. MRL/104/01
SAMPLE RECD ON 24-03-2009
TEST START DATE 24/03/2009
TEST END DATE 01/04/2009

Table with 3 columns: TESTS, PROTOCOL, RESULT. Lists pesticides like Alpha-BHC, Beta-BHC, Gamma-BHC, etc., with their respective protocols (AOAC 18th EDN 2006 GCMS/LC MS-MS) and results (ND[DL:0.01 mg/kg]).

Page 1 of 2

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Member of the SGS Group (SGS SA)



TEST REPORT

Report No. : CH:GL:9110012740 DATE : 01/04/2009
JOE No. : 9111033594 Report Control No.:9115011965

TESTS	PROTOCOL	RESULT
Mevinphos		ND(DL:0.01 mg/kg)
Phosalone		ND(DL:0.01 mg/kg)
Heptachlor Epoxide		ND(DL:0.01 mg/kg)
Chlordane		ND(DL:0.01 mg/kg)
OP-DDE		ND(DL:0.01 mg/kg)
PP-DDE		ND(DL:0.01 mg/kg)
OP-DDD		ND(DL:0.01 mg/kg)
PP-DDD		ND(DL:0.01 mg/kg)
Pirimiphos methyl		ND(DL:0.01 mg/kg)
Chlorpyrifos methyl		ND(DL:0.01 mg/kg)
Methoxy Chlor		ND(DL:0.01 mg/kg)
Chlorpyrifos		ND(DL:0.01 mg/kg)
Azinophos methyl		ND(DL:0.01 mg/kg)
Dicofol		ND(DL:0.01 mg/kg)
Ethion		ND(DL:0.01 mg/kg)
Parathion		ND(DL:0.01 mg/kg)
Malathion		ND(DL:0.01 mg/kg)

NOTE: ND - Not Detected, DL - Detection Limit.

**** End of Report ****

per pro SGS India Private Ltd.

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Authorised Signatory
A.V ABRAHAM
Asst Manager

Page 2 of 2

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TEST REPORT

Report No. : CH:GL:9110012743 DATE : 01/04/2009

JOE No. : 911103594

Report Control No.:9115011968

SAMPLE NOT DRAWN BY LABORATORY

SAMPLE SUBMITTED AND IDENTIFIED BY CUSTOMER AS : MARIGOLD OLEORESIN

CUSTOMER NAME OMNIACTIVE HEALTH TECHNOLOGIES LIMITED
ADDRESS PLOT NO.38/39,RAJIV GANDHI INTERNATIONAL BIO-TECH
PARK,PHASE-II,HINJEWADI,
CITY PUNE-411057
COUNTRY A/C SGS INDIA - THANE
INDIA
SAMPLING METHOD N.A.
SAMPLE DESCRIPTION MARIGOLD OLEORESIN
SAMPLE QTY. 25GM
BATCH NO. MRL/107/01
SAMPLE RECD ON 24-03-2009
TEST START DATE 24/03/2009
TEST END DATE 01/04/2009

Table with 3 columns: TESTS, PROTOCOL, RESULT. Rows include Pesticides, Alpha-BHC, Beta-BHC, Gamma-BHC, Delta-BHC, O,P'-DDT, P,P'-DDT, Endrin, Dieldrin, EndoSulfan, Heptachlor, Parathion Methyl, Diazinon, Fenitrothion, Aldrin, Chlorfenvinfos, Chlorothalonil.

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TEST REPORT

Report No. : CH:GL:9110012743 DATE : 01/04/2009
JOE No. : 911103594 Report Control No.:9115011968

TESTS PROTOCOL RESULT

Table with 3 columns: TESTS, PROTOCOL, RESULT. Lists various pesticides like Mevinphos, Phosalone, Heptachlor Epoxide, etc., with their respective detection limits (DL) and results (ND).

NOTE: ND - Not Detected, DL - Detection Limit.

**** End of Report ****

per pro SGS India Private Ltd.

(b) (6) [Redacted]
Authorized Signatory
A.V ABRAHAM
Asst Manager

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TEST REPORT

Report No. : CH:GL:9110012742 DATE : 01/04/2009

JOE No. : 911103594

Report Control No.:9115011967

SAMPLE NOT DRAWN BY LABORATORY

SAMPLE SUBMITTED AND IDENTIFIED BY CUSTOMER AS : MARIGOLD OLEORESIN

CUSTOMER NAME OMNIACTIVE HEALTH TECHNOLOGIES LIMITED
ADDRESS PLOT NO.38/39,RAJIV GANDHI INTERNATIONAL BIO-TECH
PARK,PHASE-II,HINJEWADI,
CITY PUNE-411057
A/C SGS INDIA - THANE
COUNTRY INDIA
SAMPLING METHOD N.A
SAMPLE DESCRIPTION MARIGOLD OLEORESIN
SAMPLE QTY. 25GM

BATCH NO. MRL/106/01
SAMPLE RECD ON 24-03-2009
TEST START DATE 24/03/2009
TEST END DATE 01/04/2009

Table with 3 columns: TESTS, PROTOCOL, RESULT. Rows include Pesticides, Alpha-BHC, Beta-BHC, Gamma-BHC, Delta-BHC, O.P'-DDT, P.P'-DDT, Endrin, Dieldrin, EndoSulfan, Heptachlor, Parathion Methyl, Diazinon, Fenitrothion, Aldrin, Chlorfenvinfos, Chlorothalonil.

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TEST REPORT

Report No. : CH:GL:9110012742 DATE : 01/04/2009

JOE No. : 911103594 Report Control No. 9115011967

TESTS	PROTOCOL	RESULT
Mevinphos		ND[DL:0.01 mg/kg]
Phosalone		ND[DL:0.01 mg/kg]
Heptachlor Epoxide		ND[DL:0.01 mg/kg]
Chlordane		ND[DL:0.01 mg/kg]
OP-DDE		ND[DL:0.01 mg/kg]
PP-DDE		ND[DL:0.01 mg/kg]
OP-DDD		ND[DL:0.01 mg/kg]
PP-DDD		ND[DL:0.01 mg/kg]
Pirimiphos methyl		ND[DL:0.01 mg/kg]
Chlorpyrifos methyl		ND[DL:0.01 mg/kg]
Methoxy Chlor		ND[DL:0.01 mg/kg]
Chlorpyrifos		ND[DL:0.01 mg/kg]
Azinophos methyl		ND[DL:0.01 mg/kg]
Dicofol		ND[DL:0.01 mg/kg]
Ethion		ND[DL:0.01 mg/kg]
Parathion		ND[DL:0.01 mg/kg]
Malathion		ND[DL:0.01 mg/kg]

NOTE: ND - Not Detected, DL - Detection Limit.

**** End of Report ****

per pro SGS India Private Ltd.

(b) (6)

Authorised Signatory
A.V ABRAHAM
Asst Manager

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SGS India Pvt. Ltd. | Laboratory: 1/509 A, Old Mahabaleshwar Road, Thoraipakkam, Chennai-600 097 Phone : 91-44-24963844/24962822, Fax : 91-44-24963075
Regd & Corp. Off : SGS House, 49, A.S. Marg, Vikhrali (West), Mumbai-400083. Tel : (022) 25798421 to 28 Fax : (022) 25798431 to 35 www.sgs.com
Member of the SGS Group (SGS SA)

Pages 000060-000145 of Curriculum Vitae removed under Freedom of Information Exemption 6

SUBMISSION END

000146

Mosley, Sylvester

From: Madhu Soni <sonim@bellsouth.net>
Sent: Friday, July 29, 2011 4:16 PM
To: Mosley, Sylvester
Subject: RE: Questions Regarding GRN000385 (Lutein)

Thank you, Dr. Mosley for your email and the query related to GRN 385. I am writing to acknowledge the receipt and we will provide our responses to the queries in two weeks.

Best regards. Have a nice weekend.

Madhu

Madhu Soni, *PhD, FACN*
Soni & Associates Inc.
749 46th Square, Vero Beach, FL 32968
Phone: 772-299-0746
Cell: 772-538-0104
www.soniassociates.net

From: Mosley, Sylvester [<mailto:Sylvester.Mosley@fda.hhs.gov>]
Sent: Friday, July 29, 2011 1:10 PM
To: 'msoni@soniassociates.net'
Subject: Questions Regarding GRN000385 (Lutein)

Good morning Dr. Soni,

After initial review of your client's GRAS Notice (GRN000385, Lutein), our reviewers have the following questions to ask:

- 1) Does Lutemax 2020 contain Lutein esters or Lutein because Lutein esters have been identified as the principal coloring component of Marigold flowers?
- 2) On page 7 of 11, the notifier states that fresh Marigold flowers (not just the petals), were used as their starting material. The composition of the product accounts for 94-96%. What is the composition of the rest of the material?
- 3) On page 2 of 11, section D, lines 12-13, it is stated, "The intended uses of lutein and zeaxanthin....result in the mean and 90th percentile intake of 7.3 and 13.4 mg/person/day, respectively." But these are the intake values of lutein. Please clarify.
- 4) On page 27 of 46, summary paragraph, the notifier states a 90th percentile intake of 0.6 mg zeaxanthin/person/day. However, all throughout the document it is stated as 2.4 mg zeaxanthin/person/day. Please clarify.
- 5) On page 30 of 46, Gaynes, 2007 and Flood *et al.*, 2006 have the same references from the "British Journal of Ophthalmology". Please provide the correct references and would appreciate the articles as well.

We request this information be provided within a two week timeframe. If you feel this can not be accomplished please let us know.

Sincerely,
Sylvester

Sylvester L. Mosley, Ph.D.
Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835
Phone: 240-402-1333
Fax: 301-436-2965
Email: Sylvester.Mosley@fda.hhs.gov

Mosley, Sylvester

From: Madhu Soni <sonim@bellsouth.net>
Sent: Thursday, August 11, 2011 12:33 PM
To: Mosley, Sylvester
Subject: RE: Questions Regarding GRN000385 (Lutein)
Attachments: Lutein GRN 385 FDA Query- Dr Mosley- Response.pdf; Gaynes 2007.pdf; Flood et al 2006.pdf

Dear Dr. Mosley,

Please find attached an electronic (PDF) file providing a point-by-point response to your queries. I hope the information and clarifications, along with some discussion in the response addresses your queries. If you have any questions or need additional explanation, please let me know. Thank you for the opportunity to provide this explanation.

Best regards

Madhu

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Sent: Friday, July 29, 2011 1:10 PM
To: 'msoni@soniassociates.net'
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College Park, MD 20740-3835
Phone: 240-402-1333
Fax: 301-436-2965
Email: Sylvester.Mosley@fda.hhs.gov

Dear Dr. Mosley,

RE: Lutein GRAS Notice (GRN 385)

This responds to your email of July 29, 2011 regarding additional information and clarifications required for our Lutein GRAS notice (GRN 000385). We are providing a point-by-point response to your queries along with some relevant discussion.

FDA Query 1: Does Lutemax 2020 contain Lutein esters or Lutein because Lutein esters have been identified as the principal coloring component of Marigold flowers?

Response: During the manufacturing of Lutemax 2020, the lutein esters are completely hydrolyzed in the saponification process resulting in the liberation of free lutein. The formation of free lutein is confirmed from the results of HPLC analysis of Lutemax 2020.

FDA Query 2: On page 7 of 11, the notifier states that fresh Marigold flowers (not just the petals), were used as their starting material. The composition of the product accounts for 94-96%. What is the composition of the rest of the material?

Response: In the industrial practice for the manufacturing of lutein, generally the fresh flowers are the starting material and not the petals. The use of flower is related to the difficulty of separation of petals from the large quantity of flowers as it involves considerable manual labor. Based on our understanding, there is no suitable equipment which can meet the requirements for separation of petals from flowers on a commercial scale.

As regards the composition of the product, batch analysis data reveals that total carotenoids ranges from 80 to 82%. The wax content is generally in the range of 14 to 16%. Additionally, the product also contains small amount of fatty acids (up to 1%), ash (up to 1%) and moisture (up to 1%). Considering all these parameters, the composition is fully (100%) characterized.

FDA Query 3: On page 2 of 11, section D, lines 12-13, it is stated, "The intended uses of lutein and zeaxanthin....result in the mean and 90th percentile intake of 7.3 and 13.4 mg/person/day, respectively." But these are the intake values of lutein. Please clarify.

Response: We apologize for the inclusion of zeaxanthin in the above mentioned sentence. The correct sentence is: The intended uses of lutein in the above mentioned food categories will result in the mean and 90th percentile intake of 7.3 and 13.4 mg/person/day, respectively.

FDA Query 4: On page 27 of 46, summary paragraph, the notifier states a 90th percentile intake of 0.6 mg zeaxanthin/person/day. However, all throughout the document it is stated as 2.4 mg zeaxanthin/person/day. Please clarify.

Response: Thank you for bringing this to our attention. By oversight we included the incorrect value. As indicated in your query the correct value should be 2.4 mg zeaxanthin/person/day.

FDA Query 5: On page 30 of 46, Gaynes, 2007 and Flood *et al.*, 2006 have the same references from the “British Journal of Ophthalmology”. Please provide the correct references and would appreciate the articles as well.

Response: We are sorry for the incorrect Journal details for the Gaynes, 2007 publication. The correct citation should be: Gaynes, B.I., 2007. Safety assessment of nutritional supplements for prevention and treatment of ophthalmic disease. **Invest. Ophthalmol. Vis. Sci.** published September 21, 2007 as eLetters (Available at: http://www.iovs.org/content/47/12/5227/reply#iovs_el_3127). The Flood et al. (2006) citation is correct. As per your email, please find attached both these articles.

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let me know.

Thank you for the opportunity to provide this explanation.

Best regards

Madhu Soni, PhD

Pages 000153-000157 have been removed in accordance with copyright laws. The removed reference is:

Gaynes, B.I., 2007. Safety assessment of nutritional supplements for prevention and treatment of ophthalmic disease. *Invest. Ophthalmol. Vis. Sci.* published September 21, 2007 as eLetters (Available at: http://www.iovs.org/content/47/12/5227/reply#iovs_el_3127)

Mosley, Sylvester

From: Madhu Soni <sonim@bellsouth.net>
Sent: Thursday, August 18, 2011 10:52 AM
To: Mosley, Sylvester
Subject: RE: Questions Regarding GRN000385 (Lutein)

Dear Dr. Mosley,

I am writing to make sure that you received my previous email of August 11, 2011 (please see below) along with the attachments. If you have any questions, please let me know.

Best regards

Madhu

Madhu G. Soni, *PhD, FACN*
Soni & Associates Inc
749 46th Square
Vero Beach, FL 32968, USA
Phone: +1-772-299-0746 ; Cell: +1-772-538-0104
www.soniassociates.net

From: Madhu Soni [<mailto:sonim@bellsouth.net>]
Sent: Thursday, August 11, 2011 12:33 PM
To: 'Mosley, Sylvester'
Subject: RE: Questions Regarding GRN000385 (Lutein)

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From: Mosley, Sylvester [<mailto:Sylvester.Mosley@fda.hhs.gov>]
Sent: Friday, July 29, 2011 1:10 PM
To: 'msoni@soniassociates.net'
Subject: Questions Regarding GRN000385 (Lutein)

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Sylvester L. Mosley, Ph.D.

Consumer Safety Officer
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5100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835
Phone: 240-402-1333
Fax: 301-436-2965
Email: Sylvester.Mosley@fda.hhs.gov

**Mosley, Sylvester**

From: Madhu Soni <sonim@bellsouth.net>
Sent: Tuesday, August 30, 2011 11:51 AM
To: Mosley, Sylvester
Subject: GRN000385 (Lutein)- Clarifications

Dear Dr. Mosley,

This has reference to our telephone discussion regarding some of the information mentioned as confidential in the GRAS notification (GRN 000385, lutein) submitted on behalf of OmniActive Health Technologies Limited. In this regard, I would like to bring to your kind attention that any information described on page 1 of 46 as well as on page 35 of 46 should not be considered as confidential. Inadvertently, the word confidential was included on these two pages. Hope this addresses your query.

If you have any questions, please let me know.

Best regards

Madhu

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From: Madhu Soni [<mailto:sonim@bellsouth.net>]
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To: 'Mosley, Sylvester'
Subject: RE: Questions Regarding GRN000385 (Lutein)

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Best regards

Madhu

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From: Madhu Soni [<mailto:sonim@bellsouth.net>]
Sent: Thursday, August 11, 2011 12:33 PM
To: 'Mosley, Sylvester'
Subject: RE: Questions Regarding GRN000385 (Lutein)

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From: Mosley, Sylvester [<mailto:Sylvester.Mosley@fda.hhs.gov>]
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Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
1100 Paint Branch Parkway (HFS-255)
College Park, MD 20740-3835
Phone: 240-402-1333

Fax: 301-436-2965

Email: Sylvester.Mosley@fda.hhs.gov

Mosley, Sylvester

From: Madhu Soni <sonim@bellsouth.net>
Sent: Friday, December 16, 2011 1:00 PM
To: Mosley, Sylvester
Subject: GRN000385 (Lutein)

Dear Sylvester,

I am writing regarding our GRAS notice 385 on lutein. It is almost 6 months since the submission and as I recall, FDA generally sends a fax in response to a notice. Our fax is not working, hence I am writing to request for a scanned copy of response, if possible. Thank you for your attention.

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From: Mosley, Sylvester [<mailto:Sylvester.Mosley@fda.hhs.gov>]
Sent: Thursday, August 18, 2011 11:38 AM
To: 'Madhu Soni'
Subject: RE: Questions Regarding GRN000385 (Lutein)

Madhu,

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Thanks,
Sylvester

Sylvester L. Mosley, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
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Email: Sylvester.Mosley@fda.hhs.gov

From: Madhu Soni [<mailto:sonim@bellsouth.net>]
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Subject: RE: Questions Regarding GRN000385 (Lutein)

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Fax: 301-436-2965
Email: Sylvester.Mosley@fda.hhs.gov

Mosley, Sylvester

From: Madhu Soni <sonim@bellsouth.net>
Sent: Monday, December 19, 2011 8:31 AM
To: Mosley, Sylvester
Subject: RE: GRN000385 (Lutein)

Good morning Sylvester,

Thank you very much for the email and the response to our GRAS notice. I very much appreciate your efforts/time to provide us a scanned copy of the response. We are happy to note that the agency has no question to our conclusion that lutein is GRAS.

Best regards. Happy Holidays!

Madhu

Madhu G. Soni, *PhD, FACN*
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From: Mosley, Sylvester [mailto:Sylvester.Mosley@fda.hhs.gov]
Sent: Monday, December 19, 2011 7:40 AM
To: 'Madhu Soni'; 'msoni@soniassociates.net'
Subject: RE: GRN000385 (Lutein)

Dear Dr. Soni,

FDA has completed its review of your client's, OmniActive Health Technologies Ltd., GRAS notice (GRN000385). Our response is attached. Please confirm receipt of this email and attachment. Hardcopy will follow by U.S. mail.

Sincerely,
Sylvester

Sylvester L. Mosley, Ph.D.
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Subject: RE: Questions Regarding GRN000385 (Lutein)

Dear Dr. Mosley,

Please find attached an electronic (PDF) file providing a point-by-point response to your queries. I hope the information and clarifications, along with some discussion in the response addresses your queries. If you have any questions or need additional explanation, please let me know. Thank you for the opportunity to provide this explanation.

Best regards

Madhu

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From: Mosley, Sylvester [mailto:Sylvester.Mosley@fda.hhs.gov]
Sent: Friday, July 29, 2011 1:10 PM
To: 'msoni@soniassociates.net'
Subject: Questions Regarding GRN000385 (Lutein)

Good morning Dr. Soni,

After initial review of your client's GRAS Notice (GRN000385, Lutein), our reviewers have the following questions to ask:

- 1) Does Lutemax 2020 contain Lutein esters or Lutein because Lutein esters have been identified as the principal coloring component of Marigold flowers?
- 2) On page 7 of 11, the notifier states that fresh Marigold flowers (not just the petals), were used as their starting material. The composition of the product accounts for 94-96%. What is the composition of the rest of the material?
- 3) On page 2 of 11, section D, lines 12-13, it is stated, "The intended uses of lutein and zeaxanthin....result in the mean and 90th percentile intake of 7.3 and 13.4 mg/person/day, respectively." But these are the intake values of lutein. Please clarify.
- 4) On page 27 of 46, summary paragraph, the notifier states a 90th percentile intake of 0.6 mg zeaxanthin/person/day. However, all throughout the document it is stated as 2.4 mg zeaxanthin/person/day. Please clarify.
- 5) On page 30 of 46, Gaynes, 2007 and Flood *et al.*, 2006 have the same references from the "British Journal of Ophthalmology". Please provide the correct references and would appreciate the articles as well.

We request this information be provided within a two week timeframe. If you feel this can not be accomplished please let us know.

Sincerely,

Sylvester

Sylvester L. Mosley, Ph.D.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

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