GR

ORIGINAL SUBMISSION

000001

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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

RECEIVED
MAY 14 2012
Division of
Biotechnology and
GRAS Notice Review

Re: GRAS Notification for Lutein Diacetate

Dear Dr. Dausch:

On behalf of our client, Industrial Organica S.A. de C.V., and in response to our recent telephone conversations, we are hereby submitting a revised GRAS Notification for lutein diacetate to be used as a food ingredient when used in the applications and under the conditions of use described herein.

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

Sincerely,

(b) (6)

Mark L. Itzkoff

GRAS Notice

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May 4, 2012

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 Lutein Diacetate as a Food Ingredient**

Dear Sirs:

Industrial Organica S.A. de C.V. (IOSA) hereby notifies the agency that IOSA has determined that lutein diacetate is Generally Recognized as Safe (GRAS) for use as a food ingredient as specified in Table I, attached.

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 lutein diacetate. No Chemical Abstract Services Registration Number (CASRN) is available for this substance. It will be supplied as a corn oil suspension known by its trade name, *Micro-Mic*™.

C. Conditions of Use:

Lutein diacetate 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 lutein diacetate as a 20% solution under the trade name *Micro-Mic*™. The food applications for lutein diacetate along with corresponding lutein equivalent levels are detailed in Table 1.

D. Basis for GRAS Determination:

In October of 2011, the status of the IOSA lutein diacetate 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 lutein and lutein diacetate . The Expert Panel concluded that, based on scientific procedures, the use of lutein diacetate 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.

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 I.1

Summary of the Individual Proposed Food-Uses for Lutein Diacetate and the Corresponding Lutein Equivalent Use Levels

Food Category	Proposed Food	Maximum Concentration of *Micro-Mic* 20% Solution (mg/RACC*)	"Lutein-Equivalent Use levels (mg/RACC*)
Baked Goods and Baking Mixes	Cereal and Energy Bars	8.9	2.0
	Crackers and Crispbreads	8.9	2.0
Beverages and Beverage Bases	Bottled Water	2.9	0.5
	Carbonated Beverages	8.9	2.0
	Meal Replacements	8.9	2.0
	Tea, Read-to-Drink	3.5	0.6
Breakfast Cereals	Instant and Regular Hot Cereals	8.9	2.0
	Ready-to-Eat Cereals	8.9	2.0
Chewing Gum	Chewing Gum	4.4	1.0
Dairy Product Analogs	Imitation Milks	8.9	2.0
	Soy Milks	8.6	1.5
Egg Products**	Liquid, Frozen or Dried Egg Substitutes	8.9	2.0
Fats and Oils	Margarine-like Spreads	8.6	1.5
	Salad Dressings	8.6	1.5
Frozen Dairy Desserts and Mixes	Frozen Yogurt	4.4	1.0
Gravies and Sauces	Tomato Based Sauces	1.3	0.3
Hard Candy	Hard Candy	4.4	1.0
Infant and Toddler Foods***	Junior, Strained and Toddler-Type Baby foods	4.4	1.0
Milk Products	Dry Milk	13.3	3.0
	Fermented Milk Beverages	3.5	0.6
	Flavored Milk and Milk Drinks	13.3	3.0
	Milk-Based Meal Replacements	13.3	3.0
	Yogurt	13.3	3.0
Processed Fruits and Fruit Juices	Energy, Sport and Isotonic Drinks	8.9	2.0
	Fruit-Flavored Drinks	8.9	2.0
	Fruit Juice	8.9	2.0
	Nectars	8.9	2.0

Food Category	Proposed Food	Maximum Concentration of *Micro-Mic* 20% Solution (mg/RACC*)	"Lutein-Equivalent Use levels (mg/RACC*)
	Vegetable Juice	8.9	2.0
Soft Candy	Chewy and Nougat Candy	4.4	1.0
	Fruit Snacks	4.4	1.0
Soups and Soup Mixes	Canned Soups	2.7	0.6

* RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC. Uses listed and level same as in GRASN 000140.

** Unlike GRASN 000140, egg products and soup and soup mixes (meat and poultry) that comes under USDA jurisdiction are excluded from the present GRAS determination.

*** Does not include infant formula.

Section II

Identity of the Notified Substance

Lutein diacetate is a purified and esterified crystalline extract from the marigold *(Tagetes erecta)* flower. The lutein diacetate concentrate contains approximately 70% lutein diacetate. *Micro-Mic*™ Lutein diacetate solution is prepared from the concentrate by dispersing the concentrate in corn oil. *Micro-Mic*™ Lutein diacetate solution typically contains minimum 25% by weight total carotenoids, including about 22% lutein diacetate . The chemical and physical characteristics of lutein and zeaxanthin in free and diacetate form are detailed in Table II.1.

Table II.1 Chemical and Physical Characteristics of Lutein and Zeaxanthin free and acetate form

Property/Parameter	Lutein/ lutein diacetate	Zeaxanthin/ zeaxanthin diacetate
CAS Registry No.	127-40-2/CAS not available for lutein diacetate	144-68-3/CAS not available for zeaxanthin diacetate
Chemical names	Xanthophyll; β, ε-carotene-3,3' – diacetate/ Xanthophyll; β, ε-carotene-3,3' – diacetate	β, β -carotene-3,3'- diacetate/ β, β -carotene-3,3'- diacetate
Empirical formula	$C_{40}H_{56}O_2$ / $C_{44}H_{63}O_6$	$C_{40}H_{56}O_2$ / $C_{44}H_{63}O_6$
Molecular weight	568.88 / 699.97	568.88 / 699.97
Physical state	Crystalline / crystalline	Crystalline / crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble / insoluble	Insoluble / insoluble

*Based on information from IOSA and other publicly available sources

The structural formulas of lutein diacetate and zeaxanthin are presented in Figures II.1 and II.2.

Figure II.1

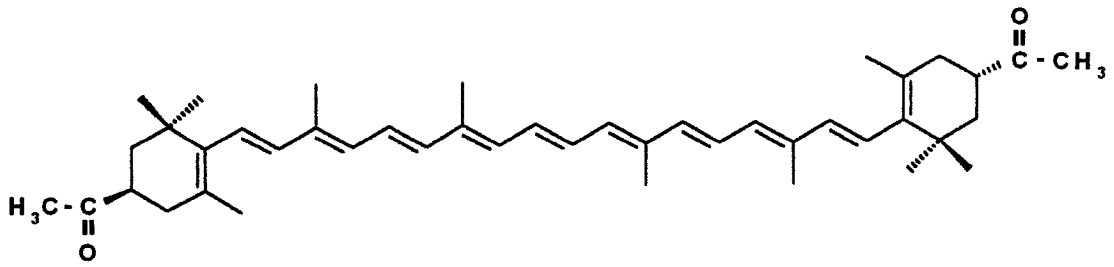
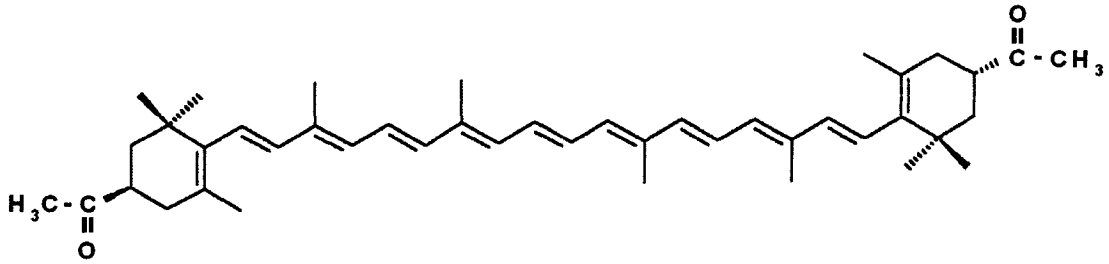


Figure II.2



Food grade specifications for *MICRO-MIC*™ Lutein diacetate solution containing minimum 22% lutein diacetate have been established by IOSA and are presented in Table II.2.

Table II.2. Specifications for *MICRO-MIC*™ Lutein diacetate

Parameters	**Specifications**
Appearance	Oil Suspension
Odor	Characteristic
Identity	1.09 – 1.11
Density	0.96
Moisture (%)	0.2
Excipient	Corn Oil
Carotenoids	
Total Carotenoid Esters (%)	25 (minimum)
Lutein Diacetate (%)	22 (minimum)
Zeaxanthin Diacetate (%)	1.5 (minimum)
Heavy Metals (ppm)	
Lead	< 2.0
Arsenic	< 1.0
Mercury	< 1.0
Cadmium	< 1.0
Microbiology Assay:	
Total plate count (cfu/g)	<1000
Yeast and molds (cfu/g)	<100
E. coli and Salmonella	Negative
Salmonella	Negative
Pseudomonas euriginosa	Negative
Staphylococcus	Negative

Manufacturing Process

Marigold oleoresin is warmed and is treated successively first with a diluted alkali to remove any free fatty acids in the oleoresin. After thoroughly rinsing with water, a centrifugation step follows in order to separate the undesirable free fatty acids, gums, waxes and water. Another treatment follows with a dilute acid to further remove other impurities. They are separated by centrifuging.

Following the centrifugation, the mass is treated with a strong potassium hydroxide water solution to saponify (hydrolyze) the lutein. (In natural form lutein occurs as the diester of fatty acids).

After the hydrolysis reaction has reached completion, the saponified mass is diluted with water and the pH is reduced with a diluted acetic acid solution. At this point, a clear separation of phases occur. The water phase is separated from the organic phase either by decanting or centrifuging the solution.

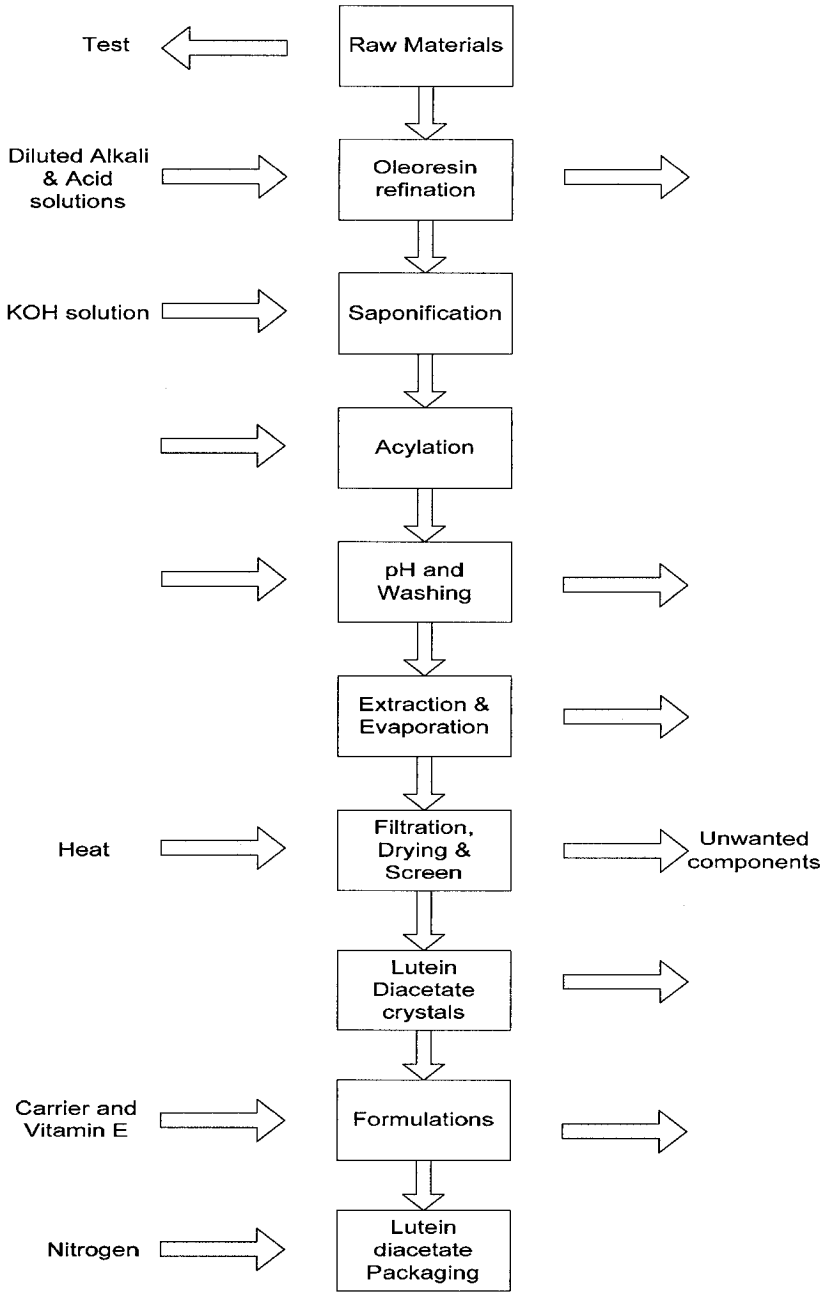
The supernatant (organic) phase contains free fatty acids and minute crystals of lutein and zeaxanthin. The supernatant phase is rinsed with water until any traces of acetic acid have been removed. At this point, the lutein is contained in the organic phase that is also called "the xanthophylls concentrate."

The xanthophylls concentrate is extracted several times (4-5) with hexane to remove the free fatty acids and any non polar impurities. Xanthophylls are not soluble in hexane and remain in the xanthophylls concentrate.

Any remaining moisture is removed from the mass of crystals which are then slowly incorporated into acetic anhydride under agitation. The esterification reaction is allowed to continue to completion. Following esterification, excess anhydride is removed by successive water rinsing.

The water is removed from the end product by filtration or centrifugation and a mass of high purity lutein crystals is obtained. The crystals are dried under vacuum and are either: (1) packed with nitrogen or vacuum, (2) dispersed in edible oil, or (3) microencapsulated by spray drying.

Figure 3.


Test
Raw Materials
Diluted Alkali & Acid solutions
Oleoresin refination
KOH solution
Saponification
Acylation
pH and Washing
Extraction & Evaporation
Heat
Filtration, Drying & Screen
Unwanted components
Lutein Diacetate crystals
Carrier and Vitamin E
Formulations
Nitrogen
Lutein diacetate Packaging

Section III

Summary of the Basis for the Notifier's Determination that Lutein Diacetate is GRAS

As discussed in the Expert Opinion, lutein diacetate is expected to dissociate in the human body resulting in free lutein. Therefore, it is reasonable to compare IOSA's lutein diacetate product with non-esterified lutein products that have been the subject of previous GRASN's. IOSA's lutein diacetate 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.

A. *FloraGlo*™ - Xanthophyllls concentrate conversion

In GRASN 000140, the lutein use levels provided are labeled "mg crystalline lutein." The crystalline lutein referred to in GRASN 140, *FloraGlo*™, is a blend containing 74% lutein. The *FloraGlo*™ crystalline lutein is analogous to IOSA's xanthophylls concentrate. As noted above, lutein diacetate will comprise 69% of the xanthophylls concentrate. We also note that due to esterification, 1.0 g lutein = 1.2 g lutein diacetate. Using this information, the equivalent use level for IOSA's xanthophylls concentrate (lutein diacetate concentrate) to the lutein use levels set forth in GRASN 140 is calculated as follows:

1.0 g *FloraGlo*™ = 0.74 g lutein

0.74 g lutein = 0.89 g lutein diacetate ((0.74 g)(1.2) = 0.889)

The IOSA xanthophylls concentrate will contain 69% lutein diacetate. Thus,

1.0 g *FloraGlo*™ = 1.29 g xanthophylls concentrate (0.89 g/69% = 1.29 g)

B. *FloraGlo*™ – *MicroMic*™ conversion:

The lutein diacetate will be supplied to processors as *MicroMic*™, a 20% suspension. Thus,

0.89 g lutein diacetate = 4.45 g *MicroMic*™.

Therefore, the lutein equivalent conversion factor from *FloraGlo*™ crystalline lutein to *MicroMic*™ lutein diacetate dispersion is 1.0 g *FloraGlo*™ is equivalent to 4.45 g *MicroMic*™.

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 lutein diacetate (in the form of the lutein diacetate suspension) will be used in place of other lutein products, not in addition to those products, the concentration of lutein in the individual foods will not increase and the proposed use of lutein diacetate will not affect the total dietary intake of lutein. 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.

Given that lutein diacetate is intended for use in the same foods and equivalent levels of addition as specified for lutein in GRASN 000140, the estimates of dietary intake for Kemin lutein are considered the same for lutein diacetate. As an added measure of assurance on this point, we note that the current predominant use for lutein is as a component of multi-vitamin supplements.

We also note that in addition to Kemin's GRAS notice, FDA has accepted five additional GRAS notifications on lutein as of March 23, 2012 (lutein ester- GRASN 000110; lutein suspension- GRASN 000221; crystalline lutein- GRASN 291; lutein free and ester- GRASN 000385; and lutein suspension GRASN 000390). FDA responded to all of these Notices stating that the agency had no questions regarding the conclusions that the use of lutein is GRAS.

In coming to the conclusion that lutein diacetate is also GRAS, IOSA relied upon scientific studies that showed that neither lutein diacetate, zeaxanthin diacetate 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 lutein diacetate 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 Opinion

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LUTEIN DIACETATE (MICRO-MIC™) 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

John A. Thomas, Ph.D., F.A.T.S., D.A.T.S.
Stanley T. Omaye, Ph.D., D.A.T.S.
Madhusudan G. Soni, Ph.D., F.A.C.N.

October, 2011

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LUTEIN DIACETATE (MICRO-MIC™) AS A FOOD INGREDIENT

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DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LUTEIN DIACETATE (MICRO-MIC™) 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 PC at the request of Industrial Organica S.A de C.V. (IOSA), to determine the Generally Recognized As Safe (GRAS) status of Lutein Diacetate (MICRO-MIC™) as a nutrient supplement [21 CFR 170.3(o)(20)][2] in selected food products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, 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, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on lutein and lutein diacetate was conducted through August 2011 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 and unanimously agreed to the decision described herein.

1.1. Background

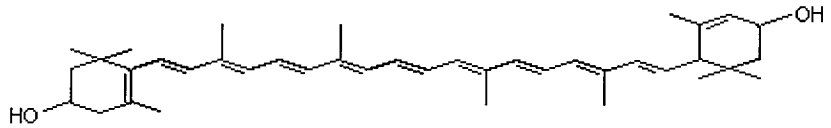
In recent years carotenoids have received considerable attention in the scientific community because of their powerful antioxidant properties, protecting the cells of the body from damage caused by free radicals. Lutein and zeaxanthin are two of the most abundant carotenoids in the North American diet (IOM, 2000). The eyes are considered as repositories for carotenoids with lutein and zeaxanthin concentrated in the retina and lens. Available evidence indicate that higher dietary intake of lutein and zeaxanthin is related to reduced risk of cataracts and age-related macular degeneration, two eye conditions for which there is minimal options for effective prevention. 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). Unlike β-carotene, lutein and zeaxanthin are not considered 'provitamin A' compounds as these carotenoids are not converted to retinol (an active form of vitamin A) in the human body. In recent years, investigators have prepared different forms of lutein with improved bioavailability. Because of unique nutritional characteristics of lutein, IOSA intends to use lutein diacetate (Lutein Diacetate- MICRO-MIC™) in a limited number of conventional foods as a dietary ingredient.

1.2. Description

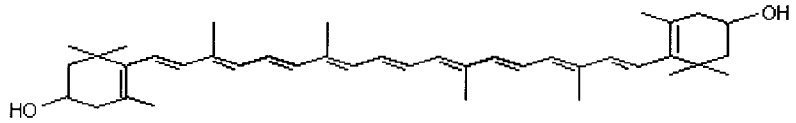
Lutein Diacetate (MICRO-MIC™) is a product rich in lutein (diacetate form) suspended in edible oil. It is obtained from a natural carotenoid source, a variety of Aztec Marigold flowers

[1]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.
[2] "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.

(*Tagetes erecta* L). The product is a red orange oily suspension with characteristic odor. The lutein diacetate concentrate is standardized and diluted into forms useful for food and beverage applications. MICRO-MIC™ for food uses will be marketed as a standardized product oil suspension with commonly used dietary oil (corn oil). The product will be marketed under the names: MICRO-MIC™ containing different levels and up to 20% lutein diacetate. The primarily marketed 20% (MICRO-MIC™) product contains minimum 20% lutein diacetate and 1% zeaxanthin diacetate. Both carotenoids in the product are in esterified form. The subject of this GRAS determination lutein diacetate (ester form) is substantially equivalent to lutein ester that has been the subject of FDA GRAS notified substance (GRN 000110; FDA, 2003) except that the FDA notified substance was primarily ester of dipalmitate (fatty acid), while the current product is ester of diacetate. Food grade antioxidant (Vitamin E) is added to the marketed product MICRO-MIC™ 20% (lutein diacetate dispersed in corn oil) in accordance with good manufacturing practices. The structural formulas of lutein and zeaxanthin are presented in Figure 1. General descriptive parameters and properties of lutein, zeaxanthin free and acetate forms are summarized in Table 1.



Lutein



Zeaxanthin

Figure 1. Chemical Structure of Lutein and Zeaxanthin

Table 1. Chemical and Physical Characteristics of Lutein and Zeaxanthin free and acetate forms

Property/Parameter	Lutein/ lutein diacetate	Zeaxanthin/ zeaxanthin diacetate
CAS Registry No.	127-40-2/CAS not available for lutein diacetate	144-68-3/CAS not available for zeaxanthin diacetate
Chemical names	Xanthophyll; β, ε-carotene-3,3' –diol / Xanthophyll; β, ε-carotene-3,3' – diacetate	β, β -carotene-3,3'-diol / β, β - carotene-3,3'- diacetate
Empirical formula	$C_{40}H_{56}O_2$ / $C_{44}H_{63}O_6$	$C_{40}H_{56}O_2$ / $C_{44}H_{63}O_6$
Molecular weight	568.88 / 699.97	568.88 / 699.97
Physical state	Crystalline / crystalline	Crystalline / crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble / insoluble	Insoluble / insoluble

*Based on information provided by IOSA (2011) and other publicly available sources

1.3. Specifications and Identity

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Typical food grade specifications of Lutein Diacetate 20% (MICRO-MIC™) have been established by IOSA and are presented in Table 2. Analytical results from five non-consecutive

lots (Appendix I) demonstrate that Lutein Diacetate 20% (MICRO-MIC™) is consistently manufactured to meet these specifications. The product is standardized to the desired contents of lutein diacetate with corn oil. Typical compositional analysis of lutein diacetate concentrate and the 20% product (MICRO-MIC™) prepared from the concentrate by dispersing in corn oil and with the addition of antioxidant is summarized in Table 3.

Table 2. Specifications of Lutein Diacetate 20% (MICRO-MIC™)*

Parameters	Specifications	Methods
Appearance	Oil Suspension	Visual
Odor	Characteristic	Organoleptic
Identity	1.09 – 1.11	UV-Vis
Density	0.96	Gravimetry
Moisture (%)	0.2	Karl Fisher
Excipient	Corn Oil	In House
Carotenoids		
Total Carotenoid Esters (%)	25 (minimum)	UV-Vis
Lutein Diacetate (%)	22 (minimum)	UV-Vis/HPLC
Zeaxanthin Diacetate (%)	1.5 (minimum)	UV-Vis/HPLC
Heavy Metals (ppm)		
Lead	< 2.0	Atomic absorption (AA)
Arsenic	< 1.0	A.A
Mercury	< 1.0	A.A
Cadmium	< 1.0	A.A
Microbiology Assay:		
Total plate count (cfu/g)	<1000	In House
Yeast and molds (cfu/g)	<100	In House
E. coli and Salmonella	Negative	In House
Salmonella	Negative	In House
Pseudomonas euriginosa	Negative	In House
Staphylococcus	Negative	In House

*Based on information provided by IOSA (2011)

Table 3. Typical Compositional Analysis of Lutein Diacetate (MICRO-MIC™) (IOSA, 2011)

COMPOUNDS	Concentrate (% weight)	MICRO-MIC™ 20% (% weight)
Lutein Diacetate	69.0%	20.0-22.0%
Zeaxanthin Diacetate	2.1%	1.0- 3.5%
Lutein	0-5%	0-2%
Zeaxanthin	0-2 %	0-1 %
Total Fat*		70-80 %
Waxes **	15-30%	4-8%
Total Carbohydrate	0-5%	0-5 %
Proteins	0- 1%	0- 1%
Moisture	0-5%	0-3%
Ash	0-1%	0-1%
Vitamin E		0.5%

*Fatty Acid profile: Palmitic acid C16:0 = 10.7%; Stearic acid C18:0 = 2.4 %; Oleic acid C18:1 = 27.1 %; Linoleic acid C18:2 = 55.8 %; Linolenic acid: C18:3 = 1.0%. ** Miristic acid (22.6%), palmitic acid (60.4%) stearic acid (14.4%). Sterols, Hydrocarbons, pigments, waxes

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1.4. Manufacturing Process

Lutein Diacetate (MICRO-MIC™) 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*) oleoresin as the raw material. The oleoresin material is obtained by hexane extraction of dried marigold flower petals. 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.

The saponified mass is acylated with food grade acetic acid and acetic anhydride. The acylated product is diluted with water, pH adjusted and the water is used to wash away salts and other water soluble impurities. Lutein diacetate concentrate is extracted (few times) with hexane to further remove unwanted components. The solvent is separated by filtration, decanting and centrifugation. Lutein diacetate crystals are then treated under high vacuum and temperature evaporation to remove remaining solvent. Lutein diacetate crystals are filtered and dried to remove water and screened to collect the lutein diacetate crystal of appropriate size. Processing aids such as potassium hydroxide, acetic acid, and the solvent (hexane) used during the manufacturing process are food grade quality as specified in the Food Chemical Codex (5th Edition).

The crystals of appropriate size are formulated with excipient (corn oil) to desired concentration along with vitamin E (antioxidant). The final Lutein Diacetate product (MICRO-MIC™) is packed under high vacuum and inert nitrogen atmosphere in food-grade plastic pouches. The residual concentration of hexane in the final product is below 25 ppm. Heavy metal levels and microbiological contaminants are checked to meet the specifications (Table 2). The lutein diacetate concentrate is routinely checked for common pesticide residues to make sure that none were detected at detection limits of < 0.01 mg/kg.

[redacted]

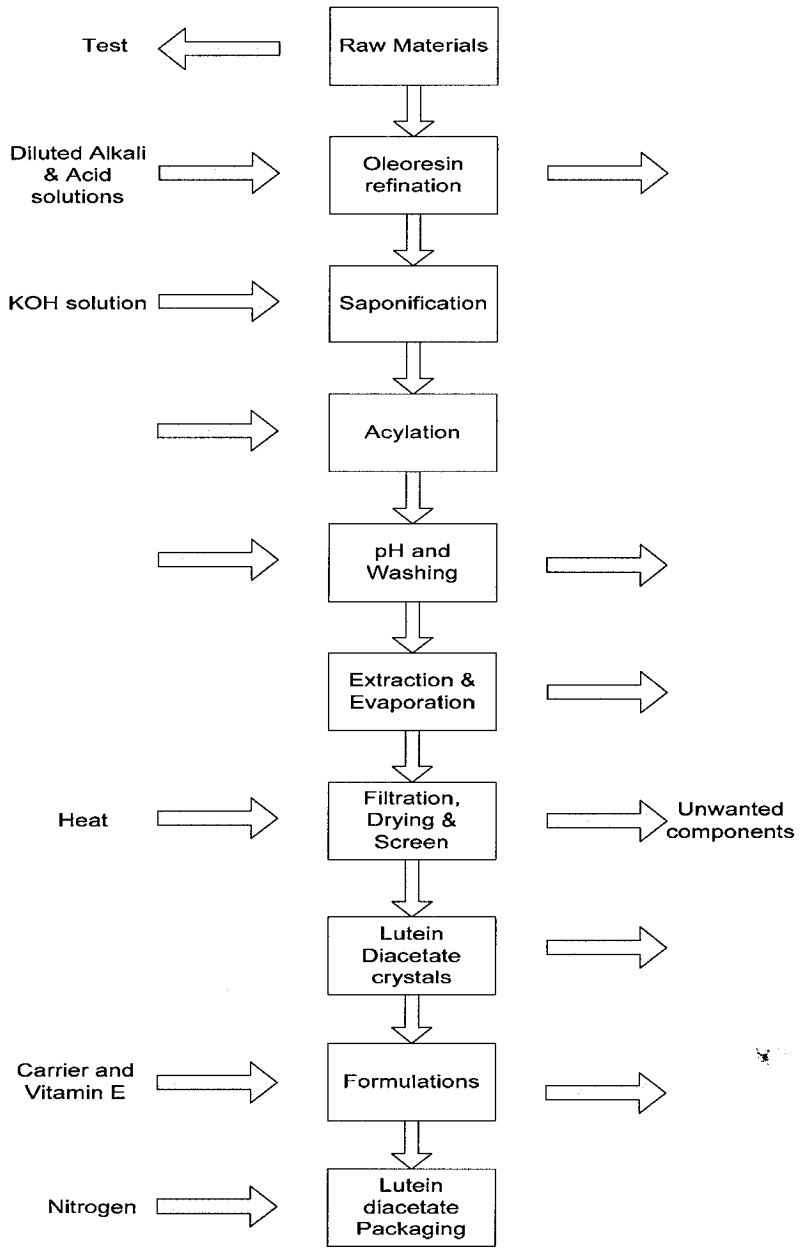

Figure 2. Manufacturing process of Lutein Diacetate (MICRO-MIC™) (IOSA, 2011)

1.5. Occurrence and Uses

Lutein (Latin luteum = egg yolk, from luteus = yellow) is a yellow pigment in the chemical family of fat soluble carotenoids. Over 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, but also in maize, carrots, green peppers and peas), yellow orange fruits, yellow orange vegetables and egg yolks. Kusaba et al. (2011) reported accumulation of lutein 3-acetate in senescent leaves of rice. Presence of lutein and zeaxanthin has been reported in human breast milk (GRN 000110; FDA, 2003). Predominant form of lutein in human breast milk is reported to exist in free form. Exposure of lutein from

human breast milk depends on stage of lactation, geographical location and individual diets. The presence of lutein in human milk ranges from 3 to 232 μg/L (Canfield et al., 2003). Inside the human body lutein can not be synthesized and hence humans depend entirely on dietary sources such as vegetables, eggs or dietary supplement in the form of lutein pills.

Lutein in free and ester form is commonly marketed as a dietary supplement around the world. Based on the 1987 National Health Interview Survey from the US, 51.1% adults (aged 19-99 years) consumed a vitamin/mineral supplement during the past year. Of these only 23.1% consumed these supplements daily (Subar and Block, 1990). Commercially available multivitamins such as Centrum® (American Home Products, Madison, NJ) contain a recommended daily dose of 250 μg unesterified lutein (~500 μg lutein ester equivalents). Another supplement retailer, General Nutrition Centers, Inc. (GNC) markets a lutein ester supplement under the brand name Natural Brand™ with a recommended daily dose of 12 mg lutein ester. Based on this information and the levels recommended in labeling, theoretical lutein ester consumption from dietary supplements may range from 0.5 mg to 12 mg/day.

In four separate GRAS notifications on lutein [lutein ester- GRN 000110 (FDA, 2003); crystalline lutein- GRN 000140 (FDA, 2004); suspended lutein- GRN 000221 (FDA, 2007); crystalline lutein- GRN 291 (FDA, 2009)], FDA responded that they had no questions regarding the conclusions that the use of lutein is GRAS under the conditions described in the notices. In these notices, use of lutein in specified food categories was estimated to result in levels of up to 13.4 mg/day and was considered as safe. In the first GRAS notice (GRN 000110) to FDA the notified substance was in the form of lutein ester (dipalmitate).

1.6. Intended Technical Effects

Lutein Diacetate 20% (MICRO-MIC™) derived from Marigold flowers (*T. erecta* L) is intended for addition to a limited number of conventional foods as a nutritional ingredient. Its use is intended for the general population at the levels identified in this document for addition to the following food categories: Baked Goods and Baking Mixes; Beverages and Beverage Bases; Breakfast Cereals; Chewing Gum; Dairy Product Analogs; Fats and Oils; Frozen Dairy Desserts and Mixes; Gravies and Sauces; Hard Candy; Infant and Toddler Foods; Milk Products; Processed Fruits and Fruit Juices; Soft Candy; Soups and Soup Mixes. It is recognized that there are Standard of Identity requirements for some of these foods, and as such, IOSA does not intend to refer them by the commonly recognized names such as milk, or yogurt.

While the use of Lutein Diacetate 20% (MICRO-MIC™) derived from Marigold flowers in the above described food categories may also impart a color to the product, the intended use would fall outside the definition of "color additive" for the following reasons: Lutein Diacetate 20% (MICRO-MIC™) is solely added for its intended technical effect as a nutrient supplement and thus may constitute an "unimportant color" [21 CFR 70.3(g)] and it does not relate to any use of the ingredient as a color additive [21 CFR 70.3(f)].

1.7. Intended Use Levels and Food Categories

The Lutein Diacetate 20% product (MICRO-MIC™) is intended for use in the same foods and at levels proportional to those notified by Kemin Foods for the FloraGLO® Crystalline Lutein in the GRN 000140 (FDA, 2004). The FloraGLO® Crystalline Lutein has been reported to contain >74% lutein and >2% zeaxanthin, while Lutein Diacetate 20% (MICRO-MIC™) contains >20% lutein diacetate and >1% zeaxanthin diacetate. On the basis of lutein content,

Lutein Diacetate 20% (MICRO-MIC™) can be added at level of 3.7 time that of FloraGLO® Crystalline Lutein. Additionally, lutein in FloraGLO® Crystalline Lutein is in free form, while Lutein Diacetate 20% (MICRO-MIC™) is in diacetate form. On molecular weight basis 1 g of free lutein is equivalent to 1.2 g of lutein diacetate. Thus to reach equivalent level of lutein found in FloraGLO® Crystalline Lutein, 4.44 times higher levels of Lutein Diacetate 20% (MICRO-MIC™) can be used. Thus, in order to deliver equivalent levels of lutein to that of FloraGLO® Crystalline Lutein, addition of the Lutein Diacetate 20% product (MICRO-MIC™) will be approximately 4.44 times the overall bulk food addition compared to FloraGLO® Crystalline Lutein.

The proposed food uses of lutein diacetate with small amounts of zeaxanthin diacetate are as a food ingredient, 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, 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. The intended food uses and use levels are presented in Table 4. The application of lutein diacetate to the same foods and at the same (lutein equivalent) levels as those in GRN 000140 (FDA, 2004) is not expected to notably affect the intake of lutein in the diet of the public from introduction into the market by another supplier who will have to compete in essentially the same market and foods. IOSA specifically excludes food categories such as egg products and soup and soup mixes that come under USDA jurisdiction.

The dietary analysis below was presented by Kemin in GRN 000140 (FDA, 2004) and was not questioned by FDA in their response letter of June 14, 2004. "The consumption of FloraGLO® Crystalline Lutein, lutein and zeaxanthin, from all proposed food-uses of FloraGLO® Crystalline Lutein, was estimated using the 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). On an all-user basis, the mean and 90th percentile intakes of FloraGLO® Crystalline Lutein by the total U.S. population from all proposed food-uses of FloraGLO® were 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. Based on the composition of FloraGLO® Crystalline Lutein as 76% lutein and 7% zeaxanthin, 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 earlier, lutein diacetate is intended to be added to food on a "lutein equivalent" basis, i.e., 1.2 g lutein diacetate replacing 1.0 g lutein. Based on the above information, 90th percentile lutein intake from the use of Lutein Diacetate 20% (MICRO-MIC™) will be similar (13.4 mg lutein/person/day). A summary of use levels and food categories is presented in Table 4. As Lutein Diacetate 20% (MICRO-MIC™) contains about half the amount of zeaxanthin (in the form of diacetate) found in FloraGLO® Crystalline Lutein, the resulting 90th percentile intake of zeaxanthin diacetate will be 0.6 mg/person/day. Given that the Lutein Diacetate 20% (MICRO-MIC™) product is similar in composition and is intended for use in the same foods at levels of addition as notified by Kemin Foods for the FloraGLO® Crystalline Lutein in GRN 000140, the estimates of intake for the FloraGLO® crystalline lutein are considered to be the same (on lutein

equivalent basis) for Lutein Diacetate 20% (MICRO-MIC™) and would not be additive. In addition to lutein diacetate and zeaxanthin diacetate, the Lutein Diacetate 20% product (MICRO-MIC™) also contains up to 0.5% d,l-α-tocopherol. For the Lutein Diacetate 20% product (MICRO-MIC™) containing up to 0.5% d,l-α-tocopherol, comparable mean intakes of d,l-α-tocopherol will be on the order of 0.48 mg/person/day (0.008 mg/kg body weight/day) with the 90th percentile intake of 0.98 mg/person/day (0.016 mg/kg body weight/day).

Table 4. Summary of the Individual Proposed Food-Uses for Lutein Diacetate 20% (MICRO-MIC™) and corresponding use levels for lutein (equivalent) in the US.

Food Category	Proposed Food	Use levels as lutein (mg/RACC[1])
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
Soups and Soup Mixes	Canned Soups	0.6

[1]RACC Reference amounts customarily consumed per eating occasion (21 CFR §101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC. Uses listed and level same as in GRN 000140. *Unlike GRN 000140, egg products and soup and soup mixes (meat and poultry) that comes under USDA jurisdiction are excluded from the present GRAS determination. **Excludes infant formula.

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2. SAFETY RELATED STUDIES

In four separate GRAS notifications on lutein [lutein ester- GRN 000110 (FDA, 2003); crystalline lutein- GRN 000140 (FDA, 2004); suspended lutein- GRN 000221 (FDA, 2007); crystalline lutein- GRN 291 (FDA, 2009)] to FDA, toxicity-related studies on lutein from the literature were presented which support the safety in use of lutein ester. The FDA did not question the acceptability and suitability of these studies to establish the safety of lutein ester, crystalline lutein and suspended lutein for the proposed food uses. Because the Lutein Diacetate 20% (MICRO-MIC™) is substantially equivalent to the products of FDA notification, studies cited and described in these notifications can be utilized for safety assessment of the lutein diacetate (MICRO-MIC™) that is the subject of this GRAS determination.

In these GRAS notices to FDA, the safety of lutein and its esters has been established in 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) support the safety of lutein and zeaxanthin. Additional studies published subsequent to the FDA GRAS notices continue to support safety of lutein as a food ingredient.

2.1. FDA Excerpt and Discussion

As safety of lutein has been extensively presented and discussed in the four 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 before considering the recent safety-related publications of lutein. The excerpts and discussion below indicate that the FDA is comfortable with the safety of the lutein, as long as consumption is below 20 mg/person/day. Additionally, JECFA has also undertaken safety assessment of lutein to determine acceptable daily intake. These assessments are fully applicable to IOSA's use of lutein diacetate in terms of its safety. A summary of the FDA findings from the GRAS notices is presented below and in Table 5.

> **GRN 000110- Lutein esters (Cognis Corporation)**: "Cognis discusses published and unpublished studies conducted in humans and various animal species regarding the absorption, distribution, metabolism, excretion, bioavailability and toxicity of lutein esters. Cognis concludes that results of these studies show no toxic or adverse effects from the consumption of lutein esters and that long-term consumption of lutein esters is well tolerated. Cognis notes, however, that carotenodermia was observed in one study in which subjects consumed supplements containing 30 mg/person/day of a mixed lutein esters product for 112 days. In contrast, Cognis notes that carotenodermia was not observed in an ongoing study in which subjects consumed supplements containing up to 40 mg/person/day of Cognis' lutein esters product for more than 90 days."[3]
>
> "As part of its notice, Cognis includes the report of a panel of individuals (Cognis' GRAS panel) who evaluated the data and information that are the basis for Cognis' GRAS determination. Cognis considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. Cognis' GRAS panel evaluated estimates of dietary exposure, published information on the safety and toxicity

[3]Literature search of scientific databases did not reveal publication of this study.

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of lutein esters and related compounds, and other available data and information. Based on this review, Cognis' GRAS panel established an acceptable daily intake (ADI) of 40 mg/person/day of lutein esters (or lutein ester equivalents), a dose that has been administered without evidence of carotenodermia."

GRN 000140- Crystalline lutein (Kemin Foods): "Using its proposed use levels and data from the United States Department of Agriculture 1994-1996 Continuing Survey of Food Intakes by Individuals and 1998 Supplemental Children's Survey, Kemin estimates that the intake of crystalline lutein would be approximately 10 milligrams per person per day (mg/person/day) at the mean and approximately 18 mg/person/day at the 90th percentile. Based on the carotenoid composition of crystalline lutein, Kemin also estimates that the intake of lutein and zeaxanthin would be approximately 7 mg/person/day (lutein) and 0.7 mg/person/day (zeaxanthin) at the mean and approximately 13 mg/person/day (lutein) and 1.2 mg/person/day (zeaxanthin) at the 90th percentile, respectively. Kemin notes that the average dietary intake of lutein and zeaxanthin from plant sources ranges from 2 to 4 mg/person/day. However, Kemin notes that there are no consumption data from which to reliably estimate the intake of lutein and zeaxanthin from dietary supplements.'

"Kemin discusses published studies conducted in humans and various animal species regarding the absorption, distribution, metabolism, excretion, bioavailability, toxicity and mutagenicity of crystalline lutein and its primary components, lutein and zeaxanthin. Kemin concludes that results of these studies show no toxic or adverse effects (including ocular damage) from the consumption of crystalline lutein or lutein and zeaxanthin from other sources. Kemin notes that carotenodermia was reported in a study in which healthy subjects consumed 15 mg/person/day of lutein (from mixed ester forms extracted from marigold) for 4 months. Kemin notes, however, that carotenodermia is considered a harmless and reversible biological effect of high carotenoid intake, and that no signs of its occurrence have been reported in other populations (e.g., patients with cataracts or patients with age-related macular degeneration) following exposure 3 times per week for 13 months of approximately 25 mg/person/day of lutein."

GRN 000221- Suspended lutein (Kemin Health): "In GRN 000221, Kemin (manufacturer of lutein) describes the use of suspended lutein in term infant formula at levels not to exceed 250 μg/l, which Kemin notes is equivalent to 37 micrograms per 100 kilocalories. Kemin estimates the mean and 90th percentile intake of dietary carotenoids for children from 2 through 6 months to be 200 and 820 micrograms per day (μg/d) respectively, and for children between 7 months to 11 months of age to be 460 and 1,100 μg/d, respectively. For the use in term infant formula, as described in GRN 000221, Kemin estimates a daily intake of 195 μg/d of suspended lutein."

"GRN 000221 discusses published and unpublished studies in GRN 000140, as well as several published animal studies that became available after the agency review of GRN 000140, and two unpublished growth studies conducted in healthy term infants. Kemin concludes that the growth studies provide corroborative evidence for the safety of suspended lutein."

GRN 000291- Crystalline lutein (IOSA): The subject of this most recent notice reviewed by FDA is a mixture of carotenoids, lutein, and zeaxanthin referred as crystalline lutein by the notifier Industrial Organica S.A. (IOSA) of Mexico (FDA, 2009). As part of this notice, IOSA included two reports by separate panels (GRAS panel 2006 and GRAS panel 2008) that evaluated the data and information that formed the basis for GRAS determination of crystalline lutein. As regards the estimated daily intake (EDI), the calculation from GRN 000140 was used in the notice. In the GRN 000140 an estimate of the current intake of crystalline lutein from the diet and the intended intake of lutein from the foods was provided.

IOSA concluded that, due to the substantial similarity in composition with the crystalline lutein that was the subject of GRN 000140 and the fact that the intended use would be substitution and not an additive, the EDI calculations would be considered the same for the uses of crystalline lutein in both GRN 000140 and its notice. The mean intake of lutein and zeaxanthin from specified foods was estimated to be 7.3 and 0.7 mg/person/day, respectively, while the 90th percentile intake was determined as 13.4 and 1.2 mg/person/day, respectively. In its notice, IOSA discussed published and unpublished studies that were summarized in GRN 000140, as well as several published animal and human studies that became available after the agency review of GRN 000140. Based upon the totality of the available evidence, IOSA concluded that crystalline lutein in the diet at the estimated levels is not considered to pose any safety concerns.

In addition to the above described GRAS notices that were reviewed by FDA and did not raise any concern, JECFA also completed a safety evaluation of lutein from *T. erecta* L. and assigned a acceptable daily intake (ADI) of 0 to 2 mg lutein and zeaxanthin/kg body weight (JECFA, 2006). The JECFA AD1 was established on the basis of a no observed adverse effect level (NOAEL) of 200 mg lutein/kg body weight/day (the highest dose tested) reported in a 90-day rat study (Pfannkuch et al., 2000, 2001; Kruger et al., 2002), to which a safety factor of 100 was applied. This safety factor was considered appropriate given the lack of adverse effects reported at much higher doses than 200 mg/kg body weight/day (up to 1,000 mg/kg body weight/day in a study of developmental toxicity). The committee noted that there were no adverse effects documented in any of the studies of toxicity in animals, including mice, rats, and monkeys, or in humans.

The JECFA determination of ADI of 120 mg/person based on animal studies may be high given the metabolism-related differences in carotenoid between humans and animals. It is known that at low levels of exposure (physiological levels), rats do not transport β-carotene intact but rather metabolizes it to vitamin A in intestinal cells. Available evidence suggest that in order to raise blood carotenoid levels, rats need to be exposed to very high doses (>0.02% of the diet) of β-carotene (White, et al., 1993, Lee, et al., 1999; Wolf, 2002). Although lutein does not serve as a vitamin A precursor, there is limited comparative data on lutein between rodents and humans. Rats and mice may not be the most appropriate models for studying carotenoid absorption and bioavailability (Krinsky et al., 1990; Lee et al., 1999). Generally, high levels of dietary carotenoid are fed in these studies to achieve adequate tissue levels. Thus, relevance to human must be considered with caution.

From the foregoing analysis and the FDA review of the submitted GRAS Notices where the agency had no objection to the conclusions of lutein and its esters being GRAS including one specific submission on lutein ester, consumption of lutein or its ester as an ingredient from conventional foods resulting in daily intake of up to 40 mg/person/day of lutein ester (or lutein ester equivalent; 20 mg free lutein) is considered as safe. Additionally, JECFA evaluation further support the safety determinations of lutein and its esters assessed in the GRAS notices. A comparison between GRAS notices, JECFA repot and current GRAS determination is presented in Table 5.

Table 5. Summary of safety assessments of lutein reported in GRAS notices, JECFA and current GRAS determination.

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

*For an individual weighing 60 kg

2.2. Lutein Diacetate (MICRO-MIC™) Bioavailability

In a recent study, Bone et al. (2011) compared changes in serum lutein following administration of lutein diacetate and crystalline lutein to human subjects. In this study, ten subjects were assigned to each of three groups and were given lutein diacetate (20 mg/day), lutein (20 mg/day) or placebo for 24 weeks. Serum samples were collected at 0 (baseline), 6, 12 and 24 weeks for lutein analyses. The average serum lutein for weeks 6 to 24 expressed as a ratio to baseline was 5.52±2.88 for lutein diacetate group, 4.43±1.61 for crystalline lutein and 1.03±0.25 for placebo group. Both groups receiving lutein showed significant increase in serum lutein levels as compared to placebo group. The difference between groups receiving lutein diacetate and crystalline lutein was not significant. Although not statistically significant, the average serum lutein response was higher (~25%) for lutein diacetate group compared to crystalline lutein group. However, further studies in larger population are needed to confirm this observation. The results of this study also suggest that lutein diacetate is readily dissociated following ingestion and free lutein is absorbed.

2.3. Absorption, Distribution, Metabolism and Elimination

Available information suggests that daily consumption of lutein and zeaxanthin in Americans from dietary sources (mainly fruits and vegetables) ranges from 1.35-1.97 mg (median intake) (CDC, 1998). This intake results in serum lutein concentrations of ~0.4 μmol/L. Oral single dose administration of lutein to human subjects showed peak concentrations in the plasma at 16 hours (Kostic et al., 1995). Supplementation of human with 10 mg of lutein daily for 18 days revealed an increase in plasma lutein concentrations over time (Khachik et al., 1995). In humans consuming supplements containing 16-62 mg/day of lutein esters (equivalents of 8-31 mg/day of unesterified lutein) steady state serum lutein levels of 0.26-1.76 μmol/L were achieved. Lutein appears to be absorbed intact following intestinal hydrolysis of lutein esters.

Khachik et al. (1995) reported identification of at least six lutein metabolites, four of which resulted from oxidation and two from non-enzymatic dehydration.

As fat-soluble compounds, lutein and zeaxanthin are likely to follow the same digestion and intestinal absorption pathways as dietary fat. Following absorption, both lutein and zeaxanthin are incorporated into chylomicrons, and are approximately evenly distributed between high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in the circulation (Olson, 1996; Furr and Clark, 1997; Goulinet and Chapman, 1997). Lutein from lipoprotein fraction appears to be distributed to extra-hepatic tissue following the interaction of lipoprotein particles with receptors, and degradation of lipoproteins by extra- hepatic enzymes such as lipoprotein lipase (Boileau et al., 1999). Yemelyanov et al. (2001) isolated a specific xanthophyll-binding protein from human macula that mediates the uptake of lutein and zeaxanthin from the bloodstream. Information from other carotenoid investigations suggests that lutein is likely to be excreted primarily through the bile in to feces (Leo et al., 1995), with some excretion by subcutaneous glands and sweat but not urine (Bendich, 1988). These and other studies on the metabolism of lutein and lutein esters are extensively described in the FDA GRAS notices.

In summary, available metabolism studies indicate that administration of lutein or lutein esters results in lutein being bioavailable. Lutein esters are hydrolyzed in the gut to unesterified lutein and it is the unesterified lutein that enters the circulation. Following absorption, lutein is incorporated into chylomicrons, and distributed in HDL and LDL in the circulation. Extra-hepatic tissue uptake of lutein appears to be receptor binding and mediated by degradation by lipase. Lutein is likely to be excreted primarily through the bile in to feces. Available metabolism-related information suggest that the results from studies that used lutein esters or unesterified lutein are equally valid for the safety assessment of lutein esters (in this case lutein diacetate).

2.4. Recent Toxicological Studies

In recent years, because of its potentials to the healthy structure and function of human body, lutein or its esters has become the subject of intense investigations. As a result, there have been considerable efforts to elucidate the biological role and safety of lutein and its esters. As a result, there is a vast published literature on lutein esters or unesterified lutein. Since the JECFA evaluation and most recent GRAS notification (GRN 000221), several publications have appeared on this subject in the publicly available databases. Relevant safety-related findings from these recent publications are summarized in the following sections.

2.4.1. Animal Studies

Khachik et al. (2006b) investigated the effects of lutein, or zeaxanthin, or a combination of the two, on changes in plasma levels of these carotenoids as well as any effects on ocular and renal toxicity in a one year study in monkeys. In this study, female rhesus monkeys were divided into control (n = 3), lutein-treated (n = 5, 9.34 mg lutein/kg and 0.66 mg zeaxanthin/kg), zeaxanthin-treated (n = 5, 10 mg zeaxanthin/kg), and lutein/zeaxanthin-treated (n = 5, lutein and zeaxanthin, each 0.5 mg/kg). The animals were supplemented with these levels daily for a period of 12-months. Supplementation with lutein or zeaxanthin increased the plasma and ocular tissue concentrations of these carotenoids and their metabolites. Supplementation did not cause ocular toxicity and had no effect on biomarkers and indicators of renal toxicity such as urinary creatinine and protein. The results of this study suggest that administration of either lutein or

zeaxanthin to monkeys for 1 year at a dose of approximately 10 mg/kg body weight/day did not cause ocular or renal toxicity.

Harikumar et al. (2008) investigated toxicity profile of lutein and lutein esters isolated from marigold flowers (*T. erecta*) in Wistar rats. In two separate dose-response investigations, lutein and its ester form (fatty acid esters) dissolved in sunflower oil were administered daily orally (gavage) at doses of 4, 40, and 400 mg/kg body weight for 4 weeks and for 13 weeks to Wistar rats (5/sex/group). The control group was treated with sunflower oil (vehicle only). The dose of lutein ester was prepared as molar equivalent of lutein. Administration of lutein or its ester did not result in any mortality, or changes in body weight, food consumption, and organ weight. Administration of lutein and its ester form did not alter the hepatic and renal function as evaluated by serum chemistry parameters, and did not produce any treatment-related alterations in the hematological parameters and lipid profile. Histopathological examination of the organs did not reveal any treatment-related changes. The results of this study suggest that the no-observed adverse effect level (NOAEL) of lutein (unesterified or in ester form) was 400 mg/kg body weight/day, the highest dose tested.

2.4.2. Lutein Diacetate (MICRO-MIC™) Subchronic Study

In a 90 day dose-response study, Kumar et al. (2009) evaluated the safety of lutein diacetate 20% (the subject of this GRAS assessment; MICRO-MIC™) at doses up to 210 mg/kg/day in rats. In this study, 120 Sprague-Dawley rats (10/sex/group; 6-7 week old) were administered (gavage) once daily with lutein acetate at dose levels of 0 (vehicle control), 2.1 (low dose), 22.5 (mid dose), and 210 (high dose) mg/kg body weight for 90 days. In addition to these groups, control and high dose recovery group were also included as part of the study. The test substance was dissolved in corn oil. The study was conducted in accordance with the Principles of Good Laboratory Practice for the testing of Chemicals OECD [C(97)/186- Final)]. All rats were observed for clinical signs, mortality, ophthalmic abnormalities, neurological changes (functional observation battery), body weight changes and food consumption changes. At the end of treatment and recovery period, hematological and clinical chemistry parameters were investigated. At termination, rats were subjected to detailed necropsy and weights were recorded for liver, adrenal glands, kidney, ovaries, testes, epididymides, uterus, thymus, spleen, brain and heart. Over 40 tissues and organs were harvested at the necropsy and fixed in 10% buffered neutral formalin. Histopathological examination was carried on full set of tissues collected from the high dose and control groups. All gross lesions from all rats irrespective of group were also evaluated for histological changes.

There were no treatment-related clinical signs observed during the course of study in the low and mid dose group animals. In the high dose group, stained (light brown colored) feces were noted. In the high dose recovery group, stained feces were noted during the first four days of the recovery. The feces coloration was likely due to the direct contact of the test article with the contents of the gastrointestinal tract. Administration of lutein diacetate did not reveal any changes in ophthalmologic or neurological examinations. Compared to control group, no changes in mean body weight, net body weight gains or food intake were noted. Lutein diacetate administration did not affect organ weights or relative (to body weight) organ weights ratios. Lutein diacetate treatment did not affect hematology and clinical chemistry parameters. Histological examinations did not reveal any treatment-related microscopic changes. A single incidence of deciduoma observed in a high dose treated female rat was not considered as treatment-related. Minor changes noted in some of the parameters in the study were not

considered as treatment-related. The results of this study suggest that oral administration of lutein diacetate at doses up to 210 mg/kg/day did not cause toxicological or histopathological abnormalities. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for lutein diacetate is determined as 210 mg/kg/day, the highest dose tested (Kumar et al., 2009).

2.4.3. Mutagenicity and Carcinogenicity Studies

In two separate *in vitro* genotoxicity tests, Wang et al. (2006) investigated the mutagenic and clastogenic potentials of lutein from marigold flowers in Ames test and chromosome aberration. The mutagenicity of lutein was examined at concentrations of 334, 668 and 1335 μg/plate using the standard Ames test (*Salmonella typhimurium* strains TA97, TA98, TA100 and TA102) in the presence and absence of S9 mix. In this assay, lutein was not mutagenic at any of the tested concentrations. In anti-mutagenic experiments using *S. typhimurium* strains TA98 and TA100 following addition of the known mutagens (2-aminofluorene and dexon for TA98 and cyclophosphamide and sodium azide for TA100) and lutein, a dose-related anti-mutagenic effect of lutein was noted. Similar results were obtained in a standard chromosome aberration test using Chinese hamster ovary (CHO) cells to evaluate the clastogenicity and anti-clastogenicity of lutein (66.8, 133.5 and 267.0 mg/L). The results of these most commonly used methods (Ames test and the CHO chromosomal aberration) for the evaluation of genetic mutation and chromosome damage caused by mutagens and clastogenic compounds, provide supportive data that lutein is neither mutagenic nor clastogenic.

Santocono et al. (2006) examined the ability of carotenoids, including lutein, to protect against UVA-induced DNA damage in rat tracheal epithelial cells and in human neuroblastoma cells using the "comet assay," a rapid and sensitive single-cell gel electrophoresis technique used to detect primary DNA damage in individual cells. In both cell lines, the irradiation with UVA resulted in time-dependent DNA damage. The rate of DNA damage was different in these two cell lines with neuroblastoma cells more resistant to the oxidative irradiation insult. In the case of neuroblastoma cells, the presence of carotenoid during UVA exposure increased the damage. The addition of carotenoids to epithelial cells after 2 min of UVA exposition did not improve the kinetics of DNA repair, but lutein (after 180 min incubation) showed a genotoxic effect. The addition of carotenoids, including lutein, to neuroblastoma cells after 30 min of UVA exposure positively influenced the kinetics of DNA repair in the first 15 min of incubation. At longer exposure times, while the behavior measured was not constant, a genotoxic effect was not observed. The investigators concluded that the effectiveness of carotenoids, including lutein, as antioxidants depends on a number of factors and it can also act as prooxidant. Available evidence from other studies indicate that the effect of lutein and other carotenoids as antioxidants depends on number of factors such as concentration, cell type, cell status, timing of insult exposure, location in the cell, interaction with other antioxidants, etc, and the results of this *in vitro* study are difficult to interpret.

In an *in vivo* study, Moreno et al. (2007) reported that treatment with lutein during the promotion phase of carcinogenesis in a rat model of hepatocarcinogenesis inhibited the size of hepatic macroscopic nodules and DNA damage. In this study, the effects of lutein on hepatic preneoplastic lesions and DNA strand breakage induced in Wistar rats (initiation with diethylnitrosamine and promotion with 2-acetylaminofluorene coupled with partial hepatectomy) were investigated following lutein (70 mg/kg; alternate day) administration (gavage) specifically during the initiation or promotion phase, for 2 and 6 weeks, respectively. Administration of

lutein during the initiation phase neither inhibited nor induced hepatic preneoplastic lesions and DNA damage. On the other hand, lutein administration during the promotion phase inhibited the size of hepatic macroscopic nodules and DNA damage. The results of this study suggest that lutein acts as an inhibitor during promotion, but not initiation of hepatocarcinogenesis.

2.5. Human Clinical Studies

In a randomized trial, forty-five subjects with no age-related macular degeneration (AMD), large drusen, or advanced AMD, received one of three doses of lutein (2.5, 5, or 10 mg) daily for 6 months (Rosenthal et al., 2006). Besides collecting information about adverse events, safety was assessed by: visual acuity, comprehensive ophthalmic examination, fundus photography, liver function tests, visual field tests, and the "Age-Related Eye Disease Study" (AREDS) side-effect questionnaire. Supplementation with lutein resulted in a dose-related increase in serum lutein concentration. The increases in serum lutein levels did not vary with AMD disease severity. No toxicity was observed with these dose levels of lutein. No adverse side effects were recorded on the AREDS side-effects questionnaire or in visual function. Liver function test results remained unchanged and normal. The antioxidant vitamin levels in the serum were not suppressed by lutein supplementation. The results of this study indicate that administration of lutein at doses up to 10 mg/day was safe (Rosenthal et al., 2006).

Following the publication of the above described study (Rosenthal et al., 2006), Gaynes (2007), in a Letter to Editor, questioned the conclusion drawn by Rosenthal et al. (2006) that "the highest dose of lutein (10 mg) was safe as a supplement." Gaynes (2007) claimed that on the basis of both the content and internal study validity, the conclusion drawn by Rosenthal et al. (2006) is a result of oversimplification of essential concepts in clinical drug testing as related to study statistical power and detectable event rate. In this communication, Gaynes (2007) also suggested that animal studies have identified three target organs for lutein bioaccumulation following intravenous administration, and suggested that lutein may be metabolized resulting in liver toxicity. Chew et al (2007) in a rebuttal, agreed with Gaynes (2007) that the study design has low statistical power to detect adverse side effects. However, they claimed that the totality of evidence based on other studies suggests that the dose of lutein of 10 mg/day is safe.

Chew et al. (2007) indicated that oral lutein supplementation may result in different metabolic patterns than if administered *via* an intravenous route. They noted that the oral supplementation of lutein to female monkeys increased concentrations of lutein in both plasma and tissues (liver, lung, colon, kidney, breast, ovaries, spleen, cervix, and other ocular tissue) without detectable toxicity. Although lutein is a member of the carotenoid family, it does not share the metabolic pathways of β-carotene because it is not a substrate for the 15,15'-monooxygenase enzyme that cleaves β-carotene into vitamin A. Lutein, therefore, does not possess provitamin A activity and is unlikely to cause liver toxicity.

In a randomized, double-blinded clinical trial, Khachik et al. (2006a) investigated the effect of lutein (with 6% zeaxanthin) supplementation at doses of 2.5, 5.0, and 10 mg/day for 6 months on serum carotenoid distribution in 45 elderly human subjects (> 60 years of age), with or without age-related macular degeneration. Supplementation with lutein (10 mg/day) resulted in a significant increase in serum lutein and its metabolite levels. The increase in the serum levels of lutein/zeaxanthin correlated with increases in the serum levels of their metabolites. Although data were not presented, the investigators stated in the manuscript that based on results of liver function tests and visual function examinations, no toxicity or side effects were

associated with supplementation with lutein at dose levels up to 10 mg/day. These investigators concluded that elderly human subjects with and without AMD can safely take lutein supplements at doses up to 10 mg/day for 6 months with no apparent toxicity or side effects.

In two separate reports, a group of researchers from Australia (Vu et al., 2006; Robman et al., 2007) reported complex interactions between lutein/zeaxanthin and ω-3 fatty acids intake with the *progression* of AMD. In the study by Robman et al. (2007), 254 subjects identified with early AMD were re-examined to determine over a seven year period, the progression of AMD. Intakes of lutein/zeaxanthin and fatty acids were estimated from food frequency questionnaires. Energy-adjusted lutein/zeaxanthin intake as a continuous variable was associated with AMD progression in the worst affected eye when defined by the most stringent criterion. Energy-adjusted ω-3 fatty acid intake was also found to be associated with AMD progression. In the previous study by this group, Vu et al. (2006) also reported (in the letter section of the British Journal of Ophthalmology) a marked increase in the risk of both early and late AMD among people who consumed greater than the median intake of linoleic acid and higher dietary intakes of lutein/zeaxanthin. These investigators stated that "based on these data, lutein/zeaxanthin supplementation could not be recommended". This second report used cross-sectional data based on photographic macular assessments of 71.9% of their sample of 2448 persons, who attended follow-up examinations. Several factors as discussed below may have affected the outcome of these reports, and the available evidence does not result in a clear picture of the association of lutein/zeaxanthin and lipid intake with AMD.

Contrary to the findings of Vu et al. (2006) and Robman et al. (2007), Flood et al. (2006; reported as Letter to Editor) did not find any association with energy adjusted lutein/zeaxanthin intake (n = 986) and the incidence of early, late or any AMD, whether or not this was stratified by linoleic acid intake. The median linoleic acid intake in this study was less than the median used by Vu et al. (2006) (6.6 g verses 7.2 g). However, stratification of the data by the highest tertile of linoleic acid intakes (cut-point 8.5 g) also did not reveal any association between lutein/zeaxanthin and incidence of AMD. These investigators suggested that while the examination of cross-sectional data to investigate associations with disease may be useful, conclusions drawn from such data need to be made with care, in light of other known literature.

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

Satia et al. (2009) examined associations of supplemental beta-carotene, retinol, vitamin A, lutein, and lycopene with lung cancer risk among participants, aged 50-76 years, in the VITamins And Lifestyle (VITAL) cohort study in Washington State. For this study, eligible individuals (n = 77,126) completed a 24-page baseline questionnaire, including detailed questions about supplement use (duration, frequency, dose) during the previous 10 years from multivitamins and individual supplements/mixtures. Incident lung cancers (n = 521) were

identified by linkage to the Surveillance, Epidemiology, and End Results cancer registry. Longer duration of use of individual beta-carotene, retinol, and lutein supplements (but not total 10-year average dose) was associated with statistically significantly elevated risk of total lung cancer and histologic cell types. The investigators concluded that long-term use of individual beta-carotene, retinol, and lutein supplements should not be recommended for lung cancer prevention, particularly among smokers. Similarly, earlier in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial also it was noted that a sub population (alcohol users) seemed to be more associated with risk of cancers (Omaye et al., 1997). Satia et al. (2009) also noted that generalizability from their study is limited by the fact that the VITAL cohort is predominantly white and generally healthy, which is reflected in the fact that there were fewer current smokers than in the US population as a whole. In other reports by van den Brandt et al. (2002) and Vandenbroucke and Psaty (2008) have extensively discussed limitation of observational studies. Vandenbroucke and Psaty (2008) raised concerns on the credibility of observational research about harms. The available evidence indicate that intake of lutein is safe for general healthy population.

In a double-blinded, randomized, placebo-controlled clinical trial with a cross-over design, 34 adult patients with retinitis pigmentosa were randomized into two groups (Bahrami et al., 2006). One group (n = 16), received lutein supplementation (10 mg/day for 12 weeks followed by 30 mg/day) for the first 24 weeks and then placebo for the following 24 weeks, while the other group (n = 18) received placebo treatment (24 weeks) prior to lutein (received placebo during the first half and lutein in the second half). Supplementation with lutein did not result in any significant adverse effects. One participant on lutein, and two participants on placebo had impaired liver function tests at one of their 6-week visits, but in all three of these subjects the serum liver enzyme levels (markers of liver function test) returned to the normal range when tests were repeated. The investigators concluded that lutein supplementation at 10-30 mg/day for up to 6 months is safe.

In a 16-week prospective, randomized, double-blinded, controlled and with parallel groups study, healthy term infants were fed either control formula or experimental formula with lutein at 200 μg/l (Capeding et al., 2010). In this study 232 infants ≤14 days postnatal age were randomized and 220 (94.8%) completed the study. At 4, 8, 12, and 16 week, weight, head circumference, and length were measured. Safety was assessed through monitoring of adverse events throughout the study and evaluation of selected blood chemistry tests (albumin, alkaline phosphatase, total bilirubin, blood urea nitrogen, calcium, creatinine, glucose, phosphorus, and total protein) were performed at the end of the study. No differences between treatment groups were found in any of the measures of growth at any of the measurement time points. Study formulas with and without lutein were well tolerated. The mean values of all measured blood chemistry parameters fell within the modified normal ranges for infants, and the values for both groups for any measured parameter were similar. The frequency and severity of the adverse events recorded in the study were similar between treatment groups, were not judged by the investigators to be formula related. The results of this study revealed that infants fed lutein-fortified formula demonstrated growth equivalent to that of infants fed unfortified lutein formula.

In a recently published, randomized, controlled, multicenter trial, Rubin et al. (2011) compared plasma carotenoid levels among preterm infants (n=203, <33 weeks gestational age) fed diets with and without added lutein, lycopene and β-carotene with human milk-fed term infants. The investigators also assessed safety and health. In the supplemented group, plasma

carotenoid levels were higher at all time points and were similar to those of term human milk-fed infants. Infants supplemented with carotenoids had lower plasma C-reactive protein. Plasma lutein levels correlated with the full field electroretinogram-saturated response amplitude in rod photoreceptors and the supplemented group also showed greater rod photoreceptor sensitivity. The results of this study suggest that carotenoid supplementation for preterm infants raises plasma concentrations to those observed in human milk-fed term infants.

2.6. 'Observed Safe Levels'- Risk Assessment

In a review article, Shao and Hathcock (2006) undertook an extensive and systematic review of the safety database of lutein and performed a risk assessment on exposure. In this assessment, a newer method termed the "Observed Safe Level" (OSL) was utilized for risk assessment. Based on the comprehensive review of safety data, the OSL for lutein was identified as 20 mg/day. The OSL risk assessment method provided strong evidence for support of safety of lutein intake at levels up to 20 mg/day. The authors reported that although much higher levels have been tested without adverse effects and may be safe, the data for intakes above 20 mg/day are not sufficient for a confident conclusion of long-term safety. In this assessment of the thirty peer-reviewed, published human clinical trials involving lutein, 11 most relevant studies regarding safety were considered including two studies which appeared during the year 2006. Published relevant human clinical trials involved lutein doses of 8, 10, 12, 15, 20, 20.5, 30 and 40 mg/day. The trial with the highest dosage of lutein (40 mg/day) was for nine weeks followed by an additional 17 weeks at 20 mg/day in patients with retinitis pigmentosa. The longest duration trial was 12 months at a lutein dose of 10 mg/day in AMD patients. No adverse effects were observed in any of the clinical trials. The authors stated that the absence of any pattern of adverse effects in any of the published human trials provides support for a high level of confidence in the safety of lutein. Based on the complete absence of adverse effects in all the published human trials using lutein doses above, at, and below the 20 mg level and other considerations, 20 mg/day was designated as the OSL. In addition to these human clinical trials, Shao and Hathcock (2006) also considered animal data to determine the NOAEL. A dose of 639 mg/kg/day in rats for 4 weeks had no adverse effects, and this dose was determined as NOAEL. The application of a 1000-fold uncertainty factor (UF) by these individuals would result in an acceptable daily intake of 38 mg/kg/day. This dose is higher than that determined on the basis of human studies, and also supports the safety of lutein at 20 mg/day.

2.7. Safety of Vitamin E

Given the common natural food presence, fortification as an essential nutrient and approval for multipurpose direct food addition of vitamin E (α-tocopherol), the safety of direct food addition of this component in the Lutein Diacetate 20% product (MICRO-MIC™) is considered well established at moderate intake levels. As with most essential vitamins and minerals, health concerns may be associated with excessive intake. The recommended dietary reference intake (DRI) of vitamin E is in the range of 15-19 mg/day for adolescents, adults and lactating females. The National Academy of Sciences (NAS) has determined that the upper limit (UL) of α-tocopherol intake as a dietary supplement was 1000 mg/day in adults and 800 mg/day in adolescents and pregnant women. According to NAS, the UL is a maximal level of nutrient intake that is likely to pose no risks of adverse effects. The estimated maximal intake of vitamin E of approximately 0.98 mg/person/day (0.016 mg/kg body weight/day) from likely consumption of Lutein Diacetate 20% product (MICRO-MIC™) is a low additional intake of vitamin E in

comparison to normal dietary intake, dietary supplementation or acceptable NAS UL values and therefore, does not pose any safety concerns.

3. SUMMARY AND DISCUSSION

IOSA intends to use Lutein Diacetate (MICRO-MIC™), a standardized extract obtained from a variety of Aztec Marigold flowers (*T. erecta* L) as a nutrient supplement at concentrations of up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12) in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, 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. Lutein Diacetate 20% (MICRO-MIC™) is a red orange oily suspension with characteristic odor. Compositional analysis of Lutein Diacetate 20% (MICRO-MIC™) demonstrated that product primarily contains lutein diacetate with small amount of zeaxanthin diacetate, carbohydrate and protein. The product contains corn oil as an excipient. The intended use of Lutein Diacetate 20% (MICRO-MIC™) in the specified food categories will result in 90th percentile estimated daily intakes of 13.4 mg lutein/person/day and 0.6 mg zeaxanthin/person/day.

There is sufficient qualitative and quantitative scientific as well as history of use evidence to determine the safety-in-use of Lutein Diacetate 20% (MICRO-MIC™) and its constituents in the above mentioned applications. As constituents of Lutein Diacetate 20% are components of diet, the history of consumption of 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. Presence of lutein and zeaxanthin has been reported in human breast milk. Lutein esters are commonly marketed as dietary supplements and its recommended doses range from 0.5 to 12 mg/day. Lutein and its ester has been the subject of four GRAS notices to FDA. In these submissions, use of lutein in specified food categories was estimated to result in levels of up to 13.4 mg/person/day.

In response to four separate GRAS notifications on lutein (lutein ester- GRN 000110; crystalline lutein- GRN 000140; suspended lutein- GRN 000221; crystalline lutein- GRN 291), FDA did not question the safety of lutein ester, crystalline lutein and suspended lutein for the specified food uses. The subject of present GRAS determination lutein diacetate is an esterified form and is substantially equivalent to lutein ester that has been the subject of FDA GRAS notified substance (GRN 000110) except that the FDA notified substance was ester of dipalmitate (fatty acid), while the current product is in the form of diacetate (ester). Following oral ingestion, lutein esters are hydrolyzed in the gastrointestinal to unesterified lutein and it is the unesterified lutein that is absorbed and enters circulation. Available human clinical and animal studies suggest that administration lutein ester or its free form results in lutein being bioavailable. Thus results from studies that used esterified or free forms of lutein are equally valid for the safety assessment of lutein ester (or diacetate).

The FDA responses to GRAS notifications on lutein and its esters indicate that the FDA 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. Recent studies

that appeared after these assessments also did not reveal any findings that affect the conclusion from previous determinations. The results of recent studies show that supplementation of rhesus monkeys with lutein at a dose level of 10 mg/kg/day for one year did not cause ocular toxicity and had no effect on biomarkers associated with kidney toxicity (urinary creatinine and protein). The results of a recent genotoxicity study suggest that lutein is neither mutagenic nor clastogenic. In an *in vitro* study, the presence of lutein reduced DNA damage when rat epithelial cells were exposed to UVA radiation, while increased DNA damage was noted in neuroblastoma cells following exposure to UVA, and in the presence of lutein. The differential effect noted in rat epithelial cells and neuroblastoma cells is difficult to interpret and data from other reports from repair rates for UVA-induced damage vary greatly. In an *in vivo* rat model of hepatocarcinogenesis, treatment with lutein during the promotion phase of carcinogenesis was found to inhibit the size of hepatic macroscopic nodules and DNA damage.

In two cross-sectional studies by the same group (Vu et al., 2006; Robman et al., 2007), an association between intake of lutein/zeaxanthin and ω-3 fatty acids with progression of age-related macular degeneration (AMD) was reported. Conversely, in another report (Flood et al., 2006) no such association was observed. The cross-sectional studies using dietary survey data have limitations such as it measures the current diet in a group of people with a disease which may well be altered by the presence of the disease. A further limitation of cross-sectional studies may be due to errors in recall of the exposure and possibly outcome. Furthermore, other clinical studies did not reveal any toxic effects of lutein. In an extensive review article on the safety and risk assessment of lutein, use of "observed safe level" methodology for risk assessment revealed a strong evidence for safety of lutein intake at levels up to 20 mg/day for human. In this assessment, the authors suggested that much higher levels (up to 40 mg/day) may be safe, but the data for intakes above 20 mg/day are not sufficient for a confident conclusion of long-term safety (Shao and Hathcock, 2006).

In a well designed dose-response subchronic toxicity study, lutein diacetate 20% (MICRO-MIC™) was administered (gavage) daily to rats at doses of 0, 2.1, 22.5 and 210 mg/kg body weight 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 lutein diacetate is determined as 210 mg/kg/day, the highest dose tested. The estimated daily intake of Lutein Diacetate 20% (MICRO-MIC™) from the intended food uses is about 1000 fold lower compared to the NOAEL determined from the subchronic toxicity study. Similarly, the estimated daily intake of lutein from the intended uses of Lutein Diacetate 20% (MICRO-MIC™) is approximately 1000 fold lower compared to the NOAEL of 200 mg/kg body weight/day from the rat study used to establish the JECFA ADI.

In summary, on the basis of scientific procedures[4], history of exposure and use, the consumption of Lutein Diacetate 20% (MICRO-MIC™) derived from Marigold flowers (*Tagetes erecta* L) as a nutrient supplement at use levels of 0.3 to 3 mg/serving in certain specified foods resulting in a 90th percentile intake of 13.4 mg lutein/person/day and 0.6 mg zeaxanthin/person/day is considered safe. The proposed uses are compatible with current regulations, *i.e.*, Lutein Diacetate 20% (MICRO-MIC™) is used as a nutrient supplement [21 CFR 170.3(o)(20)] baked goods and baking mixes, beverages and beverage bases, breakfast

[4] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

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).

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 Lutein Diacetate (MICRO-MIC™) as a nutrient supplement [21 CFR 170.3(o)(20)] in selected food products [baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes] at levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12) when not otherwise precluded by a Standard of Identity as described in this monograph and resulting in the 90th percentile all-user estimated intake of 13.4 mg lutein/person/day is 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 Lutein Diacetate (MICRO-MIC™), when used as described, is GRAS based on scientific procedures.

Signatures

(b) (6)

John A. Thomas, Ph.D., F.A.C.T., D.A.T.S. — Date 4/20/12

(b) (6)

Stanley T. Omaye, Ph.D., D.A.T.S. — Date 4/17/12

(b) (6)

Madhusudan G. Soni, Ph.D., F.A.C.N. — Date 4/24/12

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6. APPENDIX I

Analytical data from five non-consecutive manufacturing lots of lutein diacetate 20% (MICRO-MIC™) (IOSA, 2011)*

Parameters	908J1MD2085	910FMA2085	915M2MC2085	923M1MB2085	930EM2085
Appearance	Oil Suspension	Oil Suspension	Oil Suspension	Oil Suspension	Oil Suspension
Odor:	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Identity	1.09 – 1.11	1.09 – 1.11	1.09 – 1.11	1.09 – 1.11	1.09 – 1.11
Density	0.96	0.96	0.96	0.96	0.96
Moisture (%)	0.2%	0.2 %	0.2 %	0.2 %	0.2 %
Excipient	Corn Oil	Corn oil	Corn oil	Corn oil	Corn oil
Composition: Carotenoids					
Total Carotenoid Esters (%)	24.68	24.75	24.40	24.91	23.45
Lutein Diacetate (%)	21.88	21.42	21.53	21.06	20.60
Zeaxanthin Diacetate (%)	1.42	1.39	1.40	1.37	1.34
Heavy metals (ppm)					
Lead	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
Arsenic	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Mercury	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Microbiology assay:					
Total plate count (cfu/g)	<1000	<1000	<1000	<1000	<1000
Yeast and molds (cfu/g)	<100	<100	<100	<100	<100
E. coli and Salmonella	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
Pseudomonas euriginosa	Negative	Negative	Negative	Negative	Negative
Staphylococcus	Negative	Negative	Negative	Negative	Negative

*Based on information and data provided by IOSA

Reference Materials

Pages 000046-000097 have been removed in accordance with copyright laws. The removed references are:

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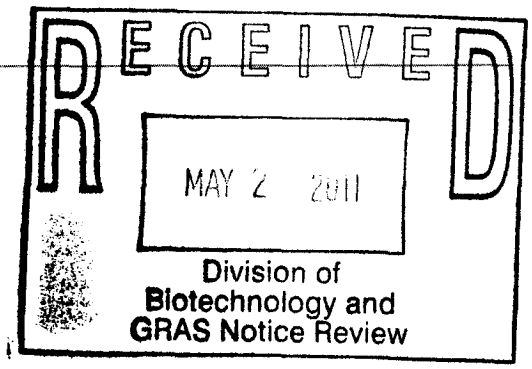
Ramos-Valle, Moraima



From: Dausch, Judith
Sent: Tuesday, May 22, 2012 10:10 AM
To: Ramos-Valle, Moraima
Subject: FW: GRSN for lutein Diacetate
Attachments: May 4 Claim revised page 2.pdf

Hi Moraima,

Here is the revised page you requested from Mark Itzkoff regarding the resubmission of GRN 416 lutein diacetate. Thank you.

Judy

From: Mark Itzkoff [mailto:mitzkoff@ofwlaw.com]
Sent: Tuesday, May 22, 2012 10:03 AM
To: Dausch, Judith
Subject: GRSN for lutein Diacetate

Dr. Dausch,

I received a phone call from someone at FDA asking that we make a minor change in page 2 of the GRAS Notice we submitted on IOSA'a behalf two weeks ago. Unfortunately, our voice mail had a glitch and I was unable to hear the person's name.

The revised page 2 is attached. If you know who requested the amendment, could you please forward it to her?

If you have any questions, please let me know.

Mark

Mark L. Itzkoff
Olsson Frank Weeda Terman Matz PC
202 518-6327
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.

B. Common or Usual Name of Substance:

The common or usual name of the substance is lutein diacetate. No Chemical Abstract Services Registration Number (CASRN) is available for this substance. It will be supplied as a corn oil suspension known by its trade name, *Micro-Mic*™.

C. Conditions of Use:

Lutein diacetate 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 lutein diacetate as a 20% solution under the trade name *Micro-Mic*™. The food applications for lutein diacetate along with corresponding lutein equivalent levels are detailed in Table 1.

D. Basis for GRAS Determination:

In October of 2011, the status of the IOSA lutein diacetate 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 lutein and lutein diacetate . The Expert Panel concluded that, based on scientific procedures, the use of lutein diacetate 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.

SUBMISSION END