GR

ORIGINAL SUBMISSION

000001

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August 5, 2013

BY FEDERAL EXPRESS

RECEIVED
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OFFICE OF
FOOD ADDITIVE SAFETY

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Re: GRAS Notification for meso-Zeaxanthin

Dear Sirs:

On behalf of our client, Industrial Organica S.A. de C.V., we are hereby submitting the enclosed GRAS Notification for meso-xeaxanthin to be used as a food ingredient when used in the applications and under the conditions of use described herein. In compliance with 21 C.F.R. §170.36 (b) (proposed), we are enclosing an original and three copies of this notice.

Should you have any questions regarding this Notice, please do not hesitate to contact me.

Sincerely,

(b) (6)

Mark L. Itzkoff

MIL:amt

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August 5, 2013

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Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied
Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Re: **GRAS Claim for the Use of Meso-Zeaxanthin as a Food Ingredient**

Dear Sirs:

Industrial Organica S.A. de C.V. (IOSA) hereby notifies the agency that IOSA has determined that meso-zeaxanthin rich extract (*Hi-Fil Z*™) is Generally Recognized as Safe (GRAS) for use as a food ingredient as specified in Table I, attached.

Section I

A. Name and Address of Notifier:

Industrial Organica S.A. de C.V.
Ave. Almazán No. 100 Col. Topo Chico,
Monterrey, N.L., Mexico

All communications regarding this notice should be sent to:

Mark L. Itzkoff
Counsel for IOSA
Olsson Frank Weeda Terman Matz PC
600 New Hampshire Ave, NW
Washington, DC 20037

B. Common or Usual Name of Substance:

The common or usual name of the substance is meso-zeaxanthin. The Chemistry Abstract Services number for this substance is 31272-50-1. It will be supplied in powder form under its trade name, *Hi-Fil Z*™.

C. Conditions of Use:

Meso-zeaxanthin-rich extract (Hi-FIL Z™) that is the subject of this Notice will be used as a food ingredient in a variety of food and beverage applications specified in Table 1. Currently, IOSA plans to market *Hi-Fil Z*™, primarily containing meso-zeaxanthin, as a powder.

D. Basis for GRAS Determination:

During July of 2013, the status of the IOSA meso-zeaxanthin-rich extract was reviewed by a panel of scientists (the Expert Panel) assembled by Soni and Associates Inc for IOSA. The Expert Panel was comprised of three noted toxicologists with expertise in food safety. The Panel independently and critically reviewed the information and the available toxicology literature on meso-zeaxanthin and lutein. The Expert Panel concluded that, based on scientific procedures, the use of meso-zeaxanthin as detailed in Table 1 is GRAS.[1]

Thus, the basis for our GRAS determination is the scientific procedures detailed in the Expert Panel Statement.

E. **Data Availability Statement:**

The data and information that are the basis for the Notifier's GRAS determination is available for agency review during normal business hours at the office of Olsson Frank Weeda Terman Matz PC provided above or will be forwarded to the agency upon request.

Respectfully Submitted,

(b) (6)

Mark L. Itzkoff
Counsel for Industrial Organica S.A. de C.V.

[1] A copy of the Expert Panel Statement is attached as Appendix I.

Table 1

Summary of the Individual Proposed Food-Uses for Meso-Zeaxanthin

Food Category	Proposed Food	Max. Conc. of *Hi-Fil Z* (mg/RACC*)
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.0
	Crackers and Crispbreads	2.0
Beverages and Beverage Bases	Bottled Water	0.5
	Carbonated Beverages	2.0
	Meal Replacements	2.0
	Tea, Read-to-Drink	0.6
Breakfast Cereals	Instant and Regular Hot Cereals	2.0
	Ready-to-Eat Cereals	2.0
Chewing Gum	Chewing Gum	1.0
Dairy Product Analogs	Imitation Milks	2.0
	Soy Milks	1.5
Egg Products**	Liquid, Frozen or Dried Egg Substitutes	2.0
Fats and Oils	Margarine-like Spreads	1.5
	Salad Dressings	1.5
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.0
Gravies and Sauces	Tomato Based Sauces	0.3
Hard Candy	Hard Candy	1.0
Infant and Toddler Foods***	Junior, Strained and Toddler-Type Baby foods	1.0
Milk Products	Dry Milk	3.0
	Fermented Milk Beverages	0.6
	Flavored Milk and Milk Drinks	3.0
	Milk-Based Meal Replacements	3.0
	Yogurt	3.0
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	2.0
	Fruit-Flavored Drinks	2.0
	Fruit Juice	2.0
	Nectars	2.0
	Vegetable Juice	2.0
Soft Candy	Chewy and Nougat Candy	1.0
	Fruit Snacks	1.0

Section II

Identity of the Notified Substance

Meso-zeaxanthin is a purified and esterified crystalline extract from the marigold *(Tagetes erecta)* flower. The meso-zeaxanthin concentrate contains approximately 70% meso-zeaxanthin. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table II.1.

Table II.1

Property/Parameter	Lutein/ meso-zeaxanthin
CAS Registry No.	127-40-2 / 31272-50-1
Chemical names	Xanthophyll; β, ε-carotene-3,3' / Xanthophyll; 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
Density	0.35-0.40 g/mL
Solubility in water at 5°C	Insoluble / insoluble

The structural formulas of lutein, meso-zeaxanthin and zeaxanthin are presented in Figures II.1.

Section II

Identity of the Notified Substance

Meso-zeaxanthin is a purified extract from the marigold *(Tagetes erecta)* flower. The meso-zeaxanthin concentrate contains approximately 60% meso-zeaxanthin, 5% RR-zeaxanthin and 15% lutein. The chemical structure of meso-zeaxanthin, zeaxanthin and lutein are presented in Figure II.1, while physical chemical characteristics of zeaxanthin and lutein are detailed in Table II.1.

Figure II.1

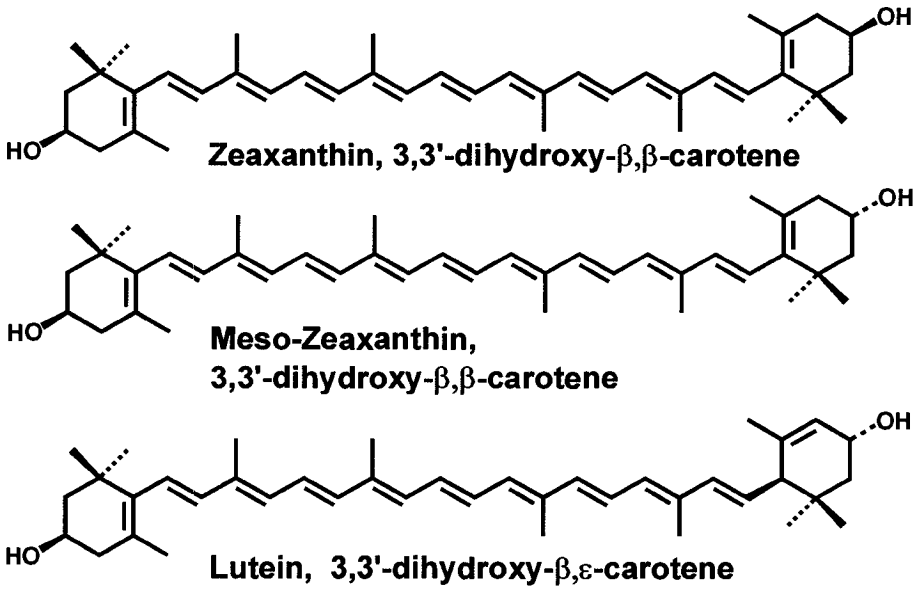

Table II. 1. Chemical and Physical Characteristics of Zeaxanthin and Lutein

Property/Parameter	Zeaxanthin	Lutein
CAS Registry No.	144-68-3	127-40-2
Chemical names	β, β -carotene-3,3'-diol	β, ε-carotene-3,3' –diol
Chemical isomers	1. (3R,3'R)-β, β-carotene-3,3'-diol; 2. (3R,3'S)-β, β-carotene-3,3'-diol (meso); 3. (3S,3'S)-β, β-carotene-3,3'-diol	No stereoisomers
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	207-215.5°C	177-178°C
Density	0.38-0.41 g/mL	0.35-0.40 g/mL
Solubility in water at	Insoluble	Insoluble

Table II. 1. Chemical and Physical Characteristics of Zeaxanthin and Lutein

Property/Parameter	Zeaxanthin	Lutein
5°C		

*Based on information provided by IOSA (2012) and other publicly available sources.

Specifications for Hi-Fil Z are presented in Table II.2

Table II.2
Specifications of Meso-Zeaxanthin Product (Hi-FIL Z™)*

Parameters	Specifications	Methods
Appearance	Orange red powder	Visual
Odor	Characteristic	Organoleptic
Taste	Characteristic	Organoleptic
Moisture (%)	< 5	Karl Fisher
Carotenoids		
Zeaxanthin (%)	65 (minimum)	UV-Vis/HPLC
Lutein (%)	15 (minimum)	UV-Vis/HPLC
R,R-zeaxanthin (%)	5.0 (maximum)	UV-Vis/HPLC
Heavy Metals		
Lead (ppm)	< 1.0	Atomic absorption (AA)
Arsenic (ppm)	< 1.0	A.A
Mercury (ppm)	< 1.0	A.A
Cadmium (ppm)	< 1.0	A.A
Residual solvents		
Methanol (ppm)	50	Gas Chromatography (GC)
Hexane (ppm)	50	GC
Acetone (ppm)	100	GC
Microbiology Assay:		
Total plate count (cfu/g)	<1000	NOM092-SSA1-1994
Yeast and molds (cfu/g)	<100	NOM111-SSA1-1994
Escherichia coli	Negative	NOM113-SSA1-1994
Salmonella	Negative	NOM114-SSA1-1994

*Based on information provided by IOSA (2012); NOM = NORMA OFICIAL MEXICANA (Official Mexican Standard); SSA = SECRETARIA DE SALUBRIDAD Y ASISTENCIA (Mexican Health Secretariat)

Manufacturing Process

As outlined in Figure II.2, meso-zeaxanthin rich extract (Hi-FIL Z™) is manufactured according to current good manufacturing practices (cGMP). The manufacturing process starts with marigold (*T. erecta*) petals as the raw material. The petals are washed with water, pressed mechanically to remove any excess water and dried with air. The dried petals are ground, then pelletized with steam and the pellets are cooled. The pellets are extracted with hexane followed by evaporation under high vacuum and heat leading to the formation of marigold oleoresin. The purification process starts by treating the marigold oleoresin with diluted alkali solution, followed by diluted acid solutions to eliminate impurities and to remove unwanted fatty acids, waxes, gums and other plant materials from the oleoresin. Following this, the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls and also cause isomerization.

The pH of the isomerized extract is adjusted and the product is washed with water followed by extraction with a solvent blend (hexane, acetone and methanol). The solvent mixture is removed from the oleoresin via evaporation under high vacuum and heat. The residue is filtered and dried to remove water, then screened to collect the meso-zeaxanthin rich extract. In the final step meso-zeaxanthin rich product (Hi-FIL Z™) is packed under inert nitrogen atmosphere in food-grade plastic pouches.

The residual concentration of solvents in the final extract is as follows: hexane < 50 ppm, acetone <100 ppm, methanol <50 ppm. Heavy metal levels and microbiological contaminants are checked to meet the specifications (Table II.2). The meso-zeaxanthin rich concentrate is routinely checked for common pesticide residues to make sure that none are detected at detection limits of < 0.01 mg/kg. Processing aids such as potassium hydroxide and the solvents (hexane, acetone, methanol) used during the manufacturing process are food grade quality as specified in the Food Chemical Codex (5th Edition).

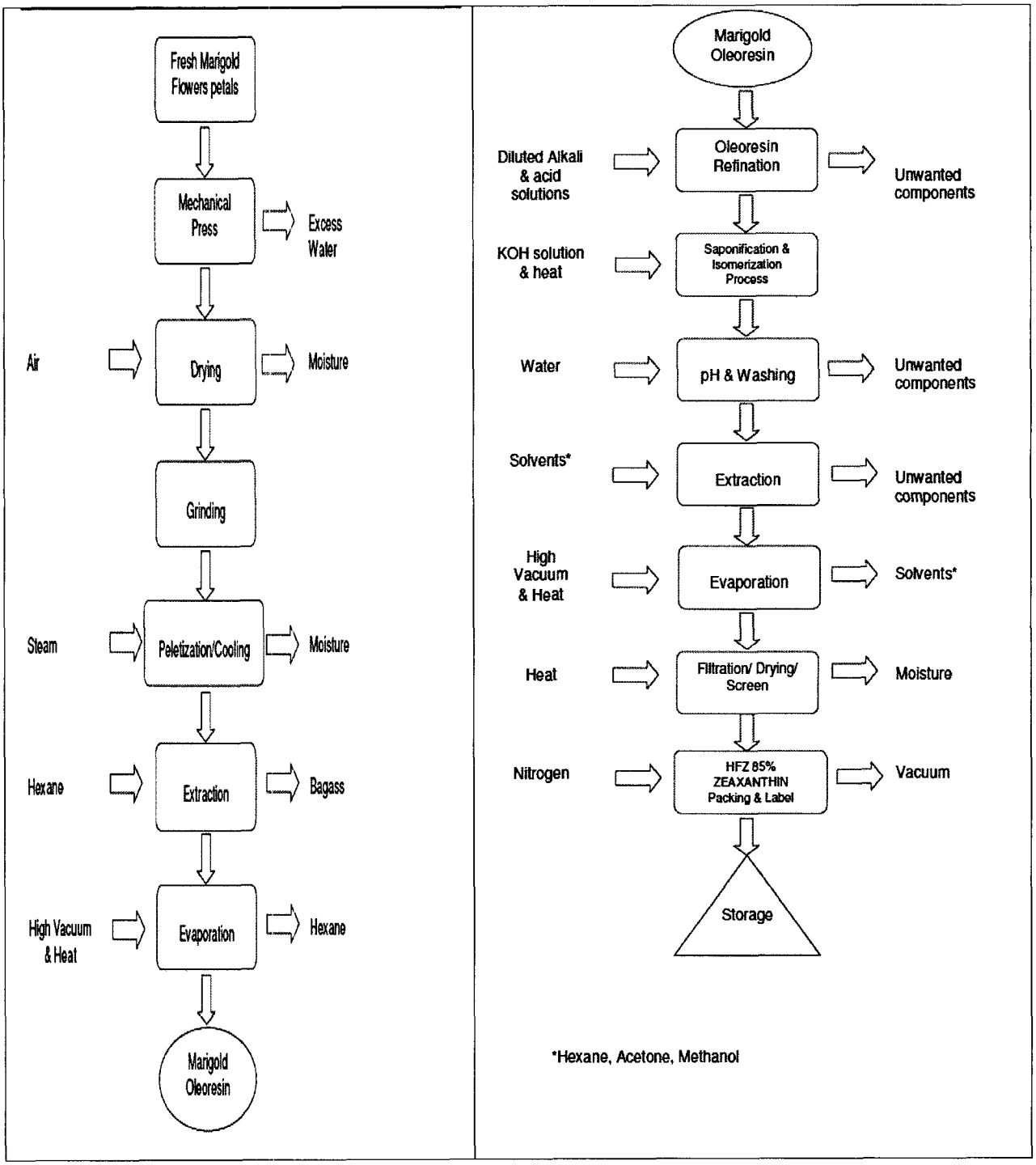

Figure II.2. Manufacturing Process of Meso-Zeaxanthin (Hi-FIL Z™) (IOSA, 2012)

Section III

Summary of the Basis for the Notifier's Determination that Meso-zeaxanthin is GRAS

As discussed in the Expert Opinion, meso-zeaxanthin is expected to dissociate in the human body resulting in free lutein. Therefore, it is reasonable to compare IOSA's meso-zeaxanthin product with non-esterified lutein products that have been the subject of previous GRASN's. IOSA's meso-zeaxanthin product is intended for use in the same foods and at levels equivalent to the lutein addition levels specified by Kemin Foods for crystalline lutein in GRASN 000140.

The proposed food uses are as a food ingredient and as a dietary source of lutein and zeaxanthin in foods such as 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. Since meso-zeaxanthin containing product will be used in place of other lutein/zeaxanthin products, not in addition to those products, the concentration of lutein/zeaxanthin in the individual foods will not increase and the proposed use of meso-zeaxanthin will not affect the total dietary intake of lutein/zeaxanthin.

Using the dietary analysis presented by Kemin in GRASN 000140, IOSA concludes that the mean all-user intakes of lutein and zeaxanthin from the consumption of proposed food-uses will be 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 will remain 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.

An independent panel of recognized experts, qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by IOSA to determine the Generally Recognized As Safe (GRAS) status of meso-zeaxanthin. A comprehensive search of the scientific databases for safety and toxicity information on meso-zeaxanthin, zeaxanthin and lutein was conducted through June 2013. Additionally, safety and regulatory evaluations by national and international agencies were also searched and considered for the present assessment. Based on a critical evaluation of the pertinent data and information summarized herein and employing scientific procedures, the Expert Panel members have individually and collectively determined that the addition of meso-zeaxanthin to the specified foods such as 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, soft candy, and soups and soup mixes (Table I.1.), when not otherwise precluded by a Standard of Identity, meeting the specification cited above and manufactured in according with current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use, as specified herein.

In response to six separate GRAS notifications on lutein, FDA did not question the safety of lutein for the specified food uses. Along with lutein, the presence of R,R-zeaxanthin and meso-zeaxanthin (GRN 385) was also noted in these notices. The subject of this GRAS determination is similar to these GRAS notices particularly as regards the presence of lutein and R,R-zeaxanthin. It is also similar to one of the notices (GRN 385) as regards the presence meso-zeaxanthin. The FDA responses to the GRAS notifications on lutein products indicate that the agency is satisfied with the safety-in-use of the lutein, as long as consumption is below 20 mg/person/day.

In coming to the conclusion that meso-zeaxanthin-rich extract is GRAS, IOSA relied upon scientific studies that showed that neither meso-zeaxanthin, nor any of their degradation products pose any toxicological hazards or safety concerns at the intended levels of use and on published toxicology studies and other articles relating to the safety of the product. The Expert Panel convened by IOSA also concluded that the intended use of meso-zeaxanthin is GRAS. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

APPENDIX I

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MESO-ZEAXANTHIN (Hi-FIL Z™) AS A FOOD INGREDIENT

Olsson Frank Weeda Terman Matz PC
600 New Hampshire Ave NW
Washington D.C., 20037
USA

Prepared for
Industrial Organica S.A de C.V.
Ave. Almazán No. 100 Col. Topo Chico, Zip Code: 64260
Monterrey, N.L.
Mexico

Panel Members

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

July 2013

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MESO-ZEAXANTHIN (Hi-FIL Z™) AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MESO-ZEAXANTHIN (Hi-FIL Z™) 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 Olsson Frank Weeda Terman Matz PC at the request of Industrial Organica S.A de C.V. (IOSA), to determine the Generally Recognized As Safe (GRAS) status of meso-zeaxanthin-rich extract (Hi-FIL Z™) as a food ingredient 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, soft candy, and soups and soup mixes] at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on zeaxanthin and lutein was conducted through June 2013 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by IOSA and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on July 22, 2013 and unanimously agreed to the decision described herein.

1.1. Background

In recent years, carotenoids have received considerable attention in the scientific community because of their powerful antioxidant properties, protecting the cells of the body from damage caused by free radicals. Lutein and zeaxanthin are two of the most abundant carotenoids in the North American diet (IOM, 2000). The eyes are considered as repositories for carotenoids with lutein and zeaxanthin concentrated in the retina and lens. Macular pigment, initially identified as a combination of lutein and zeaxanthin, was subsequently shown to be characterized by the presence of specific stereoisomers of these two carotenoids. While lutein is present as a single stereoisomer, zeaxanthin exists as three stereoisomers called (R,R-), (S,S-) and (R,S- or meso-) zeaxanthin. All three occur in nature and also in macular pigment. Available evidence indicates that higher dietary intake of lutein and zeaxanthin is related to reduced risk of cataracts and age-related macular degeneration (AMD). Lutein and zeaxanthin occur in high concentrations 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).

Zeaxanthin is the major carotenoid in the macula and low serum zeaxanthin concentrations is inversely correlated with the risk of AMD. It has been also reported that meso-zeaxanthin provides slightly better protection against lipid membrane oxidation than zeaxanthin (Bhosale and Bernstein, 2005). As zeaxanthin, including the meso-isomer and lutein, have the ideal anatomic, optical and biochemical properties to confer protection against AMD, supplements containing these macular carotenoids have been recommended by eye-care

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

professionals for the last decade. Studies have confirmed their benefit in terms of visual improvement and morphological progression of the condition (Bone et al., 2011a). Given the synergistic antioxidant properties of lutein, R,R-zeaxanthin and meso-zeaxanthin, and that meso-zeaxanthin is a major carotenoid component in the foveal center where a lack of macular pigment is associated with increased risk of AMD, it can be concluded that a supplement must contain all three macular carotenoids. For this reason, IOSA intends to supply a blend of meso-zeaxanthin, R,R-zeaxanthin and lutein (Hi-FIL Z™) for use in a limited number of conventional foods as a dietary ingredient.

1.2. Description

Meso-zeaxanthin-rich extract (Hi-FIL Z™), the subject of this GRAS assessment, is obtained from a natural carotenoid source, a variety of Aztec Marigold flowers (*Tagetes erecta* L). The extract is an orange red powder with a characteristic taste and odor. The extract contains zeaxanthin isomers (referred to as R,R- and meso-zeaxanthin in this document) and lutein. The structural formulas of zeaxanthin (R,R- and meso-) and lutein are presented in Figure 1. The extract will be marketed under the names: Hi-FIL Z™ containing 65% zeaxanthin (~60% meso-zeaxanthin, 5% R,R-zeaxanthin) and 15% Lutein. Food grade antioxidant (Vitamin E- 0.5%) is added to the marketed product (Hi-FIL Z™) in accordance with good manufacturing practices. General descriptive parameters and properties of zeaxanthin and lutein are summarized in Table 1.

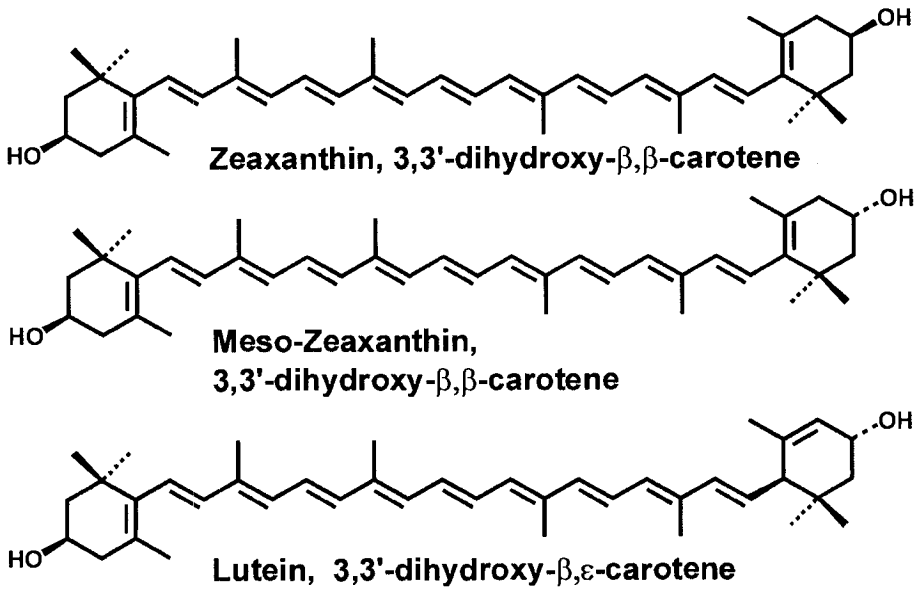

Figure 1. Chemical Structure of Zeaxanthin and Lutein
Zeaxanthin, 3,3'-dihydroxy-β,β-carotene is synonymously referred to as 3R,3'R Zeaxanthin, while meso-Zeaxanthin, 3,3'-dihydroxy- β,β-carotene is referred as 3R,3'S meso-Zeaxanthin

000017

Table 1. Chemical and Physical Characteristics of Zeaxanthin and Lutein

Property/Parameter	Zeaxanthin	Lutein
CAS Registry No.	144-68-3	127-40-2
Chemical names	β, β -carotene-3,3'-diol	β, ε-carotene-3,3' –diol
Chemical isomers	1. (3R,3'R)-β, β-carotene-3,3'-diol; 2. (3R,3'S)-β, β-carotene-3,3'-diol (meso); 3. (3S,3'S)-β, β-carotene-3,3'-diol	No stereoisomers
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	207-215.5°C	177-178°C
Density	0.38-0.41 g/mL	0.35-0.40 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

*Based on information provided by IOSA (2012) and other publicly available sources.

1.3. Specifications and Identity

Typical food grade specifications of Hi-FIL Z™, a product rich in meso-zeaxanthin, have been established by IOSA and are presented in Table 2. Analytical results from four non-consecutive lots are provided in Appendix I, which demonstrate that meso-zeaxanthin preparation (Hi-FIL Z™) is consistently manufactured to meet the standard specifications, except for hexane. For three of the four batches (Appendix I) the hexane levels are higher than the standard specification of 50 ppm. However, for all future batches IOSA assures that the hexane specification will be below 50 ppm. IOSA plans to market the meso-zeaxanthin rich extract primarily as a powder (trade name Hi-Fil Z™), beadlet, or as a suspension in a food oil, e.g., corn oil or soybean oil. The final products may also include other FDA-compliant materials such as maltodextrin or modified corn starch. Typical compositional analysis of meso-zeaxanthin-rich product (Hi-FIL Z™) with the addition of antioxidant is provided in Table 3. The residual solvent levels for methanol, hexane and acetone are low and considered as safe. These solvents are commonly used in the production of food ingredients.

Table 2. Specifications of Meso-Zeaxanthin Product (Hi-FIL Z™)*

Parameters	Specifications	Methods
Appearance	Orange red powder	Visual
Odor	Characteristic	Organoleptic
Taste	Characteristic	Organoleptic
Moisture (%)	< 5	Karl Fisher
Carotenoids		
Zeaxanthin (%)	65 (minimum)	UV-Vis/HPLC
Lutein (%)	15 (minimum)	UV-Vis/HPLC
R,R-zeaxanthin (%)	5.0 (maximum)	UV-Vis/HPLC
Heavy Metals		
Lead (ppm)	< 1.0	Atomic absorption (AA)
Arsenic (ppm)	< 1.0	A.A
Mercury (ppm)	< 1.0	A.A
Cadmium (ppm)	< 1.0	A.A
Residual solvents		
Methanol (ppm)	50	Gas Chromatography (GC)
Hexane (ppm)	50**	GC
Acetone (ppm)	100	GC
Microbiology Assay:		
Total plate count (cfu/g)	<1000	NOM092-SSA1-1994
Yeast and molds (cfu/g)	<100	NOM111-SSA1-1994
Escherichia coli	Negative	NOM113-SSA1-1994
Salmonella	Negative	NOM114-SSA1-1994

*Based on information provided by IOSA (2012); NOM = NORMA OFICIAL MEXICANA (Official Mexican Standard); SSA = SECRETARIA DE SALUBRIDAD Y ASISTENCIA (Mexican Health Secretariat); ** IOSA has decided that the hexane specifications for all future batches will be < 50 ppm.

Table 3. Typical Compositional Analysis of Meso-Zeaxanthin-rich product (IOSA, 2012)*

COMPOUNDS	Concentrate (% weight)
Zeaxanthin	62.00
Lutein	15.00
R,R-Zeaxanthin	3.00
Total Fat	9.51
Waxes**	1.77
Total Carbohydrate	3.22
Proteins	0.00
Moisture	5.00
Ash	0.50

*Based on information provided by IOSA (2012);
**Sterols, Hydrocarbons, pigments, waxes

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1.4. Manufacturing Process

Meso-zeaxanthin rich extract (Hi-FIL Z™) is manufactured according to current good manufacturing practices (cGMP), as outlined in Figure 2 at IOSA's Monterrey, Mexico plant. The manufacturing process starts with marigold (*T. erecta*) petals as the raw material. The petals are washed with water, pressed mechanically to remove any excess water and dried with air. The dried petals are ground, then pelletized with steam and the pellets are cooled. The pellets are extracted with hexane followed by evaporation under high vacuum and heat leading to the formation of marigold oleoresin. The purification process starts by treating the marigold oleoresin with diluted alkali solution, followed by diluted acid solutions to eliminate impurities and to remove unwanted fatty acids, waxes, gums and other plant materials from the oleoresin. Following this, the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls and also cause isomerization.

The pH of the isomerized extract is adjusted and the product is washed with water followed by extraction with a solvent blend (hexane, acetone and methanol). The solvent mixture is removed from the oleoresin via evaporation under high vacuum and heat. The residue is filtered and dried to remove water, then screened to collect the meso-zeaxanthin rich extract. In the final step meso-zeaxanthin rich product (Hi-FIL Z™) is packed under inert nitrogen atmosphere in food-grade plastic pouches. The residual concentration of solvents in the final extract is as follows: hexane <50 ppm, acetone <100 ppm, methanol <50 ppm. Heavy metal levels and microbiological contaminants are checked to meet the specifications (Table 2). The meso-zeaxanthin rich concentrate is routinely checked for common pesticide residues to make sure that none are detected at detection limits of < 0.01 mg/kg. Processing aids such as potassium hydroxide and the solvents (hexane, acetone, methanol) used during the manufacturing process are food grade quality as specified in the Food Chemical Codex (5th Edition).

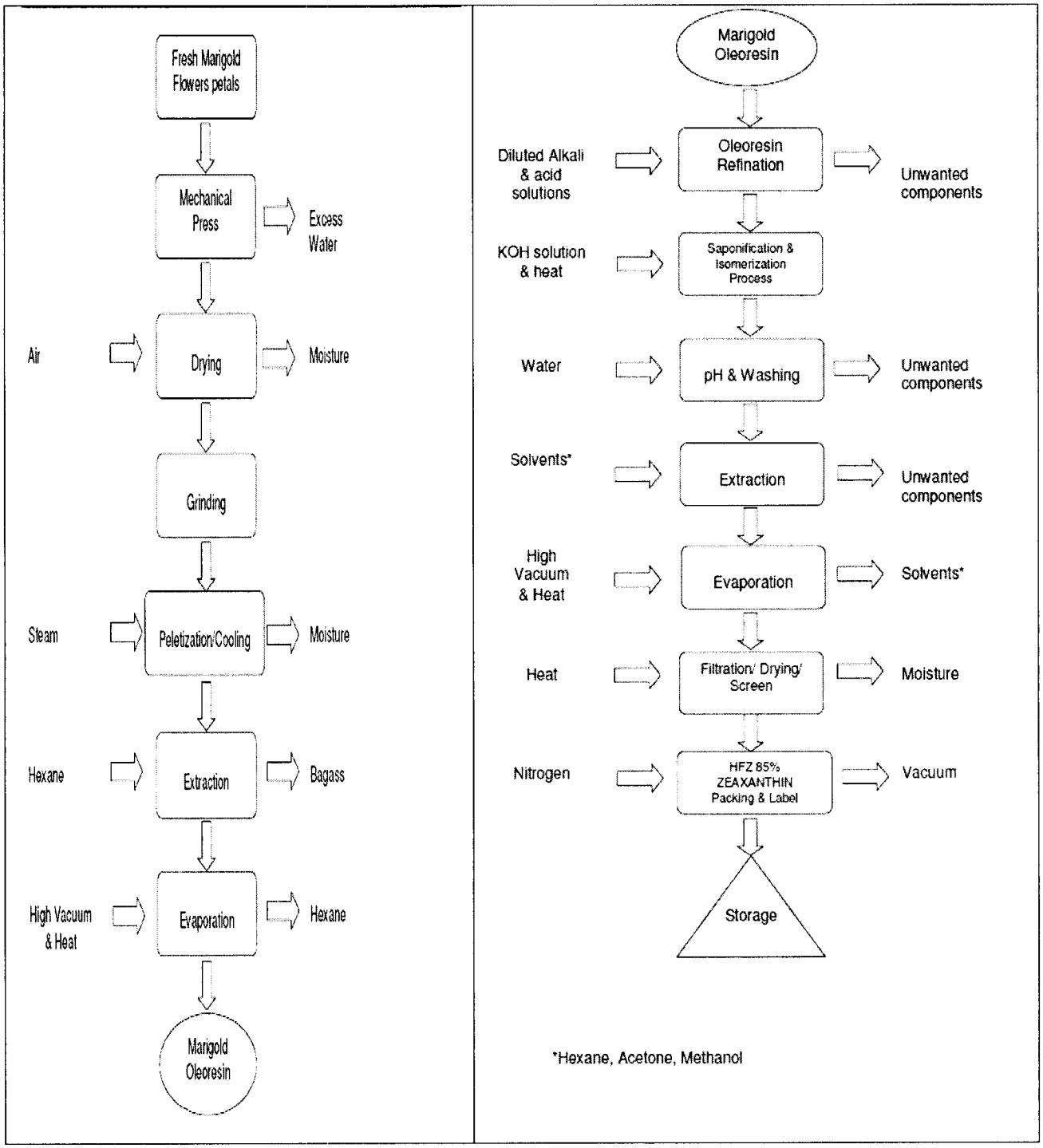

Figure 2. Manufacturing Process of Meso-Zeaxanthin (Hi-FIL Z™) (IOSA, 2012)

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1.5. Chemistry

The common structure in all carotenoids is a linear, conjugated chain of double bonds that is responsible for the brilliant red, orange and yellow pigments found in nature (Bone et al., 2011b). Lutein and zeaxanthin consist of the basic C40 isoprenoid structure common to all carotenoids along with two cyclic end groups, one β- and one α-ionone ring. The polyene chain double bonds 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. Zeaxanthin and its stereoisomers have identical chemical formulas to lutein but their absorption spectrum is shifted noticeably to the red. The structural formulas of lutein and two prominent zeaxanthin isomers are presented in Figure 1.

As mentioned earlier, zeaxanthin occurs primarily as a mixture of three isomers [(3R,3'R)-β, β-carotene-3,3'-diol; (3R,3'S)-β, β-carotene-3,3'-diol; (3S,3'S)-β, β-carotene-3,3'-diol] (Sajilata et al., 2008). In the literature, the two predominant zeaxanthin (R,R- and RS-) isomers are commonly referred to as zeaxanthin and meso-zeaxanthin, respectively (Bone et al., 2007). The hydroxyl groups on the 3 and 3' carbon atoms of the carotenoid end-groups are identical in the lutein and meso-zeaxanthin molecules. However, during the conversion of lutein to meso-zeaxanthin, there is a shift of one carbon-carbon double bond in the ε-ring of lutein. A corresponding change in optical activity occurs resulting in achirality also increasing the conjugation. Alternatively, meso-zeaxanthin may be formed from the metabolite, dehydrolutein via an enzymatic reduction pathway (Bone et al., 2007). Available evidence indicates that the keto-carotenoid canthaxanthin will undergo reduction in the human and primate retina lending credence to this possibility. However, in the plasma, dehydrolutein is formed from both lutein (Thurnham 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, the enzymatic reaction is the most likely formation route. Although R,R-zeaxanthin is the most commonly found isomer in the diet, the human macular pigment contains all three.

1.6. Occurrence and Uses

Over the years approximately 600 naturally occurring carotenoids have been identified (Moeller et al., 2000; Rice-Evans et al., 1997). Lutein and its esters are found primarily in green leafy vegetables such as spinach, lettuce, and broccoli. They are also found in maize, carrots, green peppers and peas), yellow orange fruits, yellow orange vegetables and egg yolks. The presence of lutein and zeaxanthin has been reported in human breast milk (GRN 000110; FDA, 2003). The presence of lutein in human milk ranges from 3 to 232 μg/L (Canfield et al., 2003). 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). Bone et al. (2011b) reported that birds produce meso-zeaxanthin and concentrate it along with other carotenoids in their retinas within brightly colored oil-droplets. In chicken retinas, 47% of the total zeaxanthin is meso-zeaxanthin and in turkeys it is 28%. In a recent scientific opinion on the substantiation of health claims related to meso-zeaxanthin, the European Food Safety Authority (EFSA) Panel stated, "Meso-zeaxanthin is a dietary carotenoid and is measurable in foods by established methods" (EFSA, 2010). It has been demonstrated that macular pigment is composed

principally of three isometric carotenoids, lutein, R,R-zeaxanthin and meso-zeaxanthin, present in the approximate proportions 2:1:1 in the retina. These three pigments represented 72% of the total carotenoid content in the eye (Thurnham, 2007).

The presence of meso-zeaxanthin is reported in food sources such as shrimp carapace, fish skin, and turtle fat (Maoka et al., 1986; Connolly et al., 2010a). Meso-zeaxanthin was found in the skin of commonly consumed fish such as tilapia, catfish, halibut, and Alaska Pollock. Some of these sources from marine animals are parts of the animal that are rarely consumed. However, the carotenoids in these sources are fat-soluble and may dissolve in oil during cooking, get transferred, and become part of the food. This results in the consumption of meso-zeaxanthin along with the shrimp, fish, or turtle meat. In Mexico, meso-zeaxanthin (as part of a natural lutein and zeaxanthin mixture) is used in chicken feed to improve the color of chicken flesh and egg yolks (Bone et al., 2007; Thurnham, 2007). As a result of this, meso-zeaxanthin is found in significant quantities in the resultant poultry products, which are used for human food. Thurnham (2007 reported that there is a wide variation in xanthophyll pigments in eggs, with values ranging from 1.4 to 41·5 μg/g egg yolk for zeaxanthin and 1·9 to 129 μg/g egg yolk for lutein assuming a yolk size of 17 g.

Both lutein and zeaxanthin in free and esterified forms are marketed as dietary supplements individually or along with other dietary supplements. Compared to lutein, the amount of zeaxanthin in these products is considerably low. In recent years, supplements containing meso-zeaxanthin have been marketed. In addition to its use as a dietary supplement, meso-zeaxanthin as part of lutein formulation is also used as a food ingredient in some selected foods (FDA, 2011). In the lutein GRAS Notice 385, OmniActive Health Technologies Ltd. reported that the lutein blend that was the subject of the notice contains 13.5% zeaxanthin isomers R,R- and meso-, at a 50:50 ratio. In this notice, the resulting 90th percentile meso-zeaxanthin intake was reported as 1.2 mg/person/day (FDA, 2011). This notice received a "no questions" letter from FDA. In a New Dietary Ingredient Notification on zeaxanthin that was accepted and filed by FDA (2001), the dietary ingredient in beadlet form was reported to contain approximately 2 % meso-zeaxanthin.

1.7. Intended Technical Effects

Meso-zeaxanthin rich extract (Hi-FIL Z™) derived from Marigold flowers (*T. erecta* L) is intended for addition to a limited number of conventional foods as a nutritional ingredient to provide a supplementary source of meso-zeaxanthin in consumers' diets. Its use is intended for the general population 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; Milk Products; Processed Fruits and Fruit Juices; Soft Candy; Soups and Soup Mixes at the levels identified in this document. IOSA does not intend for meso-zeaxanthin to be used as a component of foods that are intended to be consumed by infants and toddlers, such as infant formulas, or in meat and poultry. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, IOSA does not intend to refer to them by the commonly recognized names such as milk, or yogurt.

While the use of meso-zeaxanthin-rich extract (Hi-FIL Z™) in the food categories listed above may impart a color to the product, Hi-FIL Z™ is not a "color additive" as defined at § 70.3(f). Hi-FIL Z™ is solely intended for a purpose other than coloring, *i.e.,* to provide

consumers with a supplementary source of meso-zeaxanthin. It is not intended to color the foods to which it has been added, and any color imparted is "unimportant insofar as the appearance, value, marketability or consumer acceptability is concerned." Thus, meso-zeaxanthin is exempt from the definition of "color additive" under 21 CFR 70.3(g).

1.8. Intended Use Levels and Food Categories

The meso-zeaxanthin-rich extract (Hi-FIL Z™) 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, milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes at use levels of 0.3 to 3 mg meso-zeaxanthin/serving (reference amounts customarily consumed, 21 CFR 101.12). The intended uses are in the same foods and at levels proportional to those notified by Kemin Foods for the FloraGLO® Crystalline Lutein in the GRN 140 (FDA, 2004). Unlike GRN 140, egg products and soup and soup mixes that also come under USDA jurisdiction are not among the food categories for the present GRAS determination. In addition, this Notice does not include the addition of meso-zeaxanthin to infant formula and other infant and toddler foods.

The ratio of lutein to zeaxanthin in GRN 140 is different than the ratio in the subject of present GRAS assessment. However, given that the compounds will be used in the same food products the previous intake assessment can be used to estimate the dietary intake of meso-zeaxanthin by correcting for the different concentrations of meso-zeaxanthin and lutein. In GRN 140, FloraGLO® Crystalline Lutein was reported to contain >74% lutein and between 2% and 9% zeaxanthin; meso-zeaxanthin-rich extract (Hi-FIL Z™) contains >65% zeaxanthin and >15% lutein. It can be assumed that the combined lutein and zeaxanthin content in both these products is approximately 80%. The only significant difference is in the ratio of the lutein and zeaxanthin.

In GRN 140 (FDA, 2004), the combined intake of lutein and zeaxanthin from all applications described in the notification for mean and 90th percentile all-users was estimated to be 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 an 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 noted above, the ratio or compositional contents of lutein to zeaxanthin in GRN 140 and the subject of the present GRAS assessment are different. Based on the above information and the assumption that HI-FIL Z™ will be used at the same level as FloraGlo®, the mean and 90th percentile zeaxanthin intake from the use of Hi-FIL Z™ will be 6.24 and 11.46 mg/person/day, respectively. Since Hi-FIL Z™ contains 60% meso-zeaxanthin and 5% R,R-zeaxanthin, the 90th percentile intake of these isomers will be 10.58 and 0.88 mg/person/day, respectively. Hi-FIL Z™ also contains 15% lutein; therefore, the 90th percentile lutein intake

from the proposed uses will be 2.57 mg/person/day. For safety assessment purposes the 90th percentile meso-zeaxanthin intake from the proposed uses is considered as 10.58 mg/person/day.

2. SAFETY RELATED STUDIES

2.1. Preamble

In recent years, lutein and zeaxanthin has become the subject of investigations for their potential health benefits. There has been a significant effort by researchers to elucidate the biological role of these carotenoids. The safety of lutein that also contains small amount of zeaxanthin has been extensively investigated by researchers and extensively reviewed by national and international regulatory agencies such as FDA, EFSA, JECFA, etc. In recent years, FDA has reviewed six GRAS notices on lutein (containing small amounts of zeaxanthin). FDA did not question the acceptability and suitability of the available evidence to support the proposed uses in these notices. In the first notice, the acceptable daily intake of lutein was determined as 20 mg free lutein/person/day. This suggests that FDA is comfortable with the conclusion that the consumption of 20 mg/person/day of free lutein is safe.

In one of the recent GRAS notifications (GRN 385), the subject of the notification also contained meso-zeaxanthin (about 7%) resulting in daily estimated intake of 1.2 mg meso-zeaxanthin/person/day. This also suggests that FDA is comfortable with the conclusion that the consumption of lutein with daily intake of meso-zeaxanthin at low levels is safe. Meso-zeaxanthin was not identified or reported in the other GRAS notices. The fact that meso-zeaxanthin content was not reported in the other GRAS notices does not mean that it was not present. As meso-zeaxanthin is likely to be formed during processing/manufacturing, it is probable that small amounts of meso-zeaxanthin may be present. The subject of the present GRAS assessment contains 60% meso-zeaxanthin, 5% R,R-zeaxanthin and 15% lutein resulting in the daily 90th percentile estimated intake of 10.58, 0.88 and 2.57 mg/person/day, respectively. The intake of lutein and R,R-zeaxanthin from the proposed uses is lower than that accepted by FDA in the notifications. Although FDA has accepted a notice that reported low intake of meso-zeaxanthin (1.2 mg/person/day) is safe, the relatively high intake from the proposed uses of this isomer needs further discussion and support. The EFSA (2010) document on Scientific Opinion on the substantiation of health claims related to meso-zeaxanthin and maintenance of vision stated, "In the human clinical trials, a dosage of 14.9 mg per day of meso-zeaxanthin has been administered for 120 days, without any adverse effects. Rat studies with more than 200 mg per kg per day have shown no – observed-adverse-effect (NOAEL). Ames tests have shown that meso-zeaxanthin is anti-mutagenic" (Bone et al., 2007). Generally, high levels of dietary carotenoid are fed in these studies to achieve adequate tissue levels. Thus, relevance to human must be considered with caution.

Given all this, the following section briefly describes the safety of lutein and R,R-zeaxanthin. The safety data of meso-zeaxanthin is discussed at length to support its intake from the intended uses.

2.2. Safety of Lutein and R,R-Zeaxanthin

2.2.1. FDA, JECFA Excerpt and Discussion

Since 2003, the FDA has received six separate GRAS notifications on lutein [lutein ester-GRN 110 (FDA, 2003); crystalline lutein- GRN 140 (FDA, 2004); suspended lutein- GRN 221 (FDA, 2007); GRN 291 (FDA, 2009); GRN 385 (FDA, 2011); GRN 390 (FDA, 2012)]. Among

these GRAS notices, GRN 291 was submitted by IOSA, the manufacturer of Hi-FIL Z™, the subject of this GRAS assessment. In all of these submissions, extensive data from published literature on lutein and zeaxanthin in free and esterified form was presented. The FDA did not question the acceptability and suitability of the available evidence to support the use of lutein ester, crystalline lutein, and suspended lutein all of which contained small amounts of zeaxanthin (primarily R,R-isomer). Additionally, JECFA has also extensively reviewed the biological data on lutein containing small amounts of zeaxanthin. As the subject of present GRAS assessment also contains small amounts of lutein and R,R-zeaxanthin, studies described in these notifications are directly applicable to the present GRAS determination, particularly with regards to the safety of lutein and R,R-zeaxanthin.

In these GRAS notices, the safety of lutein containing small amounts of R,R-zeaxanthin has been established through toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. Additionally, the safety is corroborated by additional studies conducted with other sources of lutein, lutein-rich foods, and lutein supplements. Furthermore, historical consumption of eggs and fruits and vegetables where these carotenoids predominate (*e.g.*, green leafy vegetables, such as spinach and kale) confirm the safety of lutein and zeaxanthin. Additional studies published subsequent to the FDA GRAS notices continue to support safety of lutein that also contains small amounts of R,R-zeaxanthin as a food ingredient.

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

As the safety of lutein containing products has been extensively presented and discussed in the six GRAS notices to FDA, it is instructive to review the FDA's review and responses to these notices on the use of lutein in conventional foods to support the safety-in-use from the proposed uses of Hi-FIL Z™. The available information suggests that the FDA has no questions regarding the safety of the lutein and R,R-zeaxanthin, provided consumption is below 20 mg/person/day. These assessments are applicable to the proposed use of Hi-FIL Z™, particularly to its constituents lutein and R,R-zeaxanthin. A summary of the data presented to FDA in these GRAS notices is summarized in Table 4. Based on the data submitted in these Notices, and supported by FDA's determination that it had no questions regarding the determinations in these Notices, it can be concluded that consumption of lutein and R,R-zeaxanthin from conventional foods that results in daily intake of up to 20 mg/person/day of lutein is safe. This determination is also supported by the JECFA lutein evaluation and the fact, as recognized in the literature, that lutein and R,R-zeaxanthin are common components of foods such as fruits and vegetables, and eggs that are known to contain this carotenoid.

Table 4. Summary of Regulatory Assessment of Lutein FDA GRAS notices and JECFA and Comparison with IOSA Product

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

*For an individual weighing 60 kg

2.3. Safety of meso-Zeaxanthin

2.3.1. Human Studies

Given its low presence in the human diet, meso-zeaxanthin has not been found to naturally occur in human blood. The presence of high levels of meso-zeaxanthin in the eye despite being a minor component of the diet supports the theory that it is produced from lutein. Initially this view was proposed on the basis of chemical reactions. Recent studies in monkeys support this hypothesis (Johnson et al., 2005). In this study, 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 detectable

meso-zeaxanthin in the macular pigment. These observations support the hypothesis that lutein is converted to meso-zeaxanthin in the body. The exact mechanism of the conversion remains to be elucidated. Available evidence suggests that a significant fraction of lutein in the central retina is converted into meso-zeaxanthin. In the center of the retina, the ratio of meso-zeaxanthin to lutein is the highest and approaches 1:1. Data from autopsy eyes indicate that the (lutein + meso-zeaxanthin): zeaxanthin ratio varies throughout the retina from 2:1 in the center, where most of the conversion occurs, to 3:1 in the periphery (Bone et al., 1997; Sajilata et al., 2008).

Bone et al. (2006) reported the serum concentrations of R,R-zeaxanthin and meso-zeaxanthin in two human subjects who were given a mixed suspension of lutein (5.5 mg), zeaxanthin (1.4 mg) and meso-zeaxanthin (14.9 mg) in soybean oil in gelatin capsules (5.5:1.4:14.9 mg/day, respectively; provided by IOSA) that was taken daily with a meal for 6 weeks. The serum concentrations of zeaxanthin and meso-zeaxanthin in one subject increased during this period by 0.043 and 0.044 mmol/l, respectively, and in the second subject by 0.133 and 0.145 mmol/l, *i.e.*, the increase in the serum concentrations were approximately equimolar in both subjects for the two isomers of zeaxanthin. The concentration of the meso-isomer was ten times the concentration of the R,R-isomer of zeaxanthin in the supplement. This suggests that the plasma uptake of meso-zeaxanthin in human subjects is only 10% of the R,R-form of zeaxanthin that is commonly present in the diet.

In an intervention study in human subjects, Thurnham et al. (2008) investigated the blood uptake of meso-zeaxanthin. In this study, volunteers (ten male and nine female) received one capsule containing 20 mg carotenoids consisting of lutein (10.8 mg), R,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 about 50% more R,R-zeaxanthin (0.088 mmol/l per mg) was actively retained by the body than lutein (0.056 mmol/L per mg) (although the difference was not significant in female subjects) 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 greater in females than males. These investigators also stated that the findings from their study indicate that the plasma responses from lutein and R,R-zeaxanthin were lower than the literature values of the pure substances. The uptake of these carotenoids appeared to be depressed slightly 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 to R,R-zeaxanthin probably indicates that meso-zeaxanthin is less well absorbed than R,R-zeaxanthin.

In a recent publication, Connolly et al. (2011) compared the differing serum carotenoid and macular pigment responses reported in several published studies. These investigators reported that the differences noted in the serum levels may be due to several factors, such as dose of carotenoids consumed per day, type of carotenoids in the supplement (*e.g.,* free versus ester), matrix in which carotenoids are consumed (*e.g.*, oil versus microencapsulated), whether the supplement was consumed alone or in the presence of other antioxidants, and noncompliance with the study supplement regimen.

Bone et al. (2007) studied macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. In this 4-month study, 19 volunteers participated. For this study, the first group of 10 participants were given gelatin capsules containing unesterified carotenoids (14.9 mg of meso-zeaxanthin, 5.5 mg of lutein and 1.4 mg of R,R-zeaxanthin) as a

suspension in soybean oil. The carotenoid mixture used in the study was produced by IOSA. A second group of 9 subjects were given gelatin capsules containing a placebo for the same 4 month period. Prior to and during the supplementation period, blood serum samples were analyzed by high performance liquid chromatography for carotenoid content. Similarly, macular pigment optical density was measured by heterochromatic flicker photometry. Supplementation with the carotenoids revealed the presence of all three carotenoids in the blood. Macular pigment optical density, measured at 460 nm, rose at an average rate of 0.59 ± 0.79 milli-absorbance unit/day in the 10 supplemented subjects. This was significantly different from the placebo group (9 subjects) for whom the average rate was -0.17 ± 0.42 milli-absorbance units/day. No adverse effects were reported by the investigators.

In a double-blind, randomized, placebo controlled trial published as abstract, Connolly et al. (2010b) investigated changes in macular pigment optical density (MPOD), and serum concentrations of the macular carotenoids in response to supplemental meso-zeaxanthin, lutein and R,R-zeaxanthin in healthy subjects. In this study, 44 healthy subjects (mean age = 43.54 ± 12.2) were recruited of which 22 subjects were randomized to consume a formulation containing 10.6 mg of meso-zeaxanthin, 5.9 mg of lutein and 1.2 mg of R,R-zeaxanthin (Intervention group), and 22 subjects consumed a placebo everyday over a six month period (Placebo group). At each study visit (Baseline, 3 months, and 6 months) the spatial profile of MPOD was measured and a blood sample was collected to analyze serum concentrations of lutein and zeaxanthin. A statistically significant increase in serum concentrations of lutein and zeaxanthin and MPOD in the intervention group at 3 and 6 month visit was noted. No adverse effects were reported by the investigators. This study was subsequently published as a complete publication (Connolly et al., 2011), along with additional safety-related data as described below.

The additional details published by Connolly et al. (2011) revealed the composition of two groups: intervention group (n = 22; male/female = 8/14; age 43±13 years; BMI = 27.2±6.1) and placebo group (n = 22; male/female = 9/13; age 45±12 years; BMI = 26.8±5). Subjects consumed one tablet per day containing 10.6 mg meso-zeaxanthin, 5.9 mg lutein, and 1.2 mg zeaxanthin or placebo for 6 months. The formulations used in this study were manufactured by IOSA. The spatial profile of MPOD and serum concentrations of lutein and zeaxanthin were quantified. Additionally, the safety of consumption was assessed by analyzing blood samples for changes in renal and liver function, as well as lipid profile, hematologic profile, and markers of inflammation after 6 months of supplementation. A significant increase in central MPOD and in serum levels of lutein and zeaxanthin was noted in the intervention group.

As regards clinical pathology parameters investigated, Connolly et al. (2011) reported a statistically significant variation from baseline to 6 months (in both positive and negative directions) in 8 of the 25 variables assessed in the intervention group and 9 of the 25 biochemical parameters analyzed in the placebo group. However, all parameters remained within the normal reference range, with the exception of total cholesterol and LDL, which had a baseline value outside the accepted normal reference range in both the groups before supplementation (i.e., at baseline). During the course of study, adverse events were also monitored by asking each subject regarding any effects arising from the consumption of the supplements. There were no adverse events recorded or reported by any subject taking part in the study after supplementation with all three macular carotenoids. The results of this 6 month clinical trial suggest that consumption of meso-zeaxanthin at levels up to 10.6 mg/day along with lutein and R,R-zeaxanthin is safe.

2.3.2. Animal and *In vitro* Safety Studies

2.3.2.1. Specific Studies with Hi-FIL Z™

In two studies, publicly available at the Howard Foundation website[2] for over 5 years and also published recently by Thurnham and Howard (2013), the subchronic toxicity and mutagenicity of meso-zeaxanthin were investigated.

In the subchronic toxicity study, conducted according to OECD and FDA Good Laboratory Practice regulations for such studies, the potential effects of meso-zeaxanthin containing extract were investigated (Chang, 2006; Thurnham and Howard, 2013). The extract used in this study was provided by IOSA and was reported to contain approximately 21% meso-zeaxanthin, 5.2% R,R-zeaxanthin and 6.7% lutein. In this study, meso-zeaxanthin extract dissolved in sunflower oil was administered (gavage) once daily at dose levels of 0, 2, 20 and 200 mg meso-zeaxanthin/kg bw/day (control, low, mid, and high dose group, respectively) for at least 90 days to Han Wistar rats (10/sex/group). Additional, five males and five females were allocated to each of control and high dose groups for recovery study. Cage-side observation (≥ twice daily), clinical signs (once weekly), and body weights (once weekly) were recorded during the course of study, while organ weights, hematology, clinical biochemistry, and urine analysis were performed at the end of the treatment (after 13 weeks) and recovery (after 17 weeks). Ophthalmologic examination was conducted prior to scheduled termination. At the end of the treatment period, macroscopic and microscopic observations were performed. All tissue samples from the control and high dose group animals euthanized following the treatment phase and the liver, kidneys, spleen, and stomach from low and mid dose group animals were processed and evaluated.

No mortality or clinical signs were observed during the dosing period of 90 days and recovery period of 28 days. No compound-related body weight changes were noted. No significant differences were noted in absolute body weight or total body weight change over the course of the study for either sex. Some statistically significant increases in weekly body weight changes were noted but changes were considered incidental and unrelated to treatment because the changes were infrequent, sporadic, and not dose related. Ophthalmologic evaluation did not reveal any compound-related findings. No compound-related changes in hematology, clinical chemistry, or coagulation were noted. A significantly higher alkaline phosphatase (ALP) activity for mid and high dose group male rats as compared to male controls was noted. Examination of the individual animal data revealed a moderate degree of variability in these values in all dose groups, including the controls, indicating that the differences between the controls and mid and high dose group male rats were most likely the results of individual animal variability rather than a compound effect. Total bilirubin concentration for low dose group female rats was significantly lower when compared to female controls; the difference was minimal in nature, inconsistent with a dose response, and neither biologically or toxicologically significant. Review of the data from recover group (day 119) data revealed significantly higher sodium, total protein, and globulin concentrations for high dose group male rats as compared to the male control rats. These differences were minimal in nature and had no biological or toxicological significance. Prothrombin time in recovery animals was significantly higher for high dose group 4 female rats

[2] http://www.howard-foundation.com/research.htm

when compared to female controls; the difference was minor and had no biological or toxicological significance.

At terminal necropsy, no compound-related macroscopic findings were noted. Some findings noted were considered incidental because they occurred infrequently, in both treated and control animals, exhibited no dose relationship, and/or are associated with normal female reproductive cycling events. Histopathological examinations of the tissue samples did not reveal any compound-related findings. Lesions considered to be spontaneous and incidental were observed in treated and control rats. Some microscopic observations seen only in compound-treated animals were also considered to be spontaneous due to incidence and severity. The results of this study suggest that daily oral administration of meso-zeaxanthin at doses of up to 200 mg/kg/day was well tolerated in rats. These observations also suggest that the NOAEL of meso-zeaxanthin in rats is >200 mg/kg/day when administered orally for 13 consecutive weeks (Thurnham and Howard, 2013).

In a mutagenicity study, 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 WP2*uvr*A) was evaluated (Mecchi, 2006; Thurnham and Howard, 2013). The extract used in this study was reported to contain approximately 49% meso-zeaxanthin, 16% R,R-zeaxanthin and 35% lutein. The assay design was based on OECD Guideline 471. The tester strains used were *S. typhimurium* tester and *E. 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) (Thurnham and Howard, 2013).

2.3.2.2. Other Studies with Meso-Zeaxanthin

In a series of studies, Xu et al. (2013) investigated a comprehensive safety profile of meso-zeaxanthin for its use as a food ingredient. Assays of acute toxicity, genetic toxicity (Ames test, mice bone marrow erythrocyte micronucleus and mice sperm abnormality) and 90-day sub-chronic toxicity were performed. The findings from these investigations are summarized below:

In the acute oral toxicity tests, maximum tolerable dose was found to be more than 10 g/kg bw in Sprague Dawley rats and ICR mice, and showed no toxicological signs during the period of the study (Xu et al., 2013).

For the mutagenicity studies as evaluated by Ames test, four histidine-deficient strains of *S. typhimurium* TA97, TA98, TA100, and TA102 were used and the assay was performed on supernatant S9 obtained from PCB-induced SD rat liver homogenate. The plate incorporation method was used in Ames test. The doses tested in the mutagenicity assay with all tester strains in the presence and absence of S9 mix were 40, 200, 1000, and 5000 µg per plate. The mean number of revertants per plate of meso-zeaxanthin treatment groups at all dose levels of four strains, TA97, TA98, TA100 and TA102, with or without S9 were negative. The results of Ames test indicate that meso-zeaxanthin has no genetic toxicity (Xu et al., 2013).

For the bone marrow erythrocyte micronucleus assay, 50 mice (5/sex/group) were divided into five groups randomly. The meso-zeaxanthin dose levels of testing groups were 1.25, 2.5 and 5.0 g/ kg bw, respectively. Cyclophosphamide (60 mg/kg bw) was administered as a positive control and distilled water was used as a negative control. Testing material were administered twice at 24 hours intervals and animals were euthanized 6 hours after last dose. Sternum bone was removed and the bone marrow cells were pulled out and processed. The micronucleus of the meso-zeaxanthin treatment groups at all dose levels and the negative control group were significantly lower than those in positive control group, confirming the validity of the trial. There were no significant differences of micronucleus between the negative control group and the meso-zeaxanthin treatment groups. The PCE/RBC ratio of each group was within normal range. The result from these experiment show that meso-zeaxanthin is not mutagenic at the tested dosage range (Xu et al., 2013).

For the sperm abnormality test, 35 male mice (7/group) were divided into five groups. Meso-zeaxanthin dose levels of testing groups were 1.25, 2.5, 5.0 g/kg bw. Mitomycin C (2.0 mg/kg bw) was used as a positive control and distilled water was used as a negative control. Intubations occurred daily for 5 days. Five mice were selected randomly in each group and euthanized 35 days after the first dose administration. Epididymis was isolated and processed for sperm abnormality. The sperm abnormality of the meso-zeaxanthin treatment groups at all dose levels had no significant differences as compared with the negative control group. The sperm abnormality ratio in all treatment groups and the negative control group was significantly lower than those in positive control group treated with Mitomycin C (Xu et al., 2013).

For 90-day feeding study of meso-zeaxanthin, Sprague Dawley rats were randomly divided into four groups (10/sex/group) and animals were orally treated with meso-zeaxanthin at dose levels of 0, 300, 600 and 1200 mg/kg bw/day for 13 consecutive weeks. During the course of study and at the end all standard parameters for such type of studies were measured. Compared with the control group, administration of meso-zeaxanthin concentrate did not result in any toxicologically significant treatment-related changes in clinical observations, body weights, body weight gains, feed consumption and organ weights. No toxicologically relevant findings were noted in hematology at midway (day 42) and termination (day 90) determinations. Among the clinical chemistry parameters, at midway determination AST levels only at high dose (1200 mg/ kg bw/day) groups of male rats had significant differences compared with the negative control group, while at termination none of the parameters showed any significant changes as compared to control (Xu et al., 2013).

At termination, necropsy and pathological examination revealed histological changes in liver cells with vacuolar degeneration increased significantly in 1200 and 600 mg/kg dose group, as well as those with inflammatory cell infiltration within liver lobule and spotted liver cell necrosis with inflammatory cell infiltration increased significantly in 1200 mg/kg dose group compared with the negative control group. However, other indexes had no significant toxicity in all the tested level. These findings suggest that meso-zeaxanthin administered at the tested dosage within the range of 600-1200 mg/kg for 90 days results in hepatotoxicity. However, at the dosage level of 300 mg/kg/day in both male and female SD rats, no noticeable toxicological effects were observed (Xu et al., 2013).

In summary, the results of investigations by Xu et al. (2013) suggest that meso-zeaxanthin has no acute toxicity (practically non-toxic), no genotoxicity (as evaluated by Ames test, mice bone marrow erythrocyte micronucleus and mice sperm abnormality) and the use of

meso-zeaxanthin is safe at dose of 300 mg/kg bw/day in rats from a 90-day feeding study. These investigators reported that after the application of a 100-fold safety factor, the ADI (acceptable daily intake) value of 3 mg/kg body weight per day can be obtained.

Firdous et al. (2011) investigated anti-mutagenic and anti-carcinogenic potentials of meso-zeaxanthin in *in vitro* and *in vivo* studies. In the *in vitro* anti-mutagenic activity experiments, Ames test (*Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 1535) with direct acting mutagens like sodium azide (NaN3) (5 μg/ plate), nitro-o-phenylendiamin (NPD) (20 μg/ plate), N-methyl- N'-nitro-N-nitrosoguanidine (MNNG) (1μg/ plate) and tobacco extract 50 mg/ plate) and with a mutagen needing microsomal activation, acetamidofluorene (AAF) (20 μg/ plate) was performed. Meso-zeaxanthin was found to inhibit the mutagenicity induced by NaN3, NPD and MNNG in a concentration dependent manner, as well as that with AAF and the tobacco extract. Concentrations needed for 50% inhibition was found to be 50 μg/plate for the chemical mutagens and 100 μg/ plate for the tobacco extract.

The *in vivo* anti-carcinogenicity activity of meso-zeaxanthin was evaluated using nitroso diethyl amine (NDEA) induced hepatocellular carcinoma in rats. For these studies, groups of male Wistar rats were treated (oral gavage) 5 days a week with NDEA alone or in combination with meso-zeaxanthin at two dose levels (50 and 250 mg/kg/day) for 20 weeks. As compared to group NDEA, treatment with meso-zeaxanthin reduced the tumor incidence. The activity of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and alkaline phosphatase was significantly elevated in both serum and liver tissue of NDEA alone treated control animals. Treatment with meso-zeaxanthin significantly reduced the increase in these enzyme levels. Histopathological findings from the liver supported the observation from liver marker enzymes. The results of these experiments suggest anti-mutagenic and anti-carcinogenic potentials of meso-zeaxanthin.

Firdous et al. (2011) also investigated the hepato-protective potential of meso-zeaxanthin against paracetamol (3 g/kg body wt, orally), 20% ethanol (7.5 g/kg body wt, orally) and CCl_4 (2.5 ml /kg, ip) in male Wistar rats. In the experiments with paracetamol and CCl_4, meso-zeaxanthin was administered daily at dose level of 50, 100, 250 mg/kg bw/day for five days prior to administration of paracetamol and CCl_4. In the ethanol experiments, meso-zeaxanthin was administered daily at dose level of 50, 100, 250 mg/kg bw/day to rats for 6 months along with ethanol. As compared to rats receiving hepatotoxicant alone, meso-zeaxanthin treatment revealed a dose-dependent decrease in levels of marker enzymes of hepatic injury such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase, and serum bilirubin. Histopathological examinations of liver tissue showed the hepatoprotective potential of meso-zeaxanthin. Although in these studies groups receiving only meso-zeaxanthin were not included, the results show protective action of meso-zeaxanthin against common hepatotoxicants.

3. SUMMARY AND DISCUSSION

IOSA intends to use meso-zeaxanthin-rich standardized extract (Hi-FIL Z™) obtained from a variety of Aztec Marigold flowers (*T. erecta* L) as a food ingredient at concentrations of up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21 CFR 101.12) in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit

juices, soft candy, and soups and soup mixes. Additionally, for the proposed GRAS assessment, IOSA excludes food categories such as egg products and soup and soup mixes that come under USDA. Meso-zeaxanthin rich product (Hi-FIL Z™) is an orange red powder with characteristic odor. Compositional analysis of the extract (Hi-FIL Z™) demonstrated that the product primarily contains meso-zeaxanthin. The other constituents include lutein, R,R-zeaxanthin, carbohydrate and fats. The intended use of meso-zeaxanthin-rich extract (Hi-FIL Z™) in the specified food categories will result in 90th percentile estimated daily intakes of 10.58 mg meso-zeaxanthin, 0.88 mg R,R-zeaxanthin and 2.58 mg lutein/person/day.

There is sufficient qualitative and quantitative scientific evidence as well as history of use evidence to determine the safety-in-use of the Hi-FIL Z™ product and its constituents in the above mentioned applications. As constituents of meso-zeaxanthin-rich extract are also present in some diets, the history of consumption of the components and its preparation support the safety-in-use. Lutein and its esters are found primarily in green leafy vegetables, yellow orange fruits, yellow orange vegetables and egg yolks. The presence of lutein and zeaxanthin has been reported in human breast milk. Meso-zeaxanthin, R,R-zeaxanthin and lutein are marketed as dietary supplements and their recommended doses range from 0.5 to 12 mg/day. Lutein and its ester has been the subject of four GRAS notices to FDA. The presence of meso-zeaxanthin has been reported as part of lutein in one these notices (GRN 385). In these submissions, use of lutein in specified food categories was estimated to result in levels of up to 13.4 mg/person/day and meso-zeaxanthin intake was estimated as 1.2 mg/person/day.

In response to six separate GRAS notifications on lutein, FDA did not question the safety of lutein for the specified food uses. Along with lutein, the presence of R,R-zeaxanthin and meso-zeaxanthin (GRN 385) was also noted in these notices. The subject of this GRAS determination is similar to these GRAS notices particularly as regards the presence of lutein and R,R-zeaxanthin. It is also similar to one of the notices (GRN 385) as regards the presence meso-zeaxanthin. The FDA responses to the GRAS notifications on lutein products indicate that the agency is satisfied with the safety-in-use of the lutein, as long as consumption is below 20 mg/person/day. Additionally, JECFA also completed a safety evaluation of lutein from *T. erecta* L. and assigned a group ADI of 0 to 2 mg lutein and zeaxanthin/kg body weight.

In a well designed dose-response subchronic toxicity study, meso-zeaxanthin-rich extract (Hi-FIL Z™) was administered (gavage) daily to rats at doses of 0, 2, 20 and 200 mg/kg body weight/day for 90 days. No treatment-related adverse effects on any of the series of parameters investigated were noted. Based on the results of this study, the NOAEL for meso-zeaxanthin is determined as > 200 mg/kg/day, the highest dose tested. The estimated daily intake of meso-zeaxanthin (10.58 mg/person/day or 0.18 mg/kg bw/day for an individual weighing 60 kg) from the intended food uses of Hi-FIL Z™ is over 1000 fold lower compared to the NOAEL determined from the subchronic toxicity study. In another subchronic toxicity study, treatment of rats with meso-zeaxanthin at doses of 300, 600 and 1200 mg/kg bw/day resulted in hepatotoxicity (as evaluated by histopathology examination) at two higher doses (600 and 1200 mg/kg bw/day). In this study, the NOAEL of meso-zeaxanthin was determined as 300 mg/kg bw/day. The findings from this study are consistent with the results of above described subchronic study, in which the NOAEL of meso-zeaxanthin was >200 mg/kg/day.

In a 6 month human study, administration of zeaxanthin-rich extract at a dose of 10.6 mg meso-zeaxanthin, 5.9 mg lutein, and 1.2 mg zeaxanthin did not reveal any adverse effects on hematological and clinical chemistry parameters including adverse event reports. The totality of

available evidence supports the safety of meso-zeaxanthin at the intended use levels. The estimated daily intake of meso-zeaxanthin from the intended uses of Hi-FIL Z™ is similar to that used in the human study that did not reveal any adverse effects.

In summary, considering the totality of the evidence, on the basis of scientific procedures[3], history of exposure and use, the consumption of meso-zeaxanthin-rich extract (Hi-FIL Z™) derived from Marigold flowers (*Tagetes erecta* L) as a food ingredient at use levels of 0.3 to 3 mg/serving in certain specified foods resulting in a 90th percentile intake of 10.58 mg meso-zeaxanthin, 0.88 mg R,R-zeaxanthin and 2.58 mg lutein/person/day is considered safe. The proposed uses are compatible with current regulations, *i.e.*, meso-zeaxanthin-rich extract (Hi-FIL Z™) is used as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes, when not otherwise precluded by a Standard of Identity, and is produced according to current good manufacturing practices (cGMP).

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

4. CONCLUSION

Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that consumption of meso-zeaxanthin-rich extract (Hi-FIL Z™) as a food ingredient 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, 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 in this monograph and resulting in the 90th percentile all-user estimated intake of 10.58 mg meso-zeaxanthin, 0.88 mg R,R-zeaxanthin and 2.58 mg lutein/person/day is safe.

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 meso-zeaxanthin-rich extract (Hi-FIL Z™), when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

Stanley T. Omaye, Ph.D., F.A.T.S. — 7/22/2013 Date

(b) (6)

Robert L. Martin., Ph.D. — 7/25/2013 Date

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. — 7/28/2013 Date

5. REFERENCES

Bhosale, P., Bernstein, P.S., 2005. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. Biochimica Biophysica Acta 1740:116-121.

Bone, R.A., Landrum, J.T., Friedes, L.M., Gomez, C.M., Kilburn, M.D., Menendez, E., Vidal, I., Wang, W., 1997. Distribution of lutein and zeaxanthin stereoisomers in the human retina. Experimental Eye Research 64:211-218.

Bone R.A., Landrum, J.T., Cao, Y., Howard, A.N., Alvarez-Calderon, F., 2007. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. Nutrition & Metabolism 4:12 doi:10.1186/1743-7075-4-12.

Bone, R., Landrum, J.T., Mendez, V., Valenciaga, A., Babino, D., 2011a. Comparison of dietary supplementation with lutein diacetate and lutein: Effects on macular pigment and serum. 16th International Symposium on Carotenoids, 17-22 July, 2011, Krakow, Poland. Acta Biologica Cracoviensia 53: 100.

Bone, R.A., Landrum, J.T., Beatty, S., Nolan, J., 2011b. Targeting AMD with a critical carotenoid: Supplementing meso-zeaxanthin may prove to be an important new approach to fighting macular degeneration. Review of Ophthalmology. Available online at: http://www.revophth.com/content/d/features/i/1448/c/27340/ (website visited on June 14, 2012).

Canfield, L.M., Clandinin, M.T., Davies, D.P., Fernandez, M.C., Jackson, J., Hawkes, J., Goldman, W.J., Pramuk, K., Reyes, H., Sablan, B., Sonobe, T., Bo, X., 2003. Multinational study of major breast milk carotenoids of healthy mothers. European Journal of Nutrition 42:133-141.

Chang, C.J.G., 2006. Thirteen-week oral (gavage) toxicity of *meso*zeaxanthin in Han Wistar rats with a 4-week recovery. Gene Logic Study Number: 1567-04370, 1-344. 2006. Gaithersburg, MD, Gene Logic Laboratories Inc. Report available at: http://www.howard-foundation.com/fullreport.pdf (website visited on June 14, 2012).

Connolly, E.E., Beatty, S., Loughman, J., Howard, A.N., Louw, M.S., Nolan, J.M., 2011. Supplementation with all three macular carotenoids: response, stability, and safety. Investigative Ophthalmology & Visual Science 52:9207-9217.

Connolly, E.E., Beatty, S., Thurnham, D.I., Loughman, J., Howard, A.N., Stack, J., Nolan, J.M., 2010a. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. Curr Eye Research 35(4):335-51.

Connolly, E., Beatty, S., Loughman, J., Nolan, J., 2010b. Meso-Zeaxanthin Ocular Supplementation Trial in Normals (MOST-N). Association for research in Vision and Ophthalmology (ARVO) annual conference. Published in Investigative Ophthalmology and Visual Science. 2010. Abstract available at: http://arrow.dit.ie/scschphycon/4/ (website visited on June 14, 2012).

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 (website visited on June 14, 2012).

Ettlin, R., Steiger, A., Hummler, H., 1980a. Tolerance study of zeaxanthin administered orally as a feed admixture to mice over 13 weeks. Unpublished report No. B-93'153 from F. Hoffmann-La Roche Ltd, Basle, Switzerland (Cited in JECFA, 2006).

Ettlin, R., Steiger, A., Hummler, H., 1980b. Tolerance study of zeaxanthin administered orally as a feed admixture to rats over 13 weeks. Unpublished report No. B-93'152 from F. Hoffmann-La Roche Ltd, Basle, Switzerland (Cited in JECFA, 2006).

FDA, 2003. GRAS Notice Inventory. GRN 000110. Lutein esters. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=110 (website visited on: June 8, 2012).

FDA, 2004. GRAS Notice Inventory. GRN 000140. Crystalline lutein. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=140 (website visited on: June 8, 2012).

FDA, 2007. GRAS Notice Inventory. GRN 000221. Suspended lutein. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=221 (website visited on: June 8, 2012).

FDA, 2009. GRAS Notice Inventory. GRN 000291. Suspended lutein. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=291 (website visited on: June 8, 2012).

FDA, 2011. GRAS Notice Inventory. GRN 000385. Lutein. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=385 (website visited on: June 8, 2012).

FDA, 2012. GRAS Notice Inventory. GRN 000390. Suspended lutein. FDA response and complete GRAS notice available at website: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=390 (website visited on: June 8, 2012).

FDA, 2001. New Dietary Ingredient Notification. Zeaxanthin. Roche Vitamins Inc. FDA Memorandum and complete NDI notice available at website: http://www.fda.gov/ohrms/dockets/dailys/01/Sep01/090601/rpt0096.pdf (website visited on: June 8, 2012).

Firdous, A.P., Sindhu, E.R., Kuttan, R., 2011. Hepato-protective potential of carotenoid meso-zeaxanthin against paracetamol, CCl_4 and ethanol induced toxicity. Indian Journal of Experimental Biology 49:44-49.

Firdous, A.P., Sindhu, E.R., Ramnath, V., Kuttan, R., 2010. Anti-mutagenic and anti-carcinogenic potential of the carotenoid meso-zeaxanthin. Asian Pacific Journal of Cancer Prevention 111795-1800.

Handelman, G.J., Nightingale, Z.D., Lichtenstein, A.H., Schaefer, E.J., Blumberg, J.B., 1999. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. American Journal of Clinical Nutrition 70:247-251.

IOM, 2000. β-carotene and other carotenoids. In: IOM. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). National Academy Press (NAP); Washington, DC, pp. 325-382.

IOSA, 2012. Information on general description, specifications, composition and manufacturing of meso-zeaxanthin-rich extract (Hi FIL T™) provided by IOSA (Unpublished information).

JECFA. 2006. Lutein from *Tagetes erecta* L. In: Safety Evaluation of Certain Food Additives: Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, June 8-17, 2004, Geneva. Food and Agriculture Organization of the United Nations (FAO) / World Health Organization (WHO); Geneva, WHO Food Additives Series, No. 54, pp. 49-86 & 637-638. Available at website: http://www.inchem.org/documents/jecfa/jecmono/v54je01.pdf (website visited on: March 22, 2012).

Khachik, F., Beecher, G.R., Smith, C.J.J., 1995. Lutein, lycopene, and, their oxidative metabolites in chemoprevention of cancer. Journal of Cell Biochemistry Supp. 22:236-246.

Maoka, T., Arai, A., Sinuzu, M., Matsuno, T., 1986. The first isolation of entiomeric and meso-zeaxanthin in nature. Comparative Biochemistry and Physiology B, 83:121-123.

Mecchi, M.S., 2006. *Salmonella-Escherichia coli*/Mammalian-microsome reverse mutation assay with a confirmatory assay with mesozeaxanthin. Report no. 7609-100; Genetic Toxicology Assay No. 26471-0-409OECD, pp. 1-27. 2008. Vienna, Virginia, Covance Laboratories Inc.

Moeller, S.M., Jacques, P.F., Blumberg, J.B., 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. Journal of American College of Nutrition 19:5228-5273.

Omaye, S. T., Krinsky, N. I., Kagan, V.E., Mayne, S.T., Liebler, D.C., Bidlack, W., R., 1997. β-Carotene: friend or foe? Fundamental Applied Toxicology 40:163-173.

Pfannkuch, F., 2001. Comprehensive overview on eye examinations on: combined 52-week oral (gavage) pilot toxicity study with two carotenoids in the cynomolgus monkey. Unpublished report No. 1004238 from F. Hoffmann–La Roche Ltd, Basle, Switzerland (Cited in JECFA, 2006).

Pfannkuch, F., Wolz, E., Aebischer, C.P., Schierle, J., Niggemann, B., Zuhlke, U., 2000a. Ro 01-9509 (zeaxanthin 10%) / Ro 15-3971 (lutein 10%): Combined 52-week oral (gavage) pilot toxicity study with two carotenoids in the cynomolgus monkey. Unpublished report No. B-171423, Amendment to Final Report No. 1, dated December 18. Submitted to WHO by Roche, Basle, Switzerland (Cited in JECFA, 2006).

Pfannkuch, F., Wolz, E., Aebischer, C.P., Schierle, J., Niggemann, B., Zuhlke, U., 2000b, Ro 01-9509 (zeaxanthin 10%)/Ro 15-3971 (lutein 10%): combined 52-week oral (gavage) pilot toxicity study with two carotenoids in the cynomolgus monkey. Unpublished report No. B-

171423, Amendment to Final Report No. 1, dated December 18, Submitted to WHO by Roche, Basle, Switzerland (Cited in JECFA, 2006).

Rice-Evans, C.A., Sampson, J., Bramley, P.M., Holloway, D.E., 1997. Why do we expect carotenoids to be antioxidants *in vivo?* Free Rad. Res. 26:381-398.

Thurnham, 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 Review Research 20:163-179.

Thurnham, D.I., Howard, A.N., 2013. Studies on meso-zeaxanthin for potential toxicity and mutagenicity. Food Chemical Toxicology 59:455-463.

Thurnham, D.I., Tremel, A., Howard, A.N., 2008. A supplementation study in humans with a combination of *meso*-zeaxanthin, (3R,3'R)-zeaxanthin and (3R.3'R, 6')-lutein. British Journal of Nutrition 100:1307-1314.

Thurmann, P.A., Schalch, W., Aebischer, J.C., Tenter, U., Cohn, W., 2005. Plasma kinetics of lutein, zeaxanthin, and 3-dehydro-lutein after multiple oral doses of a lutein supplement. American Journal of Clinical Nutrition 82:88-97.

Xu, X., Zhang, L., Shao, B., Sun, X., Ho, C., Li, S., 2-13. Safety evaluation of meso-zeaxanthin. Food Control 32:678-686.

6. APPENDIX I

Analytical data from five non-consecutive manufacturing lots of meso-zeaxanthin preparation (Hi FIL Z™) (IOSA, 2012)*

Parameters	Standard Specifications	11-86-4851	10-95-4851	11-96-4851	9-99-4851
Appearance	Powder	Powder	Powder	Powder	Powder
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Moisture (%)	< 5	1.0	0.5	1.2	4.6
Particle size	Through 24 mesh 80.0% pass	99.5	98.8	99.2	98.8
Carotenoids					
Zeaxanthin (%)	65 (minimum)	65.3	65.1	68.4	69.1
Lutein (%)	15 (minimum)	16.2	18.2	16.4	15.2
R,R-Zeaxanthin (%)	5.0 (maximum)	4.1	1.9	3.8	2.5
Heavy metals (ppm)					
Lead (ppm)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Arsenic (ppm)	< 1.0	< 0.1	< 0.1	< 0.1	< 0.1
Mercury (ppm)	< 1.0	< 0.1	< 0.1	< 0.1	< 0.1
Cadmium (ppm)	< 1.0	< 0.1	< 0.1	< 0.1	< 0.1
Residual solvent levels					
Methanol (ppm)	< 50	19.5	29.6	15.7	16.5
Hexane (ppm)**	< 50**	25.3	109.0	104.5	100.1
Acetone (ppm)	< 100	101.5	7.4	35.2	40.1
Microbiology assay:					
Total plate count (cfu/g)	<1000	<300	<300	<300	<1000
Yeast and molds (cfu/g)	<100	Negative	Negative	Negative	<100
Escherichia coli	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative

*Based on information and data provided by IOSA (2012)

** IOSA has decided that the hexane specification for all future batches will be < 50 ppm.

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Shepherd, Lillian

rom: Mark Itzkoff <mitzkoff@ofwlaw.com>
Sent: Monday, August 12, 2013 4:14 PM
To: Ramos-Valle, Moraima
Cc: Shepherd, Lillian
Subject: RE: GRAS submission for meso-zeaxanthin
Attachments: Aug 5 meso-zeaxan claim.rev.pdf

Dear Ms. Ramos-Valle

As we discussed, attached is the revised statement for the IOSA GRAS Notice for meso-zeaxanthin.

Sincerely,

Mark Itzkoff

Mark L. Itzkoff
OFW Law
Olsson Frank Weeda Terman Matz PC
600 New Hampshire Avenue, N.W.
Suite 500
Washington, D.C. 20037

www.ofwlaw.com

The preceding e-mail message contains information that is confidential, may be protected by the attorney/client or other privileges, and may constitute non-public information. This message is intended to be conveyed only to the designated recipient(s). If you are not an intended recipient of this message, please notify Olsson Frank Weeda Terman Matz PC immediately at (202) 518-6327. Unauthorized use, dissemination, distribution, or reproduction of this message is strictly prohibited and may be unlawful.

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: Monday, August 12, 2013 11:49 AM
To: Mark Itzkoff
Cc: Ramos-Valle, Moraima; Shepherd, Lillian
Subject: GRAS submission for meso-zeaxanthin

Dear Mr. Itzkoff,

This message is to follow up with our conversation regarding your GRAS submission for meso-zeaxanthin. Please use this email to send us an amended statement for the "Basis for GRAS determination" as discussed.

Sincerely,

Moraima J. Ramos Valle, M.S.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Phone: (240) 402-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

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*PRACTICE WITHIN THE DISTRICT OF COLUMBIA IS LIMITED TO MATTERS AND PROCEEDINGS BEFORE FEDERAL COURTS AND AGENCIES

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WILLIAM G.IMBERGAMO

August 5, 2013

BY Federal Express

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied
Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Re: **GRAS Claim for the Use of Meso-Zeaxanthin as a Food Ingredient**

Dear Sirs:

Industrial Organica S.A. de C.V. (IOSA) hereby notifies the agency that IOSA has determined that meso-zeaxanthin rich extract (*Hi-Fil Z*™) is Generally Recognized as Safe (GRAS) for use as a food ingredient as specified in Table I, attached.

Section I

A. Name and Address of Notifier:

Industrial Organica S.A. de C.V.
Ave. Almazán No. 100 Col. Topo Chico,
Monterrey, N.L., Mexico

All communications regarding this notice should be sent to:

Mark L. Itzkoff
Counsel for IOSA
Olsson Frank Weeda Terman Matz PC
600 New Hampshire Ave, NW
Washington, DC 20037

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FOOD ADDITIVE SAFETY

B. Common or Usual Name of Substance:

The common or usual name of the substance is meso-zeaxanthin. The Chemistry Abstract Services number for this substance is 31272-50-1. It will be supplied in powder form under its trade name, *Hi-Fil Z*™.

C. Conditions of Use:

Meso-zeaxanthin-rich extract (Hi-FIL Z™) that is the subject of this Notice will be used as a food ingredient in a variety of food and beverage applications specified in Table 1. Currently, IOSA plans to market *Hi-Fil Z*™, primarily containing meso-zeaxanthin, as a powder.

D. Basis for GRAS Determination:

In accordance with 21 CFR 170.30, the intended use of meso-zeaxanthin has been determined to be generally recognized as safe (GRAS) based on scientific procedures. During July of 2013, the status of the IOSA meso-zeaxanthin-rich extract was reviewed by a panel of scientists (the Expert Panel) assembled by Soni and Associates Inc for IOSA. The Expert Panel was comprised of three noted toxicologists with expertise in food safety. The Panel independently and critically reviewed the information and the available toxicology literature on meso-zeaxanthin and lutein. The Expert Panel concluded that, based on scientific procedures, the use of meso-zeaxanthin as detailed in Table 1 is GRAS.[1]

Thus, the basis for IOSA's determination that the proposed use of meso-zeaxanthin is generally recognized as safe is the scientific procedures detailed in the Expert Panel Statement.

E. **Data Availability Statement:**

The data and information that are the basis for the Notifier's GRAS determination is available for agency review during normal business hours at the office of Olsson Frank Weeda Terman Matz PC provided above or will be forwarded to the agency upon request.

Respectfully Submitted,
(b) (6)

Mark L. Itzkoff
Counsel for Industrial Organica S.A. de C.V.

[1] A copy of the Expert Panel Statement is attached as Appendix I.

SUBMISSION END