GR

ORIGINAL SUBMISSION

OLSSON FRANK WEEDA
TERMAN BODE MATZ PC
ATTORNEYS AT LAW

SUITE 400
1400 SIXTEENTH STREET, N.W.
WASHINGTON, D. C. 20036 (202) 789-1212
www.ofwlaw.com

PHILIP C. OLSSON
RICHARD L. FRANK
DAVID F. WEEDA (1948-2001)
DENNIS R. JOHNSON
ARTHUR Y TSIEN
JOHN W. BODE*
STEPHEN D TERMAN
MARSHALL L MATZ
MICHAEL J. O'FLAHERTY
DAVID L. DURKIN
NEIL F. O'FLAHERTY
BRETT T. SCHWEMER
TISH E. PAHL
ROBERT A HAHN
EVAN P. PHELPS
GARY H. BAISE

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JOLYDA O SWAIM
KATHRYN E. BALMFORD
JONATHAN M WEINRIEB
NANCY W MATHEWSON

COUNSEL
ROGER R SZEMRAJ

OF COUNSEL
JUR T. STROBOS
JACQUELINE H EAGLE
KENNETH D. ACKERMAN
MARK L ITZKOFF
DAVID A BIEGING
ELLIOT BELILOS

SENIOR POLICY ADVISOR
JOHN R BLOCK
CHARLES W STENHOLM
GEORGE McGOVERN
SALLY S DONNER
BRENT W. GATTIS
*BARBARA J. MASTERS

Mark L. Itzkoff - Of Counsel
Direct (202)518-6327 / Fax (202)234-2686
mitzkoff@ofwlaw.com

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GRAS Claim for the Use of Crystalline Lutein as a Food Ingredient

GRAS Claim

Industrial Organica S.A. de C.V. (IOSA) has determined that crystalline lutein is generally recognized by qualified experts as having adequately shown to be safe through scientific procedures when used as a food ingredient in the applications detailed in Table 1, below. IOSA hereby submits this GRAS claim for the use of crystalline lutein as a food ingredient as specified in Table 1.

A. Name and Address of Notifier:

Industrial Organica S.A. de C.V.
Ave. Almazan No. 100
Col. Topo Chico 64260
Monterrey, Mexico

B. Common or Usual Name of Substance:

The common or usual name of the substance is lutein also known by its trade name, *Hi Fil*™ Lutein. The Chemical Abstract Services Registration Number (CASRN) for this substance is 127-40-2.

C. Conditions of Use:

Lutein that is the subject of this Notice will be used as a food ingredient in a variety of food and beverage applications. These applications are detailed in Table 1.

D. Basis for GRAS Determination:

IOSA has determined that *Hi Fil*™ Lutein is generally recognized by qualified experts as having adequately shown to be safe through scientific procedures. This determination is supported by the opinions of two scientific panels. In June of 2006, the status of the IOSA *Hi Fil*™ Lutein was reviewed by a panel of scientists (the "2006 Panel") assembled by AAC Consulting Group of Kendle International. The 2006 Panel was comprised of three noted toxicologists with expertise in food safety and lutein production. The Panel reviewed the information and the available toxicology literature on lutein and lutein derivatives. Based on this information, the Panel issued an Expert Panel Statement, "Determination Of The GRAS Status Of *Hi Fil*™ Crystalline Lutein for Addition To Select Specified Foods" in which the it

> determined by scientific procedures that addition of *Hi Fil*™ Lutein, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.[1]

In preparation for submitting this Notice, IOSA assembled a second[2] expert panel (the "2008 Panel") to review any relevant additions to the public literature that had been published after the 2006 Expert Panel Statement. The 2008 Panel concluded that the

> studies published over the last two years also do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous self affirmed GRAS determination of lutein by IOSA.[3]

Thus, the basis for our GRAS determination is the Statement of the 2006 Expert Panel and the concurring Statement of the 2008 Panel.

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1 A copy of the 2006 Expert Panel Statement is attached as Appendix I.

2 The 2008 Panel was coordinated by EAS Consulting Group, the successor to the AAC Consulting Group who conducted the 2006 Panel.

3 *Review of safety studies of lutein (2006 – present) for GRAS update,* John Thomas *et al.*, October 24, 2008. A copy of this statement is attached as Appendix II.

E. Data Availability Statement:

The data and information that are the basis for the Notifier's GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at reasonable times at Olsson Frank Weeda Terman Bode Matz PC, 1400 Sixteenth St., NW, Washington DC 20036.

Respectfully Submitted,

Mark L. Itzkoff

Table 1 Proposed Food-Uses for *Hi Fil* ™ Lutein

Food Category	Proposed Food	Use levels (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 levels same as GRN 000140.
*Does not include infant formula.

Section II

Identity of the Notified Substance

Hi Fil™ Lutein (common or usual name lutein) is a purified crystalline extract from the marigold *(Tagetes erecta)* flower. *Hi Fil*™ Lutein typically contains approximately 80-90% by weight total carotenoids, with about 90% as lutein. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table 1.

Table 2
Chemical and Physical Characteristics of Lutein and Zeaxanthin

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical names	xanthophyll; β, ε-carotene-3,3' -diol; vegetable lutein; vegetable luteol; all-trans-(+)-xanthophylls; lutein	β, β -carotene-3,3'-diol; all-trans- β, β-carotene-3,3' -diol; zeaxanthol
Empirical formula	$C_{40}H_{56}O_2$	$C_{40}H_{56}O_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

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

Figure 1 Structural Formula of Lutein

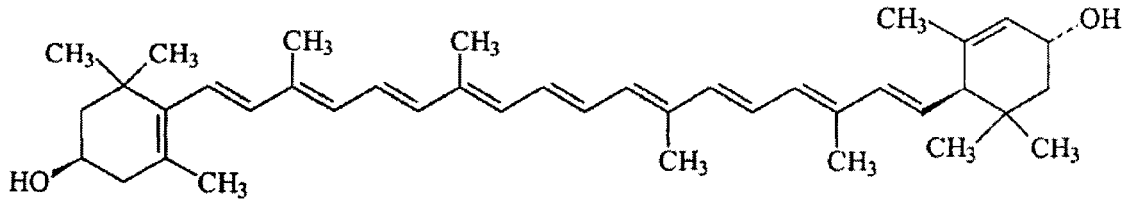
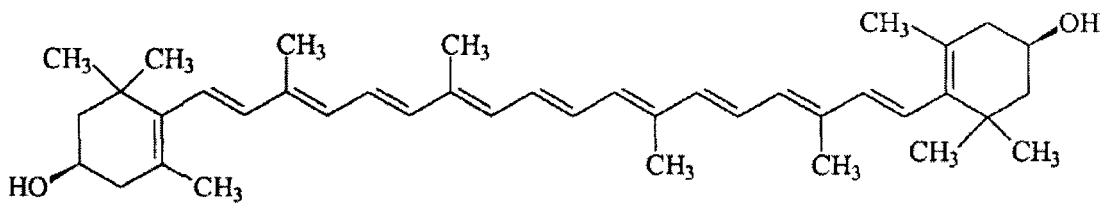


Figure 2 Structural Formula of Zeaxanthin



Specification of *Hi Fil* ™Crystalline Lutein

Crystalline lutein has been the subject of a prior GRAS notification to FDA by Kemin Foods. FDA replied with its "no question" letter on June 14, 2004 to GRN 000140. Therefore, comparisons of the *Hi Fil*™ Lutein product and the notified Kemin's FloraGLO® product are made in this GRAS determination for the purposes of showing the substantial equivalence of the two products and the direct applicability of the FDA acceptance of GRN 000140 to IOSA's *Hi Fil*™ Lutein product for specified foods. The data submitted to FDA in GRN 000140 is incorporated by reference into this Notice.

The specifications of *Hi Fil*™ Lutein are given in Table 3 and compared to Kemin's FloraGLO® Lutein in the table below. This table shows that the specifications for the two lutein products are nearly identical, with *Hi Fil*™ Lutein having a lower hexane specification as the only notable difference. Although the total carotenoid specifications are different, the lutein and zeaxanthin specifications are the same and both products are derived from marigold flowers, so actual products are likely to have same or substantially equivalent total carotenoid composition, despite the lower manufacturing specification set by Kemin on total carotenoids.

Table 3

Specifications for *Hi Fil*™ Lutein and FloraGLO® Lutein		
Principal Components	***Hi Fil*™ Lutein**	**FloraGLO® Lutein**
Total carotenoids (including lutein and zeaxanthin)	≥ 90.0% wt	≥ 80.0% wt
Lutein	≥ 74.0%	≥ 74.0% wt
Zeaxanthin	≤ 8.0% wt	≥ 2.0% wt; ≤ 9.0% wt
Waxes	≤ 7.0% wt	≤ 14.0%wt
Moisture	≤ 1.0%wt	≤ 1.0%wt
Ash	≤ 1.0%wt	≤ 1.0%wt
Other constituents	≤ 0.1%wt	≤ 0.1%wt
Pesticides and Related Potential Contaminants		
Protein	< 5000 ppm	< 100 ppm
Hexane	< 25 ppm	< 50 ppm
Thiophenes	< 300 ppm	< 300 ppm
Chlorinated and organophosphate pesticides	Not Detected	Not Detected
Heavy Metals		
Lead (Pb)	< 1.0 ppm	< 0.65 ppm
Cadmium (Cd)	< 1.0 ppm	< 1.25 ppm
Arsenic (As)	< 1.0 ppm	< 2.5 ppm
Mercury (Hg)	< 1.0 ppm	< 0.150 ppm
Microbiological Assays		
Aerobic plate count	≤ 1000 cfu/g	≤ 1000 cfu/g
E. coli	Negative/10 g	Negative/10 g
Listeria monocytogenes	Negative/25 g	Negative/25 g
Salmonella and *Shigella*	Negative/10 g	Negative/10 g
Staphylococcus aureus	Negative/100 g	Negative/100 g
Coliform	Negative/25 g	Negative/25 g
Yeast count	< 100 cfu/g	< 100 cfu/g
Mold count	≤ 100 cfu/g	< 100 cfu/g

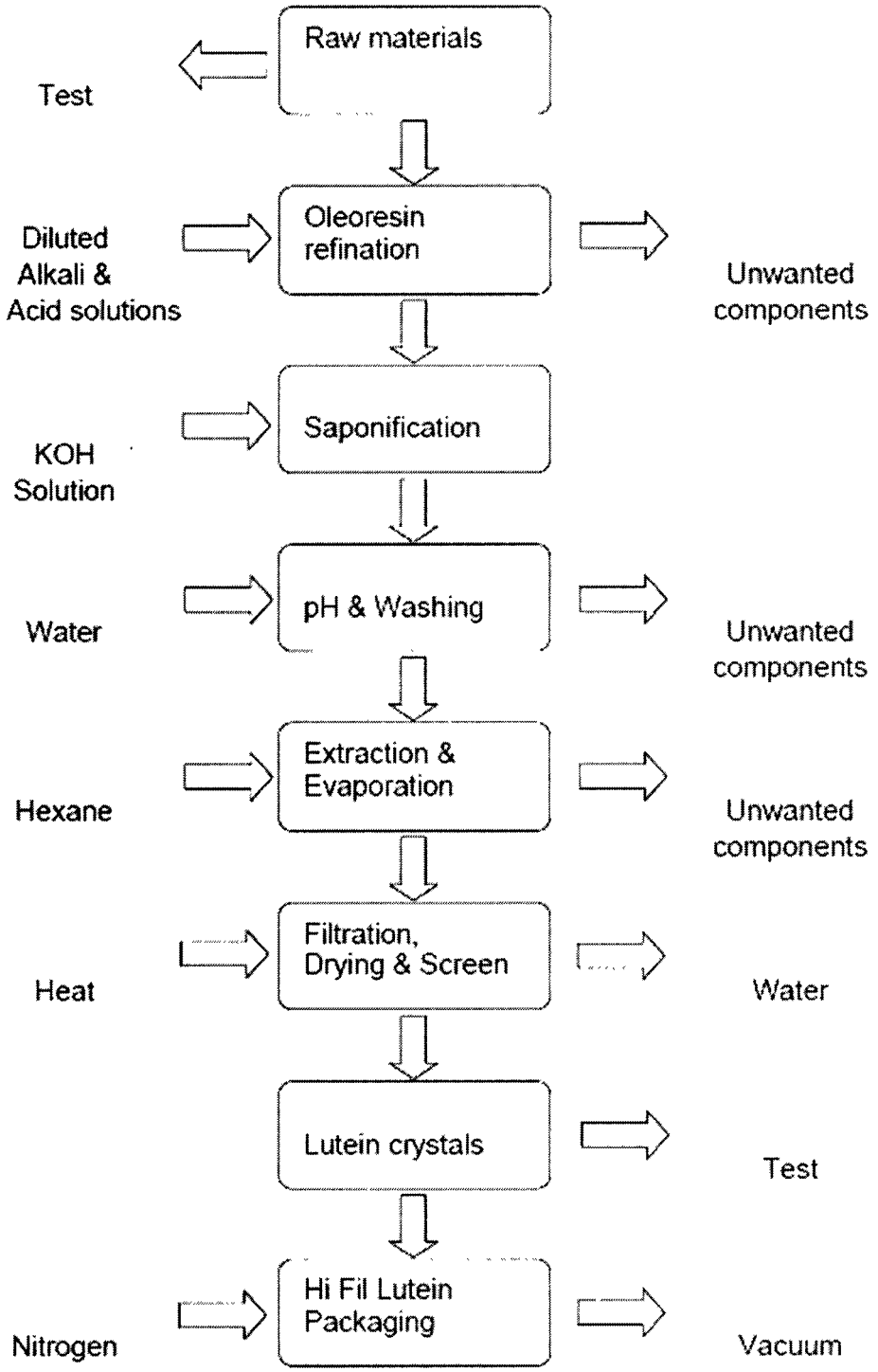
Manufacturing Process

The *Hi Fil*™ Lutein product is manufactured in accordance with current good manufacturing practice at IOSA's Monterrey México plant. The *Hi Fil*™ Lutein process starts with marigold (*Tagetes erecta*) oleoresin as the raw material. The oleoresin material is obtained by hexane extraction of dried marigold flower petals. A process diagram for *Hi Fil*™ Lutein product manufacture is presented in Figure 3.

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. Then the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls. The saponified mass is diluted with water, pH adjusted and the water is used to wash away salts and other water soluble impurities. Lutein concentrate is extracted with hexane to further remove unwanted components. The solvent is separated by filtration, decanting and centrifugation. Lutein crystals are then treated under high vacuum and temperature evaporation to remove remaining solvent. Lutein crystals are filtered and dried to remove water and screened to collect the lutein crystal product of appropriate size. The final crystalline lutein product is packed under high vacuum and inert nitrogen atmosphere in food-grade plastic pouches.

Figure 3.

Manufacturing Process Diagram



Section III

Information on Self-Limiting Levels of Use

The *Hi Fil*™ Lutein product is intended for use in the same foods and levels of addition as notified by Kemin Foods for the FloraGLO® Crystalline Lutein in GRN 000140. The proposed food uses 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, 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. The intended food uses and use levels are presented in Table 4 on the next page. The application of lutein to the same foods and at the same levels as those in GRN 000140 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.

The dietary analysis below was presented by Kemin in GRN 000140 and was not questioned by FDA in their response letter of June 14, 2004. "Lutein and zeaxanthin are among the most prevalent carotenoids in the North American diet (IOM, 2000), occurring in high concentrations in green leafy vegetables, such as spinach and kale (Khachik *et al.,* 1995; Omaye *et al.,* 1997), and in chicken egg yolks (Handelman *et al.,* 1999). In the U.S., the average daily intake of lutein and zeaxanthin from plant sources is estimated to range from 2 to 4 mg. In addition to their natural occurrence in various foods, lutein and zeaxanthin are available as dietary supplements (IOM, 2000); however, there are no consumption data from which to reliably estimate the intake of lutein and zeaxanthin by supplement users. Market share data indicate that lutein use in dietary supplements occurs predominantly in multi-vitamin type supplements."

"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. 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."

Given that the *Hi Fil*™ Lutein product is substantially equivalent in composition and is intended for use in the same foods and 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 for *Hi Fil*™ Lutein and would not be additive to FloraGLO® crystalline lutein, particularly because market use in foods is competitive and current use is predominantly in multi-vitamin supplements.

Table 4.

Summary of the Individual Proposed Food-Uses for *Hi Fil* ™ Lutein

Food Category	Proposed Food	Use levels (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 levels same as GRN 000140.

* Does not include infant formula.

Section IV

Basis for Notifier's Claim

IOSA has determined that *Hi Fil*™ Lutein is Generally Recognized as Safe based on scientific procedures. IOSA is supported in this determination by the findings of two scientific panels.

In June of 2006, the status of the IOSA *Hi Fil*™ Lutein was reviewed by a panel of scientists (the 2006 Panel) assembled by AAC Consulting Group of Kendle International. The expert panel was comprised of three noted toxicologists with expertise in food safety and lutein production. The Panel reviewed the information and the available toxicology literature on lutein and lutein derivatives. Based on this information, the Panel issued an Expert Panel Statement, "Determination Of The GRAS Status Of *Hi Fil*™ Crystalline Lutein for Addition To Select Specified Foods" in which the it

> determined by scientific procedures that addition of *Hi Fil*™ Lutein, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.[4]

In preparation for submitting this Notice, IOSA assembled a second[5] expert panel (the "2008 Panel") to review any relevant additions to the public literature that had been published after the 2006 Expert Panel Statement. The 2008 Panel concluded that the

> studies published over the last two years also do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous self affirmed GRAS determination of lutein by IOSA.[6]

Thus, our GRAS determination is supported by the Statement of the 2006 Expert Panel and the concurring Statement of the 2008 Panel.

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4 A copy of the 2006 Expert Panel Statement is attached as Appendix I.

5 The 2008 Panel was coordinated by EAS Consulting Group, the successor to the AAC Consulting Group who conducted the 2006 Panel.

6 *Review of safety studies of lutein (2006 – present) for GRAS update,* John Thomas *et al.*, October 24, 2008. A copy of this statement is attached as Appendix II.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GRAS STATUS OF *Hi Fil*™ CRYSTALLINE LUTEIN FOR ADDITION TO SELECT SPECIFIED FOODS

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Industrial Organica S.A de C.V. (IOSA)., to determine the Generally Recognized as Safe (GRAS) status of crystalline lutein sold as *Hi Fil*™ Lutein for use as an ingredient for direct addition to select specified foods. A comprehensive search of the scientific literature for safety and toxicity information on lutein was conducted through February 2006 and made available to the Expert Panel. The Expert Panel independently evaluated materials submitted by IOSA and other materials deemed appropriate or necessary. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

Identity and Composition

Hi Fil™ Lutein (common or usual name lutein) is a purified crystalline extract from the marigold *(Tagetes erecta)* flower. *Hi Fil*™ Lutein typically contains approximately 80-90% by weight total carotenoids, with about 90% as lutein. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table 1.

Table 1 Chemical and Physical Characteristics of Lutein and Zeaxanthin

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical names	xanthophyll; β, ϵ-carotene-3,3' -diol; vegetable lutein; vegetable luteol; all-trans-(+)-xanthophylls; lutein	β, β -carotene-3,3'-diol; all-trans- β, β-carotene-3,3' -diol; zeaxanthol
Empirical formula	$C_{40}H_{56}O_2$	$C_{40}H_{56}O_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

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

Figure 1 Structural Formula of Lutein

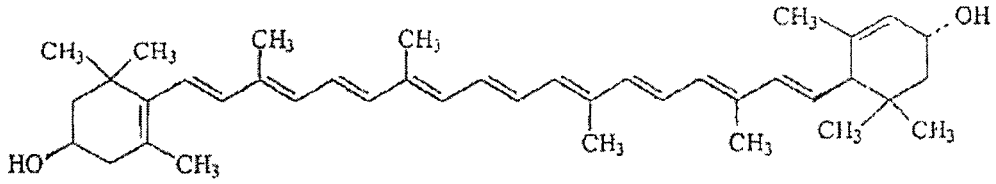
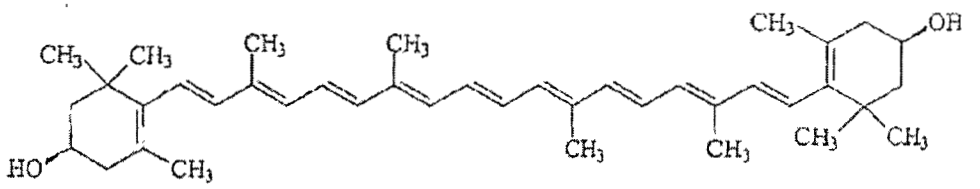


Figure 2 Structural Formula of Zeaxanthin



Typical composition for *Hi Fil*™ Lutein and Kemin FloraGLO® are provided in Table 2. As this table demonstrates, the composition of the two lutein products are substantially equivalent, with the *Hi Fil*™ Lutein having a greater carotenoid content reported as the only difference (90% *Hi Fil*™ *vs.* 83% FloraGLO®); however, the differences noted in carotenoid content are likely due to lack of accounting for other carotenoids in the FloraGLO® analysis and the greater purification and removal of non-carotenoid impurities such as waxes (<6% *Hi Fil*™ vs. <14% FloraGLO®) for the *Hi Fil*™ Lutein.

Table 2. Composition of *Hi Fil*™ Lutein Product *vs.* FloraGLO®

	***Hi Fil*™ Lutein**	**FloraGLO®**
Principal Components	**Average Amount (% dry weight)**	**Mean Amount (% dry weight)**
Total Carotenoids	≥90.0[1]	≥83.0[2]
Waxes	≤6.0	≤14.0
Free Fatty Acids	≤2.0	≤1.0
Water	≤1.0	≤1.0
Ash	≤1.0	≤1.0
Total	100	100

[1] Total Carotenoids include lutein, zeaxanthin and other carotenoids analyzed by HPLC

[2] Analysis does not include other carotenoids; likely reason for difference

Specification of *Hi Fil* ™ Crystalline Lutein

Crystalline lutein has been the subject of a prior GRAS notification to FDA by Kemin Foods. FDA replied with its "no question" letter on June 14, 2004 to GRN 000140. Therefore, comparisons of the *Hi Fil*™ Lutein product and the notified Kemin's FloraGLO® product are made in this GRAS determination for the purposes of showing the substantial equivalence of the two products and the direct applicability of the FDA acceptance of GRN 000140 to IOSA's *Hi Fil*™ Lutein product for specified foods.

The specifications of *Hi Fil*™ Lutein are given in Table 3 and compared to Kemin's FloraGLO® Lutein in the table below. This table shows that the specifications for the two lutein products are nearly identical, with *Hi Fil*™ Lutein having a lower hexane specification as the only notable difference. Although the total carotenoid specifications are different, the lutein and zeaxanthin specifications are the same and both products are derived from marigold flowers, so actual products are likely to have same or substantially equivalent total carotenoid composition, despite the lower manufacturing specification set by Kemin on total carotenoids.

Table 3. Specifications for *Hi Fil*™ Lutein and FloraGLO® Lutein		
Principal Components	***Hi Fil*™ Lutein**	**FloraGLO® Lutein**
Total carotenoids (including lutein and zeaxanthin)	≥90.0% wt	≥ 80.0% wt
Lutein	≥74.0%	≥74.0% wt
Zeaxanthin	≤8.0% wt	≥2.0% wt; ≤9.0% wt
Waxes	≤7.0% wt	≤ 14.0%wt
Moisture	≤1.0%wt	≤1.0%wt
Ash	≤1.0%wt	≤1.0%wt
Other constituents	≤0.1%wt	≤0.1%wt
Pesticides and Related Potential Contaminants		
Protein	< 5000 ppm	< 100 ppm
Hexane	< 25 ppm	< 50 ppm
Thiophenes	< 300 ppm	< 300 ppm
Chlorinated and organophosphate pesticides **	Not Detected	Not Detected
Heavy Metals		
Lead (Pb)	< 1.0 ppm	< 0.65 ppm
Cadmium (Cd)	< 1.0 ppm	< 1.25 ppm
Arsenic (As)	< 1.0 ppm	< 2.5 ppm
Mercury (Hg)	< 1.0 ppm	< 0.150 ppm
Microbiological Assays		
Aerobic plate count	≤1000 cfu/g	≤1000 cfu/g
E. coli	Negative/10 g	Negative/10 g
Listeria monocytogenes	Negative/25 g	Negative/25 g
Salmonella and *Shigella*	Negative/10 g	Negative/10 g
Staphylococcus aureus	Negative/100 g	Negative/100 g
Coliform	Negative/25 g	Negative/25 g
Yeast count	< 100 cfu/g	< 100 cfu/g
Mold count	≤100 cfu/g	< 100 cfu/g

** Confirmatory analyses of pesticide residues on three batches of product attached in Appendix A

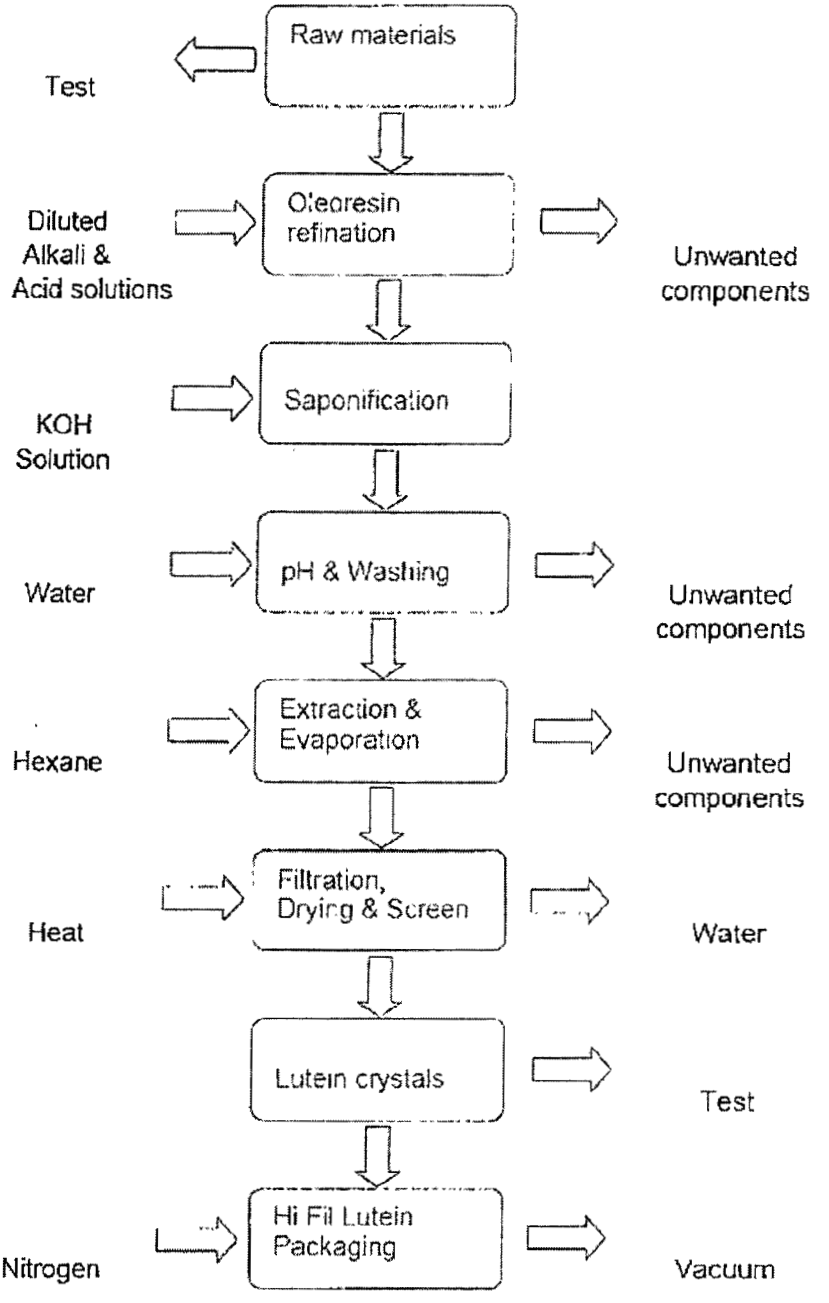
Manufacturing Process

Th *Hi Fil*™ Lutein product is manufactured in accordance with current good manufacturing practice at IOSA's Monterrey México plant. The *Hi Fil*™ Lutein process starts with marigold (*Tagetes erecta*) oleoresin as the raw material. The oleoresin material is obtained by hexane extraction of dried marigold flower petals. A process diagram for *Hi Fil*™ Lutein product manufacture is presented in Figure 3.

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. Then the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls. The saponified mass is diluted with water, pH adjusted and the water is used to wash away salts and other water soluble impurities. Lutein concentrate is extracted with hexane to further remove unwanted components. The solvent is separated by filtration, decanting and centrifugation. Lutein crystals are then treated under high vacuum and temperature evaporation to remove remaining solvent. Lutein crystals are filtered and dried to remove water and screened to collect the lutein crystal product of appropriate size. The final crystalline lutein product is packed under high vacuum and inert nitrogen atmosphere in food-grade plastic pouches.

Figure 3.

Manufacturing Process Diagram



Proposed Use and Intake/Exposure to Lutein

The *Hi Fil*™ Lutein product is intended for use in the same foods and levels of addition as notified by Kemin Foods for the FloraGLO® Crystalline Lutein in GRN 000140. The proposed food uses 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, 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. The intended food uses and use levels are presented in Table 4 on the next page. The application of lutein to the same foods and at the same levels as those in GRN 000140 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.

The dietary analysis below was presented by Kemin in GRN 000140 and was not questioned by FDA in their response letter of June 14, 2004. "Lutein and zeaxanthin are among the most prevalent carotenoids in the North American diet (IOM, 2000), occurring in high concentrations in green leafy vegetables, such as spinach and kale (Khachik *et al.*, 1995; Omaye *et al.*, 1997), and in chicken egg yolks (Handelman *et al.*, 1999). In the U.S., the average daily intake of lutein and zeaxanthin from plant sources is estimated to range from 2 to 4 mg. In addition to their natural occurrence in various foods, lutein and zeaxanthin are available as dietary supplements (IOM, 2000); however, there are no consumption data from which to reliably estimate the intake of lutein and zeaxanthin by supplement users. Market share data indicate that lutein use in dietary supplements occurs predominantly in multi-vitamin type supplements."

"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. 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."

Given that the *Hi Fil*™ Lutein product is substantially equivalent in composition and is intended for use in the same foods and 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 for *Hi Fil*™ Lutein and would not be additive to FloraGLO® crystalline lutein, particularly because market use in foods is competitive and current use is predominantly in multi-vitamin supplements.

Table 4.Summary of the Individual Proposed Food-Uses for *Hi Fil* ™ Lutein		
Food Category	**Proposed Food**	Use levels (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 levels same as GRN 000140.
*Does not include infant formula.

Safety Studies

Background

In GRN 000140, studies on lutein in the literature and on FloraGLO® Crystalline Lutein were presented which supported the safety of crystalline lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of crystalline lutein for the proposed food uses. Because the *Hi Fil*™ Lutein product is substantially equivalent in composition to the crystalline lutein product that was the subject of GRN 000140, these studies can be utilized for safety assessment of the crystalline lutein that is the subject of this notification.

"The safety of FloraGLO® Crystalline Lutein has been established in toxicological studies in rats, mutagenicity studies conducted with *Salmonella typhimurium*, and is further supported by intervention studies conducted with healthy subjects designed to measure metabolic endpoints. The safety of FloraGLO® Crystalline Lutein is also corroborated by additional animal and human studies conducted with other sources of lutein, lutein-rich foods, and lutein supplements. In addition, the safety of lutein and zeaxanthin is well established in the literature based on the historical consumption of eggs and fruits and vegetables where these carotenoids predominate (e.g., green leafy vegetables, such as spinach and kale). Lutein is the major xanthophyll found in human serum, with smaller amounts of zeaxanthin and cryptoxanthin also present (Williams *et al.*, 1998; Boileau *et al.*, 1999). Lutein is also present in breast milk (Gossage *et al.*, 2002), and, together with zeaxanthin, are the only carotenoids found in the macular region of the human retina (Bone et *al.*, 1985, 1988, 1993; Omaye *et al.*, 1997). In general, carotenoids in foods are not known to be toxic, even when ingested in large amounts (e.g., >30 mg carotenoids) (Olson, 1996; Omaye *et al.*, 1997)."

"The safety of lutein and zeaxanthin was addressed by the Institute of Medicine (IOM, 2000) following a review of the available data regarding β-carotene and other carotenoids. The IOM concluded that no adverse effects, other than carotenodermia, have been reported from the consumption of carotenoids, including lutein and zeaxanthin, in foods. Carotenodermia, characterized by a yellowish discoloration of the skin, is a harmless and reversible biological effect of high carotenoid intake (IOM, 2000). No tolerable upper intake levels were established for lutein or zeaxanthin, or for any other carotenoid, (β-carotene, α-carotene, lycopene, and β-cryptoxanthin) considered by the IOM (2000)."

Studies of Absorption and Bioavailability

"As fat-soluble compounds, lutein and zeaxanthin generally follow the same digestion and intestinal absorption pathways as dietary fat (Bendich, 1988; Furr and Clark, 1997; Boileau *et al.*, 1999; van den Berg, 1999). Incorporation into mixed bile salt micelles in the small intestine is required for mucosal uptake in the enterocyte and transport to the lymphatic and/or portal circulation (Furr and Clark, 1997; Boileau *et al.*, 1999; van den Berg, 1999). Dietary factors potentially affecting the degree of absorption of lutein and zeaxanthin following ingestion *(i.e.* factors affecting the bioavailability of lutein and zeaxanthin) include interactions with other carotenoids and/or other nutrients, the association with and digestibility of the food matrix and the isomeric form of the carotenoids *(cis versus trans)*. Absorbed 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). Distribution to extra-hepatic tissue purportedly

occurs *via* the interaction of lipoprotein particles *(e.g.*, HDL, LDL) with receptors, and degradation of lipoproteins by extra- hepatic enzymes *(e.g.*, lipoprotein lipase) (Boileau *et al.*, 1999). A specific xanthophyll-binding protein (XBP) isolated from solubilized carotenoid-rich membrane extracts from human macula has been demonstrated to mediate the uptake of lutein and zeaxanthin from the bloodstream (Yemelyanov *et al.*, 2001)."

"Intervention trials conducted with FloraGLO® Crystalline Lutein have demonstrated that, following its ingestion, lutein and zeaxanthin are absorbed intact in healthy human subjects, as evidenced by increased plasma levels (Kostic *et al.* 1995; Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999; Schalch *et al.*, 2001). In comparison with plant sources of lutein, the bioavailability of lutein from FloraGLO® Crystalline Lutein (incorporated in the diet as a suspension in oil added to salad dressing) is reported to be approximately 30 to 50% greater (Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999). However, considering that lutein from egg yolks is more bioavailable than lutein from vegetable sources (Johnson and Mayer, 2003), the increase in bioavailability of lutein from FloraGLO® Crystalline Lutein compared with all foods *(i.e.* fruits, vegetables, and eggs) may be less than that reported by van het Hof *et al.* (1999) and Castenmiller *et al.* (1999). The mean intake of lutein provided by the intended uses of FloraGLO® Crystalline Lutein is approximately 2.5 times greater than background (assuming background intake of 3 mg lutein/day); however, considering bioavailability differences (Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999; Johnson and Mayer, 2003), systemic exposure may be expected to increase to approximately 4 times above background."

"Metabolic intervention studies have indicated that carotenoid interactions may occur at the metabolic level *(e.g.*, the absorption of both β-carotene and lutein have been demonstrated to be reduced following simultaneous ingestion); however, FloraGLO® Crystalline Lutein is not expected to have a negative impact on total carotenoid balance due to the low intake levels of lutein provided under the intended conditions of use. Taken together, intervention studies indicate that lutein and zeaxanthin are effectively absorbed from FloraGLO® Crystalline Lutein, that lutein is more bioavailable from FloraGLO® Crystalline Lutein as a supplement compared with fruits and vegetables, and that lutein and zeaxanthin from FloraGLO® Crystalline Lutein are not expected to adversely affect carotenoid balance under the intended conditions of use."

As noted above, the absorption and bioavailability of the *Hi Fil*™ Lutein product is considered substantially equivalent in composition to the FloraGLO® Crystalline Lutein product, so the absorption and bioavailability would be the same for both products.

Toxicological Studies

The toxicological evaluation conducted for GRN 000140 is considered directly applicable to *HiFil*™ Lutein due to their nearly identical composition. In GRN 000140, the "Expert Panel reviewed toxicological studies conducted with FloraGLO® Crystalline Lutein, as well as safety studies of lutein and zeaxanthin, in general, from other sources *(e.g.*, marigold extracts). Based on bioavailability studies reporting up to 43% absorption, and measured tissue and/or plasma levels of lutein and/or zeaxanthin, these studies have demonstrated that each of lutein and zeaxanthin (from FloraGLO® Crystalline Lutein or as an extract from marigold) are effectively absorbed from the diet in mice, rats, cats, dogs, and monkeys, supporting the suitability of these experimental animals as appropriate models for the study of lutein and zeaxanthin absorption and safety in humans (Park *et al.*, 1998; Jewell and O'Brien, 1999; Jenkins *et al.*, 2000; Kim *et al.*,

2000 a,b; Kruger *et al.*, 2002; Schierle *et al.*, 2002)." However, of this group of animal models, only primates can concentrate lutein and zeazanthin in the retina.

"In 4-week and 13-week toxicological studies conducted with FloraGLO® Crystalline Lutein, no adverse effects were reported in terms of body weight gain, organ weights, feed intake, clinical chemistry, or histopathology in Wistar rats (Kruger *et al.*, 2002). Animals were exposed to FloraGLO® Crystalline Lutein in the diet at levels up to 773 mg/kg body weight/day (delivering 639 mg lutein plus zeaxanthin/kg body weight/day) for 4 weeks, or up to 260 mg/kg body weight/day (delivering 208 mg lutein plus zeaxanthin/kg body weight/day) for 13 weeks. The no-observed-adverse-effect-levels (NOAELs) for FloraGLO® Crystalline Lutein in Wistar rats corresponded to the highest doses tested in each toxicology study; 773 mg/kg body weight/day following 4 weeks of exposure, and 260 mg/kg body weight/day following 13 weeks of exposure. Additional toxicological studies conducted with FloraGLO® Crystalline Lutein, ranging in length from 8 to 12 weeks, have measured feed intake, body weight, organ weights, and plasma levels of cholesterol and triglycerides (Jenkins *et al.*, 2000; Kim *et al.*, 2000 a,b). No adverse effects were reported in male weanling Fischer 344 rats following exposure to up to 621 mg FloraGLO® Crystalline Lutein/kg body weight/day (31 mg lutein/kg body weight/day) in the diet for 8 weeks. Higher dose levels (1,169 and 2,383 mg FloraGLO® Crystalline Lutein/kg body weight/day) resulted in increased body weights (presumably due to an increase in caloric intake), increased plasma levels of triglycerides and cholesterol, and decreased relative lung and brain weights (2,383 mg FloraGLO® Crystalline Lutein/kg body weight/day dose group only). The reported decreased relative organ weights were considered by the Panel not to be treatment-related effects, and to be consequential of increased body weights. Furthermore, similar changes increased plasma levels of cholesterol and triglycerides, decreased relative organ weights) were not reported in toxicological studies of similar or longer duration conducted with higher doses of lutein from FloraGLO® Crystalline Lutein (Buser *et al.*, 1999; Pfannkuch *et al.*, 2000, 2001; Kruger *et al.*, 2002)."

"The genotoxic potential of FloraGLO® Crystalline Lutein (10% beadlet formulation, and without beadlet) was investigated using the reverse mutation assay (Ames test), conducted with *Salmonella typhimurium* strains TA 1535, TA 97, TA 98, TA 100, and TA 102, with and without metabolic activation (S9 fraction from rat liver) (Kruger *et al.*, 2002). No increase in the number of mutant colonies was observed for any of the five tester strains after treatment with 10% beadlet (15.8 to 5,000 pg/plate) or FloraGLO® Crystalline Lutein (15.8 to 500 pg/plate), demonstrating that neither formulation is mutagenic in S. *typhimurium* strains. The mutagenic potential of lutein from dietary sources (extracts of fruits, vegetables, and marigold) has also been investigated in various studies, indicating that lutein is non-mutagenic in bacterial test systems *in vitro*, and has no clastogenic activity or DNA damaging effects in mammalian test systems *in vivo* (Yoshikawa *et al.*, 1996; Collins *et al.*, 1998; Rauscher *et al.*, 1998)."

"Additional supportive toxicological studies conducted with rodents have investigated the effects of dietary exposure to lutein and zeaxanthin, from other sources *(e.g.*, marigold extract), for 2 to 8 weeks on body weight, feed intake, and organ weights. No changes in body weight gain, feed intake, liver weight, or spleen weight, and no external signs of toxicity were reported in BALB/c mice following 2 to 4 weeks of dietary exposure to up to 803 mg lutein/kg body weight/day and 10.9 mg zeaxanthin/kg body weight/day (Chew *et al.*, 1996; Park *et al.*, 1998, 1999). Similarly, no changes in body weights, feed intake, or organ weights were reported in Wistar rats following 16 days of dietary exposure to up to 45 mg lutein/kg body weight/day (Gradelet *et al.*, 1996;

Jewell and O'Brien, 1999), and 8 weeks of dietary exposure to 500 mg lutein/kg body weight/day was well tolerated in experimental mice models of atherosclerosis (Dwyer *et al.*, 2001)."

"The chronic toxicity of lutein and zeaxanthin (0, 0.2, or 20 mg/kg body weight/day, source not specified) was investigated in Cynomolgus monkeys following daily administration *via* gavage for 52 weeks (Schierle *et al.*, 2002). No clinical or morphological evidence of treatment-related adverse changes were reported, and it was concluded that long-term administration of lutein and zeaxanthin in Cynomolgus monkeys at dose levels up to 20 mg/kg bodyweight/day resulted in no toxic effects, as evidenced by clinical investigation parameters, necropsy, and histopathology (Schierle *et al.*, 2002). Specific to ocular toxicity, the Panel discussed ongoing long-term studies in monkeys administered up to 20 mg lutein and zeaxanthin/kg body weight/day and evaluated abstracts reporting the results of these studies. No evidence of clinical or morphological toxicity to the eyes was reported, and further, no evidence of crystal formation in the eyes of treated monkeys was noted (Schierle *et al.*, 2002; Wolz *et al.*, 2002). The Panel considered these ongoing studies to be corroborative of the overall safety of FloraGLO® Crystalline Lutein, and supportive of the shorter-term clinical studies (see below)."

Human Safety Data

"In addition to published pivotal animal toxicology studies with FloraGLO® Crystalline Lutein, the Expert Panel considered clinical data from safety/tolerance studies with FloraGLO® Crystalline Lutein, as well as with lutein and zeaxanthin from other dietary sources, to corroborate the safety of FloraGLO® Crystalline Lutein. In a 42-day study designed to evaluate plasma response to oral lutein administration (FloraGLO® Crystalline Lutein formulated as beadlets in capsules), healthy volunteers were given capsules delivering either 4 or 20 mg lutein daily (8 subjects/dose group) (Schalch *et al.*, 2001). A total of 12 subjects experienced 26 adverse events, 24 of which were reported not to be related to the study intervention, and 2 of which (mild forms of conjunctivitis and abdominal pain) were unknown in their etiology. In addition, no clinically relevant abnormalities were reported with reference to laboratory values for serum liver enzyme levels, total bilirubin, glucose, creatinine, uric acid, calcium, total cholesterol, triglycerides, sodium, potassium, total protein, prothrombin time, blood urea nitrogen, and blood cell count. Together, these findings demonstrated that exposure to up to 20 mg lutein from FloraGLO® Crystalline Lutein in healthy subjects was not associated with adverse health effects (Schalch *et al.*, 2001)."

In a study designed to examine the effect of the food matrix on carotenoid bioavailability, Castenmiller *et al.* (1999) included a measurement of serum cholesterol and triacylglycerol concentrations. Following 3 weeks of dietary intervention with 6.6 mg lutein/day from FloraGLO® Crystalline Lutein (suspension in vegetable oil with β-carotene), no significant differences were reported compared with control values, demonstrating that lutein from FloraGLO® Crystalline Lutein had no effect on plasma lipid levels in healthy non-smoking, normolipidemic volunteers (Castenmiller *et al.*, 1999). Supporting intervention studies conducted with healthy subjects have demonstrated that daily exposure to 15 mg lutein (from marigold extract) for up to 6 months results in no adverse side effects, and no changes in serum blood lipid levels (individual and total fatty acids, total saturated, monounsaturated, and polyunsaturated, total cholesterol, HDL-cholesterol, and LDL cholesterol), or in hematological or biochemical parameters (Olmedilla *et al.*, 1997, 2001; Wright *et al.*, 1999; Falsini *et al.*, 2003). Similarly, no changes in blood hemoglobin concentrations, white blood cell levels, or

serum electrolyte levels were reported in healthy subjects following 2 weeks of exposure to 11 mg lutein/day from spinach powder (Muller *et al.*, 1999), or in patients with cataract or age-related maculopathy following 13 months of dietary supplementation with capsules containing 12 mg all-trans-lutein, 13 mg of 13/15-cis-lutein and 3.3 mg vitamin E (Olmedilla *et al.*, 2002). In addition, no evidence of ocular toxicity was noted in a single healthy male volunteer receiving 20 mg lutein/day as an oil suspension prepared from marigold flowers for a total of 3 weeks (Khachik *et al.*, 1997). Similarly, clinical studies in patients with retinal degenerative diseases have reported no losses in visual acuity and no significant changes in foveal vision parameters following daily supplementation with up to 20 mg lutein for up to 6 months (Richer, 1999; Aleman *et al.*, 2001; Duncan *et al.*, 2002). Carotenodermia (yellowish discoloration of the skin) has been reported in healthy subjects exposed to 15 mg lutein/day (from mixed ester forms extracted from marigold) for 4 months (Olmedilla *et al.*, 1997, 2002; Granado *et al.*, 1998); however, carotenodermia is considered a harmless and reversible biological effect of high carotenoid intake (IOM, 2000), and 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 to approximately 25 mg lutein/day, 3 times/week for 13 months (Olmedilla *et al.*, 2001). These findings of Khachik *et al.* (1997), Olmedilla *et al.* (1997, 2002), Granado *et al.* (1998), and Muller *et al.* (1999), indicate that oral exposures to up to 20 mg lutein/day do not result in any adverse health effects, including retinal damage *(e.g.*, crystal deposition).

"In addition, studies investigating the relationship of lutein and zeaxanthin to chronic disease have reported no adverse relationships between increased serum levels of lutein and zeaxanthin, or dietary intake of lutein and zeaxanthin, and eye health *(i.e.*, risk of AMD, risk of cataracts) (EDCC Study Group, 1993; Seddon *et al.*, 1994; Mares-Perlman *et al.*, 1995; Brown *et al.*, 1999; Chasan-Taber *et al.*, 1999; Lyle *et al.*, 1999 a,b; Snellen *et al.*, 2002), and no adverse relationships between serum levels of lutein and zeaxanthin and the risk for subsequent myocardial infarction (Street *et al.*, 1994) or the progression of intima-media thickness of the common carotid arteries (Dwyer *et al.*, 2001)." In a further study, ninety patients with atrophic ARMD received 10 mg lutein daily over 12 months. Mean eye macular pigment optical density increased and visual function was improved with lutein alone or lutein together with other nutrients (Richer *et al.*, 2004).

"The Expert Panel considered the results of clinical studies with β-carotene to be of limited relevance to the evaluation of the safety of FloraGLO® Crystalline Lutein. Intervention studies conducted with β-carotene have reported that supplemental β-carotene may enhance lung tumorigenesis in cigarette smokers (ATBC, 1994; Omenn *et al.*, 1996 a,b), potentially via altered retinoid signaling (Paolini *et al.*, 1999; Wang *et al.*, 1999). Unlike β-carotene, lutein is a non-provitamin A carotenoid, and is not expected to directly alter retinoid signaling. Furthermore, the numerous epidemiological studies conducted with lutein have reported protective effects against various forms of cancer (breast, lung, adenocarcinomas of the esophagus, and gastric cardia), and have therefore provided no evidence to suggest that exposure to lutein contributes towards an enhanced risk of cancer in humans. In addition, toxicology studies conducted with FloraGLO® Crystalline Lutein have reported no adverse toxicological effects, and no mutagenic activity of FloraGLO® Crystalline Lutein has been reported *in vitro* or *in vivo* "

Additional Supporting Information on Safety of Lutein

It is well known that lutein esters are readily degraded in the GI tract by esterase enzymes to lutein and constituent compounds such as palmitate. They are absorbed by the intestinal mucosal

enterocytes as lutein. Thus, GRAS notifications on lutein esters are applicable to the safety assessment of lutein as that is the form that is present in systemic exposure. Cognis Corporation had previously submitted a GRAS Notification to FDA on July 17, 2002 (GRN 000110). This contained the unanimous concurrence from a Panel of qualified experts that Xangold® lutein esters are GRAS, based on their expert evaluation of the scientific literature for addition to conventional foods as an ingredient to provide consumers with a supplementary source of lutein in their diets. The Panel of independent qualified experts conducted and extensive review of all generally available scientific literature for safety and toxicity as well as evaluated all available data from dietary consumption of lutein and zeaxanthin. The typical daily consumption level of lutein and zeaxanthin was estimated at 1.35-1.97 mg/day. No toxicity from lutein ester supplement consumption has been reported and human clinical studies indicate that long-term consumption of lutein esters is well tolerated. Seven clinical studies ranging from 84 days to 3 years have reported that consumption of between 18 and 60 mg/day lutein ester equivalents is safe (GRN 000110). The Expert Panel established a conservative acceptable daily intake of Xangold® lutein esters of 40 mg/day.

No evidence of toxicity or safety concerns was noted by the Panel in their review of all available toxicology and clinical studies. The Expert Panel evaluated the proposed use of Xangold® lutein esters at specified levels seen in the following foods: baked goods and baking mixes, soy milk, beverages and beverage substitutes, frozen dairy desserts and mixes, processed fruit and vegetable products, egg products and egg substitutes, breakfast cereals (ready-to-eat), fats and oils, hard candy, fruit snacks and dairy products. They concluded that such use would result in an estimated daily intake that is below 40 mg/day lutein esters When the Expert Panel evaluated the combination of the 90th percentile lutein ester consumption levels from foods supplemented with Xangold® lutein esters, with the 90th percentile current consumption of lutein esters from conventional foods, the estimated daily intake of lutein esters from conventional foods is 22.3 mg/day. In addition, the potential lutein ester consumption from dietary supplements may add an additional maximum intake of 12 mg/day. They also concluded that the maximum potential theoretical lutein ester consumption at the 90th percentile may reach 34.3 mg/day and that this was a conservative estimate since it is highly unlikely that an individual would consume lutein from both conventional foods and dietary supplements at the 90th percentile level. Even this conservative estimate is well within the Acceptable Daily Intake of 40 mg/day established and was deemed to be safe.

The July 17, 2002 GRAS notification included the supporting data on which this conclusion was based. FDA responded to this GRAS notification in a letter dated January 21, 2003 stating "based on the information provided by Cognis, as well as other information available to FDA, the agency has no questions at this time regarding Cognis' conclusion that lutein esters are GRAS under the intended conditions of use."

Safety Assessment of Crystalline Lutein

As noted above, the composition and purity of the *Hi Fil*™ Lutein product is considered substantially equivalent in composition to the FloraGLO® Crystalline Lutein product, so the animal and human studies reviewed herein are applicable to both products. Subchronic studies in rats up to 208 mg/kg/day (Kruger *et al.*, 2002) and chronic studies in Cynomolgus monkeys up to 20 mg/kg/day lutein and zeaxanthin (Schierle *et al.*, 2002) did not result in any adverse effects. Lutein has not shown any genotoxic effects in multiple assays and has shown some

evidence of a protective effect against cancer. With the exception of a reversible carotenodermia seen in two studies, human clinical trials have shown no significant adverse effects in subjects taking lutein and zeaxanthin up to 20-25 mg/day for over a year. Therefore, intake of *Hi Fil*™ Lutein in the diet from specified foods at estimated mean intakes of lutein and zeaxanthin of 7.3 mg/person/day (0.14 mg/kg/day) and 0.7 mg/person/day (0.01 mg/kg/day) respectively, and 90th percentile intakes of lutein and zeaxanthin at 13.4 mg/person/day (0.28 mg/kg/day) and 1.2 mg/person/day (0.03 mg/kg/day), are not considered to pose any safety concerns.

CONCLUSION

Based on a critical evaluation of the pertinent data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively determined by scientific procedures that addition of the Lutein 10 CWD formulated product, meeting the formula for preparation and specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.

James W. Barnett, Jr., Ph.D., DABT. — 6/27/06 Date

D. Max Shodderly, Ph.D. — 27 June 06 Date

Stanley M. Tarka, Jr., Ph.D. — 6/30/06 Date

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Appendix A

Results of Pesticide Residue Analysis

EAS CONSULTING GROUP

Specializing in FDA Regulatory Matters

October 24, 2008

Mr. Mark L. Itzkoff
Olsson Frank Weeda PC
1400 - 16th St., N.W., Suite 400
Washington, DC 20036-2220

Tel: 202/518-6327
fax: 202/234-2686

RE: Review of safety studies of lutein (2006-present) for GRAS update

Executive Summary

In response to a request from Olsson Frank Weeda PC for one of their clients Industrial Organica S.A de C.V. (IOSA), this report summarizes the safety studies of lutein that have appeared in the scientific literature since the last database search performed in February 2006 for the Generally Recognized As Safe (GRAS) determination of lutein (*Hi Fil*™ crystalline lutein). In 2006, a panel of experts determined that IOSA's *Hi Fil*™ crystalline lutein was GRAS and safe for use in specified foods with user intakes determined in 90th percentile all-user intakes of lutein and zeaxanthin of 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.

Since the last database searches for the previous GRAS determination performed in February 2006, over 350 articles have appeared on lutein in these databases. Of these, about 20 articles, related to the safety of lutein, were selected for further review. In two cross-sectional studies from Australia by one group, a positive association between the intake of lutein/zeaxanthin and ω-3 fatty acids with progression of age-related macular degeneration (AMD) was reported, while another independent report did not find such an association. A critical review of the other published studies did not reveal any significant safety-related concern that will affect the previous GRAS determination. An independent panel of recognized experts reviewed the studies published over the last two years and concluded that the recent publications do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous GRAS determination of lutein by IOSA.

1. Background

Recently, Industrial Organica S.A de C.V. (IOSA) gained self affirmed Generally Recognized As Safe (GRAS) status for use of *Hi Fil*™ crystalline lutein in a variety of selected foods. IOSA now proposes to submit the self affirmed GRAS determination of lutein to the Food and Drug Administration in the form of a GRAS Notification. As the self- affirmed GRAS

assessment was conducted in 2006, IOSA asked EAS Consulting Group to update the available safety-related information on lutein that has appeared since the GRAS determination. IOSA assured EAS Consulting Group that the food categories, use levels and resulting exposure identified in the self-affirmed GRAS will remain the same. A comprehensive search of the scientific literature related to safety and toxicity of lutein was conducted since January 2006. The database searches revealed over 350 articles on lutein, of which about 20 articles related to the safety of lutein were selected for further review. These articles were obtained, reviewed and a summary of all safety-related findings from the relevant articles is presented in the following section.

2. Safety Studies

2.1. Animal studies

Khachik et al. (2006b) investigated the effects of lutein, zeaxanthin, or a combination of the two, on changes in plasma levels of these carotenoids as well as any effects on ocular and renal toxicity. 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 12-month period. 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 kidney 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.

2.2. Mutagenicity and carcinogenicity studies

Wang et al. (2006) investigated the mutagenic and clastogenic potentials of lutein from marigold flowers. The mutagenicity of lutein (334, 668 and 1335 μg/plate) was examined 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.

Santocomo 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 contrast to the *in vitro* findings reported above, 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.

2.3. 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 any dose level 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.

SUBMISSION END

AM

Mosley, Sylvester

From: Mark Itzkoff [mitzkoff@ofwlaw.com]
Sent: Monday, June 08, 2009 4:58 PM
To: Mosley, Sylvester
Subject: RE: GRN 000291
Follow Up Flag: Follow up
Due By: Wednesday, June 10, 2009 12:00 AM
Flag Status: Completed

Dear Dr. Mosley

I have asked IOSA about the crystallin lutein applications. They inform me that both the egg products and the soup and soup mix applications should be excluded from this Notice. They do not intend to market their crystalline lutein products for these applications.

Please let me know if you have any additional questions.

Regards,

Mark Itzkoff

Mark L. Itzkoff
Olsson Frank
202 518-6327

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From: Mosley, Sylvester [mailto:Sylvester.Mosley@fda.hhs.gov]
Sent: Tuesday, June 02, 2009 8:08 AM
To: Mark Itzkoff
Subject: RE: GRN 000291

Dear. Mr. Itzkoff,

We have filed your client's submission for crystalline lutein. In looking over your client's submission, we are requesting a couple of points of clarification.

1.) In table 4 on page 12, the notifier has listed egg products as one of the food categories. Do these egg products fall outside of the purview of the USDA?

2.) In table 4 on page 12, the notifier has soups and soup mixes listed in the form of canned soups. Do these canned soups contain meat and poultry?

Sincerely,

Sylvester L. Mosley, Ph.D.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety-CFSAN

U.S. Food and Drug Administration

5100 Paint Branch Parkway (HFS-255)

College Park, MD 20740-3835

Phone: 301-436-1333

Fax: 301-436-2965

Email: Sylvester.Mosley@fda.hhs.gov

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AM

From: Mark Itzkoff
To: Mosley, Sylvester;
Subject: RE: Points of Clarification
Date: Thursday, August 13, 2009 6:03:42 PM
Attachments: Aug 13 submission.pdf

Dr. Mosley

Attached is a revised submission that addresses the points discussed below. We are also forwarding a copy to you via Federal Express.

Please let me know if you have any questions or if there are any difficulties receiving this material.

Regards,

Mark Itzkoff

Mark L. Itzkoff
Olsson Frank
202 518-6327

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From: Mosley, Sylvester [mailto:Sylvester.Mosley@fda.hhs.gov]
Sent: Wednesday, July 08, 2009 4:53 PM
To: Mark Itzkoff
Subject: Points of Clarification

Dear Mr. Itzkoff,

This email is to follow-up on our phone conversation regarding the points of clarification for GRN 000291, Crystalline Lutein. The areas where we are seeking clarification are as follows:

1. Lutein is known to be orange-to-red in color. We could not find the color of the notifier's crystalline lutein in the notice. Is the crystalline lutein that is the subject of this notice colored? If so we request that the notifier present their view on whether any of the intended uses of crystalline lutein would be exempt from the definition of color additive.

2. Page 2, paragraph 3 states in part "--intended use in foods and medical foods--". Medical foods are not listed on table 1 (page 4), table 4 (page 11) nor on page 5 of the Expert Panel Statement. Please clarify whether medical foods are one of the intended uses within the notice.

3. The footnotes on page 2 refer to Appendix I and Appendix II; we were not able to locate these appendices. We think the footnotes refer to the expert panel reports of 2006 and 2008. Please clarify and/or provide the information that is located in Appendix I and Appendix II. Also, there is a header page which has Appendix A (Results of Pesticide Residue Analysis) and immediately following this page is the expert panel report of 2008. If the results of a pesticidal residue analysis are intended to follow the header page, please provide this analysis as well as a complete copy of the 2008 expert panel report. Our copy of this report seems to only have first three pages.

4. On Page 5 the text refers to chemical and physical characteristics of lutein and zeaxanthin in Table 1. We believe these characteristics are in Table 2. Please confirm that this is correct.

5. On Page 10, section III titled "Information on self-limiting levels of use". The notifier discusses exposure/EDI in section III, though we did not see information on self-limiting use in this section. Please provide information of self-limiting levels of use.

6. On Page 18: Expert panel conclusion refers to "---Lutein 10 CWD formulated product, meeting the formula for preparation---" Please confirm that this panel reviewed the crystalline lutein that is the subject of this notice.

7. On Page 5 and the first page of the 2006 expert panel page: 5 °C is listed. In GRN 000140 they list a temperature of 25 °C for the solubility in water. Please

confirm the temperature.

If you have any further questions or need further clarifications please do not hesitate to contact me.

Sincerely,

Sylvester L. Mosley, Ph.D.

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety-CFSAN

U.S. Food and Drug Administration

5100 Paint Branch Parkway (HFS-255)

College Park, MD 20740-3835

Phone: 301-436-1333

Fax: 301-436-2965

Email: Sylvester.Mosley@fda.hhs.gov

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PHILIP C OLSSON
RICHARD L. FRANK
DAVID F. WEEDA (1948-2001)
DENNIS R. JOHNSON
ARTHUR Y. TSIEN
JOHN W. BODE*
STEPHEN D. TERMAN
MARSHALL L. MATZ
MICHAEL J O'FLAHERTY
DAVID L. DURKIN
NEIL F O'FLAHERTY
BRETT T. SCHWEMER
TISH E. PAHL
ROBERT A HAHN
EVAN P PHELPS
GARY H. BAISE

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OLSSON FRANK WEEDA
TERMAN BODE MATZ PC
ATTORNEYS AT LAW

SUITE 400
1400 SIXTEENTH STREET, N.W.
WASHINGTON, D.C. 20036 (202) 789-1212
www.ofwlaw.com

JOLYDA O SWAIM
KATHRYN E. BALMFORD
JONATHAN M. WEINRIEB
NANCY W MATHEWSON
MCKAY R. TOLBOE*
COUNSEL
ROGER R. SZEMRAJ
OF COUNSEL
JUR T. STROBOS
KENNETH D. ACKERMAN
MARK L. ITZKOFF
DAVID A. BIEGING
ELLIOT BELILOS
SENIOR POLICY ADVISORS
JOHN R BLOCK
CHARLES W. STENHOLM
GEORGE McGOVERN
SALLY S. DONNER
BRENT W GATTIS
BARBARA J. MASTERS

August 13, 2009

BY E-MAIL AND FEDERAL EXPRESS

Dr. Sylvester Mosely
Office of Food Additive Safety
Center for food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch parkway
College park, MD 20740

RECEIVED
SEP 01 2009
BY: (b)(6)

Re: GRN 291

Dear Dr. Mosely,

In response to your July 8, 2009 e-mail, we are hereby forwarding the attached revised Notice regarding Industrial Organica S.A. de C.V. (IOSA) determination that crystalline lutein is generally recognized as safe (GRAS).

In response to the specific issues raised in your e-mail, please note the following:

1. We have revised the lutein specification to note the red-orange color of the lutein crystals. However, discussed in footnote 4, this color is a function of crystal structure and does not occur when lutein is dissolved. Thus, lutein will not impart color to the formulated foods.
2. The term "medical foods" was used only in the Expert Statement and is not part of the GRAS Notice. IOSA does not intend to market lutein to this application.
3. The appendices attached to this revision have been corrected.
4. The reference on Page 5 to Table 5 to "Table 1" has been amended.
5. Information on limiting the concentration of lutein in formulated foods has been added to Section III.
6. While the Expert Panel can not confirm that the IOSA lutein is the lutein that was the subject of the Panel's Statement, we can confirm on behalf of IOSA that the *Hi-Fil*™

Lutein is the product that was described to the Expert Panel and that the product is produced by the method detailed in the Statement and complies with the specifications in the Statement.

7. We have confirmed that the temperature in the solubility specification on Page 5 and the 2006 Statement is 25° C. The attached document reflects this revision.

We trust you will find the attached Notice fully supports the IOSA determination. If you have any questions, please do not hesitate to contact us.

Regards,

(b)(6)

Mark L. Itzkoff

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SUITE 400
1400 SIXTEENTH STREET, N.W.
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August 13, 2009

GRAS Claim for the Use of Crystalline Lutein as a Food Ingredient

GRAS Claim

Industrial Organica S.A. de C.V. (IOSA) has determined that crystalline lutein is generally recognized by qualified experts as having adequately shown to be safe through scientific procedures when used as a food ingredient in the applications detailed in Table 1, below. IOSA hereby submits this GRAS claim for the use of crystalline lutein as a food ingredient as specified in Table 1.

A. Name and Address of Notifier:

Industrial Organica S.A. de C.V.
Ave. Almazan No. 100
Col. Topo Chico 64260
Monterrey, Mexico

B. Common or Usual Name of Substance:

The common or usual name of the substance is lutein also known by its trade name, *Hi Fil*™ Lutein. The Chemical Abstract Services Registration Number (CASRN) for this substance is 127-40-2.

C. Conditions of Use:

Lutein that is the subject of this Notice will be used as a food ingredient in a variety of food and beverage applications. These applications are detailed in Table 1.

D. Basis for GRAS Determination:

IOSA has determined that *Hi Fil*™ Lutein is generally recognized by qualified experts as having adequately shown to be safe through scientific procedures. This determination is supported by the opinions of two scientific panels. In June of 2006, the status of the IOSA *Hi Fil*™ Lutein was reviewed by a panel of scientists (the "2006 Panel") assembled by AAC Consulting Group of Kendle International. The 2006 Panel was comprised of three noted toxicologists with expertise in food safety and lutein production. The Panel reviewed the information and the available toxicology literature on lutein and lutein derivatives. Based on this information, the Panel issued an Expert Panel Statement, "Determination Of The GRAS Status Of *Hi Fil*™ Crystalline Lutein for Addition To Select Specified Foods" in which the it

> determined by scientific procedures that addition of *Hi Fil*™ Lutein, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.[1]

In preparation for submitting this Notice, IOSA assembled a second[2] expert panel (the "2008 Panel") to review any relevant additions to the public literature that had been published after the 2006 Expert Panel Statement. The 2008 Panel concluded that the

> studies published over the last two years also do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous self affirmed GRAS determination of lutein by IOSA.[3]

---

[1] A copy of the 2006 Expert Panel Statement is attached as Appendix I. While the Expert Statement includes the use of crystalline lutein as a component of "medical foods" within the parameters of its opinion, the Notifier has no intent to market the crystalline lutein for this application and does not include it in the applications specified herein.

[2] The 2008 Panel was coordinated by EAS Consulting Group, the successor to the AAC Consulting Group who conducted the 2006 Panel.

[3] *Review of safety studies of lutein (2006 – present) for GRAS update,* John Thomas *et al.*, October 24, 2008. A copy of this statement is attached as Appendix II.

Thus, the basis for our GRAS determination is the Statement of the 2006 Expert Panel and the concurring Statement of the 2008 Panel.

E. Data Availability Statement:

The data and information that are the basis for the Notifier's GRAS determination will be sent to FDA upon request.

Respectfully Submitted,

(b)(6)

Mark L. Itzkoff
Counsel for IOSA

Table 1 Proposed Food-Uses for *Hi Fil* ™ Lutein

Food Category	Proposed Food	Use levels (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 levels same as GRN 000140.
*Does not include infant formula.

Section II

Identity of the Notified Substance

Hi Fil™ Lutein (common or usual name lutein) is a purified crystalline extract from the marigold *(Tagetes erecta)* flower. *Hi Fil*™ Lutein typically contains approximately 80-90% by weight total carotenoids, with about 90% as lutein. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table 2, below.

Table 2
Chemical and Physical Characteristics of Lutein and Zeaxanthin

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical names	xanthophyll; β, ε-carotene-3,3' -diol; vegetable lutein; vegetable luteol; all-trans-(+)-xanthophylls; lutein	β, β -carotene-3,3'-diol; all-trans- β, β-carotene-3,3' -diol; zeaxanthol
Empirical formula	$C_{40}H_{56}O_2$	$C_{40}H_{56}O_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 25°C	Insoluble	Insoluble

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

Figure 1 Structural Formula of Lutein

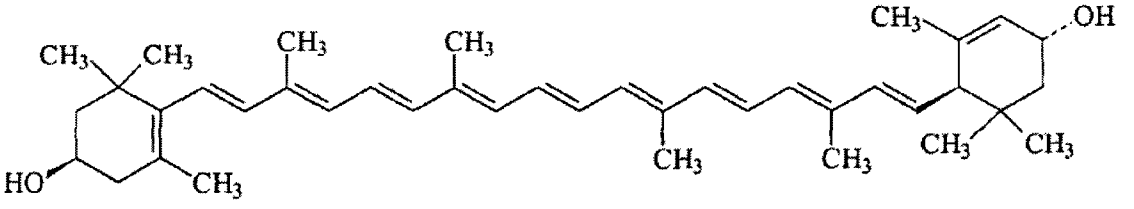
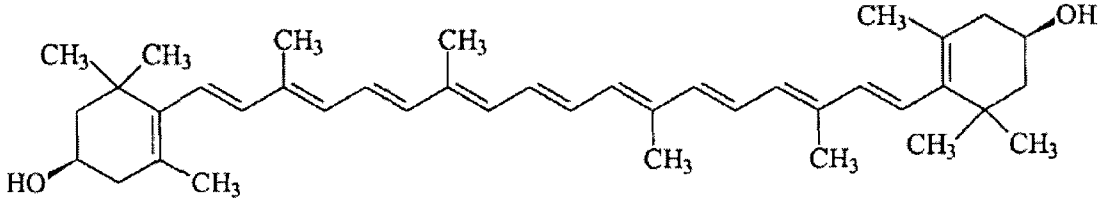


Figure 2 Structural Formula of Zeaxanthin



Specification of *Hi Fil* ™Crystalline Lutein

Crystalline lutein has been the subject of a prior GRAS notification to FDA by Kemin Foods. FDA replied with its "no question" letter on June 14, 2004 to GRN 000140. Therefore, comparisons of the *Hi Fil*™ Lutein product and the notified Kemin's FloraGLO® product are made in this GRAS determination for the purposes of showing the substantial equivalence of the two products and the direct applicability of the FDA acceptance of GRN 000140 to IOSA's *Hi Fil*™ Lutein product for specified foods. The data submitted to FDA in GRN 000140 is incorporated by reference into this Notice.

The specifications of *Hi Fil*™ Lutein are given in Table 3 and compared to Kemin's FloraGLO® Lutein in the table below. This table shows that the specifications for the two lutein products are nearly identical, with *Hi Fil*™ Lutein having a lower hexane specification as the only notable difference. Although the total carotenoid specifications are different, the lutein and zeaxanthin specifications are the same and both products are derived from marigold flowers, so actual products are likely to have same or substantially equivalent total carotenoid composition, despite the lower manufacturing specification set by Kemin on total carotenoids.

Table 3

Specifications for *Hi Fil*™ Lutein and FloraGLO® Lutein		
Principal Components	***Hi Fil*™ Lutein**	**FloraGLO® Lutein**
Total carotenoids (including lutein and zeaxanthin)	≥ 90.0% wt	≥ 80.0% wt
Lutein	≥ 74.0%	≥ 74.0% wt
Zeaxanthin	≤ 8.0% wt	≥ 2.0% wt; ≤ 9.0% wt
Waxes	≤ 7.0% wt	≤ 14.0%wt
Moisture	≤ 1.0%wt	≤ 1.0%wt
Ash	≤ 1.0%wt	≤ 1.0%wt
Other constituents	≤ 0.1%wt	≤ 0.1%wt
Color	Red - Orange	Red - Orange
Pesticides and Related Potential Contaminants		
Protein	< 5000 ppm	< 100 ppm
Hexane	< 25 ppm	< 50 ppm
Thiophenes	< 300 ppm	< 300 ppm
Chlorinated and organophosphate pesticides	Not Detected	Not Detected
Heavy Metals		
Lead (Pb)	< 1.0 ppm	< 0.65 ppm
Cadmium (Cd)	< 1.0 ppm	< 1.25 ppm
Arsenic (As)	< 1.0 ppm	< 2.5 ppm
Mercury (Hg)	< 1.0 ppm	< 0.150 ppm
Microbiological Assays		
Aerobic plate count	≤ 1000 cfu/g	≤ 1000 cfu/g
E. coli	Negative/10 g	Negative/10 g
Listeria monocytogenes	Negative/25 g	Negative/25 g
Salmonella and *Shigella*	Negative/10 g	Negative/10 g
Staphylococcus aureus	Negative/100 g	Negative/100 g
Coliform	Negative/25 g	Negative/25 g
Yeast count	< 100 cfu/g	< 100 cfu/g
Mold count	≤ 100 cfu/g	< 100 cfu/g

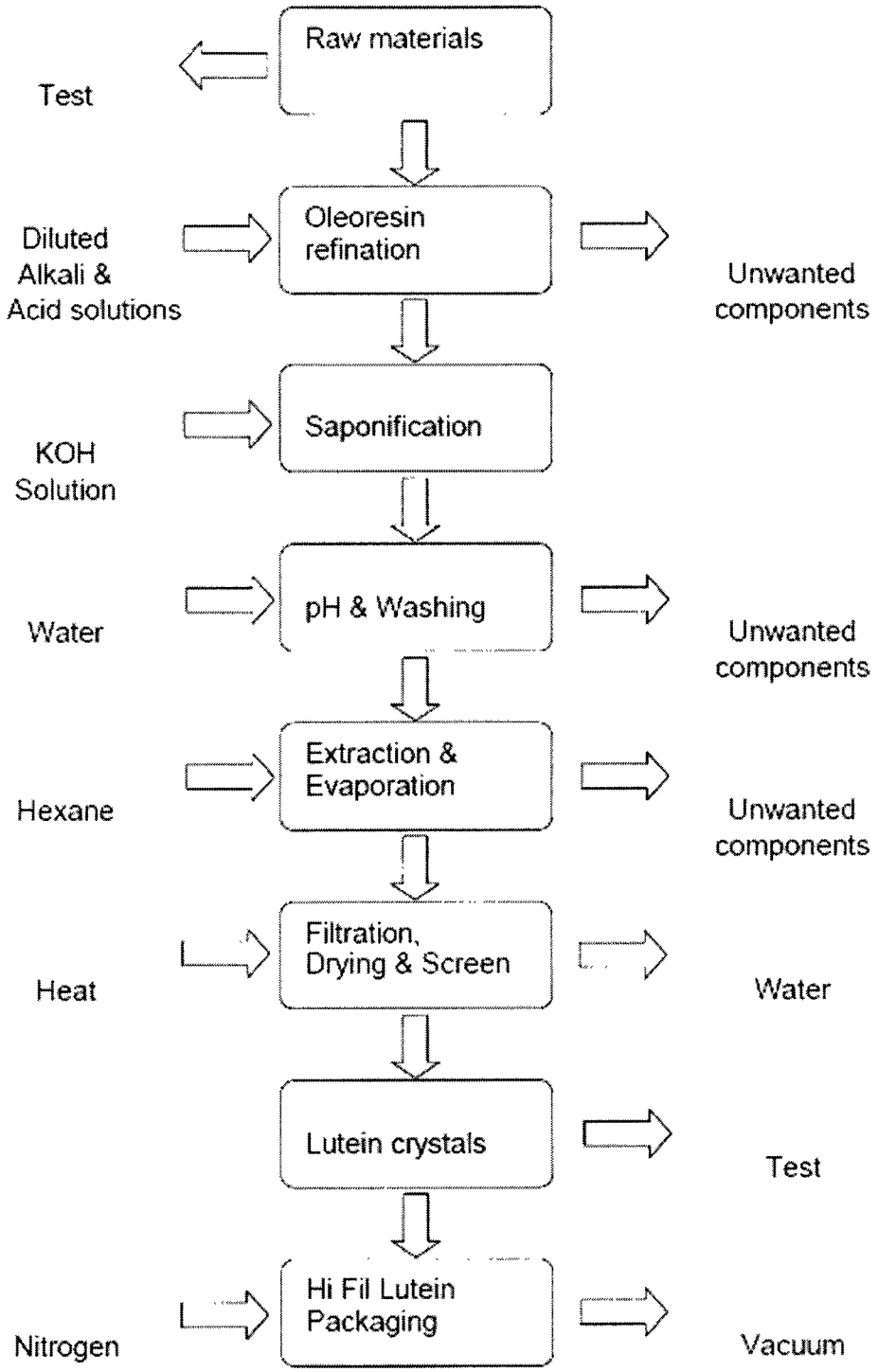
Manufacturing Process

The *Hi Fil*™ Lutein product is manufactured in accordance with current good manufacturing practice at IOSA's Monterrey México plant. The *Hi Fil*™ Lutein process starts with marigold (*Tagetes erecta*) oleoresin as the raw material. The oleoresin material is obtained by hexane extraction of dried marigold flower petals. A process diagram for *Hi Fil*™ Lutein product manufacture is presented in Figure 3.

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. Then the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls. The saponified mass is diluted with water, pH adjusted and the water is used to wash away salts and other water soluble impurities. Lutein concentrate is extracted with hexane to further remove unwanted components. The solvent is separated by filtration, decanting and centrifugation. Lutein crystals are then treated under high vacuum and temperature evaporation to remove remaining solvent. Lutein crystals are filtered and dried to remove water and screened to collect the lutein crystal product of appropriate size. The final crystalline lutein product is packed under high vacuum and inert nitrogen atmosphere in food-grade plastic pouches.

Figure 3.

Manufacturing Process Diagram



Section III

Levels of Use

The *Hi Fil*™ Lutein product is intended for use in the same foods and levels of addition as notified by Kemin Foods for the FloraGLO® Crystalline Lutein in GRN 000140. The proposed food uses 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, 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. The intended food uses and use levels are presented in Table 4 on the next page. The application of lutein to the same foods and at the same levels as those in GRN 000140 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.

Lutein has no functional effect on the manufactured food. It does not affect texture, taste or color.[4] Rather, *Hi Fil*™ Lutein is intended as an interchangeable source of supplemental lutein in the specified applications. Since the quantity of FloraGlo Lutein that may be added to the various applications is currently limited by the specifications in GRN 000140, the concentration of *Hi Fil*™ Lutein will be subject to the same limitations.

The dietary analysis below was presented by Kemin in GRN 000140 and was not questioned by FDA in their response letter of June 14, 2004. "Lutein and zeaxanthin are among the most prevalent carotenoids in the North American diet (IOM, 2000), occurring in high concentrations in green leafy vegetables, such as spinach and kale (Khachik *et al.,* 1995; Omaye *et al.,* 1997), and in chicken egg yolks (Handelman *et al.,* 1999). In the U.S., the average daily intake of lutein and zeaxanthin from plant sources is estimated to range from 2 to 4 mg. In addition to their natural occurrence in various foods, lutein and zeaxanthin are available as dietary supplements (IOM, 2000); however, there are no consumption data from which to reliably estimate the intake of lutein and zeaxanthin by supplement users. Market share data indicate that lutein use in dietary supplements occurs predominantly in multi-vitamin type supplements."

"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

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[4] While as supplied the lutein crystals are colored, the color is a function of crystal structure and is no longer present when lutein is dissolved. Thus lutein does not function as a color additive.

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. 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."

Given that the *Hi Fil*™ Lutein product is substantially equivalent in composition and is intended for use in the same foods and 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 for *Hi Fil*™ Lutein and would not be additive to FloraGLO® crystalline lutein, particularly because market use in foods is competitive and current use is predominantly in multi-vitamin supplements.

Table 4.

Summary of the Individual Proposed Food-Uses for *Hi Fil* ™ Lutein

Food Category	Proposed Food	Use levels (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 levels same as GRN 000140.
* Does not include infant formula.

Section IV

Basis for Notifier's Claim

IOSA has determined that *Hi Fil*™ Lutein is Generally Recognized as Safe based on scientific procedures. IOSA is supported in this determination by the findings of two scientific panels.

In June of 2006, the status of the IOSA *Hi Fil*™ Lutein was reviewed by a panel of scientists (the 2006 Panel) assembled by AAC Consulting Group of Kendle International. The expert panel was comprised of three noted toxicologists with expertise in food safety and lutein production. The Panel reviewed the information and the available toxicology literature on lutein and lutein derivatives. Based on this information, the Panel issued an Expert Panel Statement, "Determination Of The GRAS Status Of *Hi Fil*™ Crystalline Lutein for Addition To Select Specified Foods" in which it

> determined by scientific procedures that addition of *Hi Fil*™ Lutein, meeting the specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.[5]

In preparation for submitting this Notice, IOSA assembled a second[6] expert panel (the "2008 Panel") to review any relevant additions to the public literature that had been published after the 2006 Expert Panel Statement. The 2008 Panel concluded that the

> studies published over the last two years also do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous self affirmed GRAS determination of lutein by IOSA.[7]

Thus, our GRAS determination is supported by the Statement of the 2006 Expert Panel and the concurring Statement of the 2008 Panel.

---

[5] A copy of the 2006 Expert Panel Statement is attached as Appendix I. While the Expert Statement includes the use of crystalline lutein as a component of "medical foods" within the parameters of its opinion, the Notifier has no intent to market the crystalline lutein for this application and does not include it in the applications specified herein.

[6] The 2008 Panel was coordinated by EAS Consulting Group, the successor to the AAC Consulting Group who conducted the 2006 Panel.

[7] *Review of safety studies of lutein (2006 – present) for GRAS update,* John Thomas *et al.*, October 24, 2008. A copy of this statement is attached as Appendix II.

Appendix I

Determination of The GRAS Status Of *Hi Fil*™ Crystalline Lutein for Addition To Select Specified Foods

EXPERT PANEL STATEMENT

DETERMINATION OF THE GRAS STATUS OF *Hi Fil*™ CRYSTALLINE LUTEIN FOR ADDITION TO SELECT SPECIFIED FOODS

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Industrial Organica S.A de C.V. (IOSA)., to determine the Generally Recognized as Safe (GRAS) status of crystalline lutein sold as *Hi Fil*™ Lutein for use as an ingredient for direct addition to select specified foods. A comprehensive search of the scientific literature for safety and toxicity information on lutein was conducted through February 2006 and made available to the Expert Panel. The Expert Panel independently evaluated materials submitted by IOSA and other materials deemed appropriate or necessary. Following independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

Identity and Composition

Hi Fil™ Lutein (common or usual name lutein) is a purified crystalline extract from the marigold *(Tagetes erecta)* flower. *Hi Fil*™ Lutein typically contains approximately 80-90% by weight total carotenoids, with about 90% as lutein. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table 1.

Table 1 Chemical and Physical Characteristics of Lutein and Zeaxanthin

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical names	xanthophyll; β, ε-carotene-3,3' -diol; vegetable lutein; vegetable luteol; all-trans-(+)-xanthophylls; lutein	β, β -carotene-3,3'-diol; all-trans- β, β-carotene-3,3' -diol; zeaxanthol
Empirical formula	$C_{40}H_{56}O_2$	$C_{40}H_{56}O_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

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

Figure 1 Structural Formula of Lutein

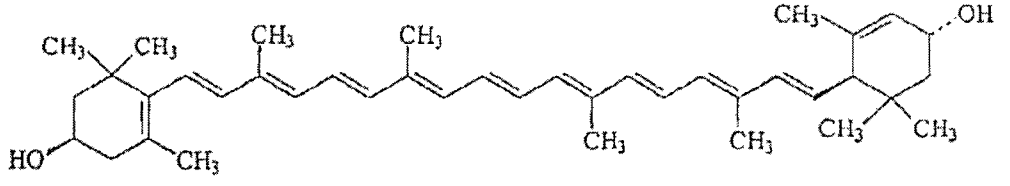
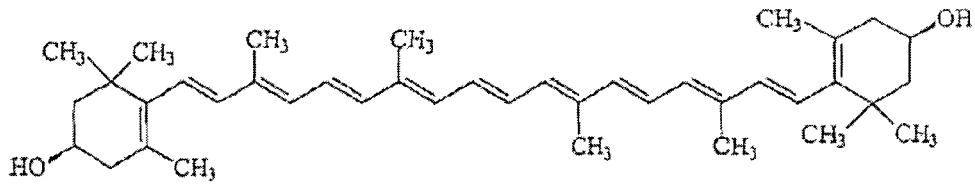


Figure 2 Structural Formula of Zeaxanthin



Typical composition for *Hi Fil*™ Lutein and Kemin FloraGLO® are provided in Table 2. As this table demonstrates, the composition of the two lutein products are substantially equivalent, with the *Hi Fil*™ Lutein having a greater carotenoid content reported as the only difference (90% *Hi Fil*™ *vs.* 83% FloraGLO®); however, the differences noted in carotenoid content are likely due to lack of accounting for other carotenoids in the FloraGLO® analysis and the greater purification and removal of non-carotenoid impurities such as waxes (<6% *Hi Fil*™ vs. <14% FloraGLO®) for the *Hi Fil*™ Lutein.

Table 2. Composition of *Hi Fil*™ Lutein Product *vs.* FloraGLO®

	***Hi Fil*™ Lutein**	**FloraGLO®**
Principal Components	**Average Amount (% dry weight)**	**Mean Amount (% dry weight)**
Total Carotenoids	≥90.0[1]	≥83.0[2]
Waxes	≤6.0	≤14.0
Free Fatty Acids	≤2.0	≤1.0
Water	≤1.0	≤1.0
Ash	≤1.0	≤1.0
Total	100	100

[1]Total Carotenoids include lutein, zeaxanthin and other carotenoids analyzed by HPLC

[2] Analysis does not include other carotenoids; likely reason for difference

Specification of *Hi Fil* ™Crystalline Lutein

Crystalline lutein has been the subject of a prior GRAS notification to FDA by Kemin Foods. FDA replied with its "no question" letter on June 14, 2004 to GRN 000140. Therefore, comparisons of the *Hi Fil*™ Lutein product and the notified Kemin's FloraGLO® product are made in this GRAS determination for the purposes of showing the substantial equivalence of the two products and the direct applicability of the FDA acceptance of GRN 000140 to IOSA's *Hi Fil*™ Lutein product for specified foods.

The specifications of *Hi Fil*™ Lutein are given in Table 3 and compared to Kemin's FloraGLO® Lutein in the table below. This table shows that the specifications for the two lutein products are nearly identical, with *Hi Fil*™ Lutein having a lower hexane specification as the only notable difference. Although the total carotenoid specifications are different, the lutein and zeaxanthin specifications are the same and both products are derived from marigold flowers, so actual products are likely to have same or substantially equivalent total carotenoid composition, despite the lower manufacturing specification set by Kemin on total carotenoids.

Table 3. Specifications for *Hi Fil*™ Lutein and FloraGLO® Lutein		
Principal Components	***Hi Fil*™ Lutein**	**FloraGLO® Lutein**
Total carotenoids (including lutein and zeaxanthin)	≥90.0% wt	≥ 80.0% wt
Lutein	≥74.0%	≥74.0% wt
Zeaxanthin	≤8.0% wt	≥2.0% wt; ≤9.0% wt
Waxes	≤7.0% wt	≤ 14.0%wt
Moisture	≤1.0%wt	≤1.0%wt
Ash	≤1.0%wt	≤1.0%wt
Other constituents	≤0.1%wt	≤0.1%wt
Pesticides and Related Potential Contaminants		
Protein	< 5000 ppm	< 100 ppm
Hexane	< 25 ppm	< 50 ppm
Thiophenes	< 300 ppm	< 300 ppm
Chlorinated and organophosphate pesticides **	Not Detected	Not Detected
Heavy Metals		
Lead (Pb)	< 1.0 ppm	< 0.65 ppm
Cadmium (Cd)	< 1.0 ppm	< 1.25 ppm
Arsenic (As)	< 1.0 ppm	< 2.5 ppm
Mercury (Hg)	< 1.0 ppm	< 0.150 ppm
Microbiological Assays		
Aerobic plate count	≤1000 cfu/g	≤1000 cfu/g
E. coli	Negative/10 g	Negative/10 g
Listeria monocytogenes	Negative/25 g	Negative/25 g
Salmonella and *Shigella*	Negative/10 g	Negative/10 g
Staphylococcus aureus	Negative/100 g	Negative/100 g
Coliform	Negative/25 g	Negative/25 g
Yeast count	< 100 cfu/g	< 100 cfu/g
Mold count	≤100 cfu/g	< 100 cfu/g

** Confirmatory analyses of pesticide residues on three batches of product attached in Appendix A

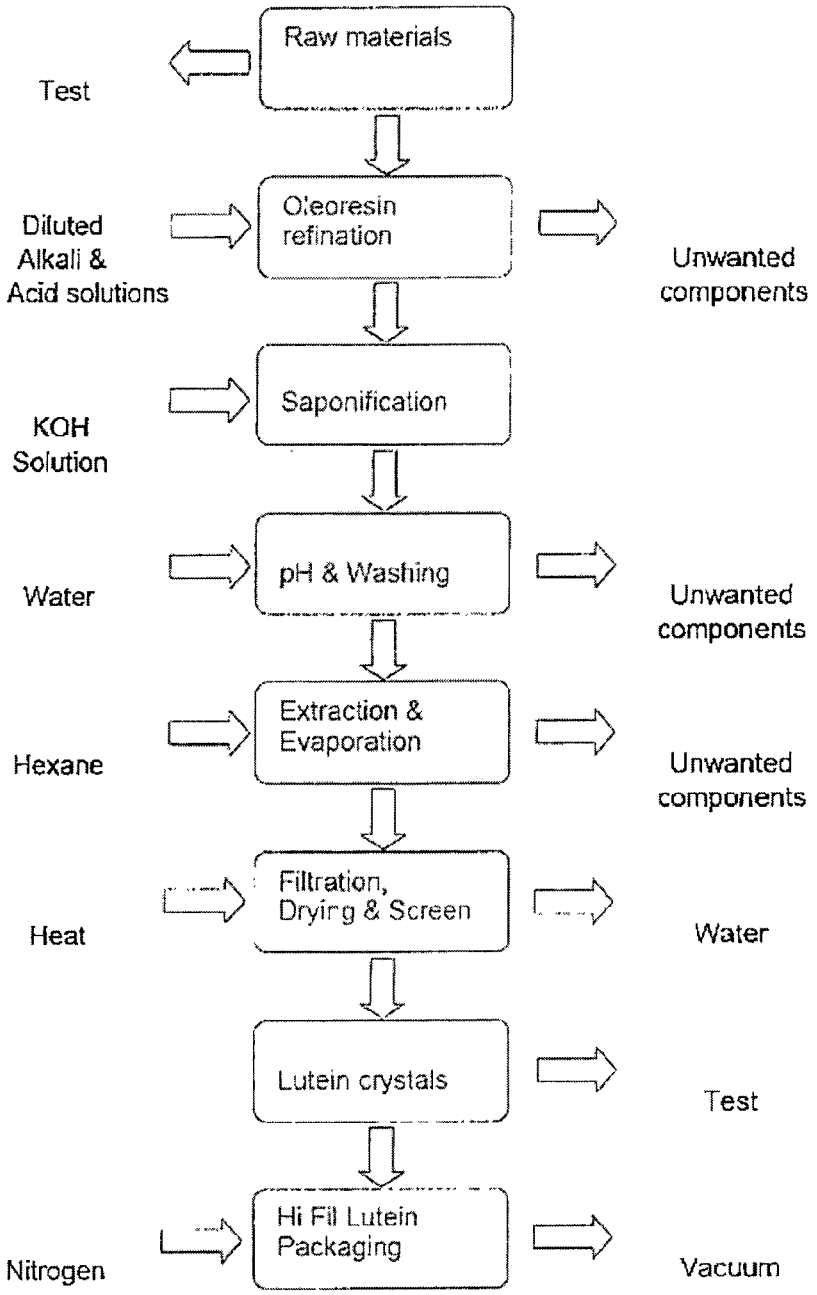
Manufacturing Process

Th *Hi Fil*™ Lutein product is manufactured in accordance with current good manufacturing practice at IOSA's Monterrey México plant. The *Hi Fil*™ Lutein process starts with marigold (*Tagetes erecta*) oleoresin as the raw material. The oleoresin material is obtained by hexane extraction of dried marigold flower petals. A process diagram for *Hi Fil*™ Lutein product manufacture is presented in Figure 3.

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. Then the oleoresin is saponified using potassium hydroxide and water with mixing under controlled temperature to free the xanthophylls. The saponified mass is diluted with water, pH adjusted and the water is used to wash away salts and other water soluble impurities. Lutein concentrate is extracted with hexane to further remove unwanted components. The solvent is separated by filtration, decanting and centrifugation. Lutein crystals are then treated under high vacuum and temperature evaporation to remove remaining solvent. Lutein crystals are filtered and dried to remove water and screened to collect the lutein crystal product of appropriate size. The final crystalline lutein product is packed under high vacuum and inert nitrogen atmosphere in food-grade plastic pouches.

Figure 3.

Manufacturing Process Diagram



Proposed Use and Intake/Exposure to Lutein

The *Hi Fil*™ Lutein product is intended for use in the same foods and levels of addition as notified by Kemin Foods for the FloraGLO® Crystalline Lutein in GRN 000140. The proposed food uses 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, 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. The intended food uses and use levels are presented in Table 4 on the next page. The application of lutein to the same foods and at the same levels as those in GRN 000140 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.

The dietary analysis below was presented by Kemin in GRN 000140 and was not questioned by FDA in their response letter of June 14, 2004. "Lutein and zeaxanthin are among the most prevalent carotenoids in the North American diet (IOM, 2000), occurring in high concentrations in green leafy vegetables, such as spinach and kale (Khachik *et al.*, 1995; Omaye *et al.*, 1997), and in chicken egg yolks (Handelman *et al.*, 1999). In the U.S., the average daily intake of lutein and zeaxanthin from plant sources is estimated to range from 2 to 4 mg. In addition to their natural occurrence in various foods, lutein and zeaxanthin are available as dietary supplements (IOM, 2000); however, there are no consumption data from which to reliably estimate the intake of lutein and zeaxanthin by supplement users. Market share data indicate that lutein use in dietary supplements occurs predominantly in multi-vitamin type supplements."

"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. 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."

Given that the *Hi Fil*™ Lutein product is substantially equivalent in composition and is intended for use in the same foods and 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 for *Hi Fil*™ Lutein and would not be additive to FloraGLO® crystalline lutein, particularly because market use in foods is competitive and current use is predominantly in multi-vitamin supplements.

Table 4.Summary of the Individual Proposed Food-Uses for *Hi Fil* ™ Lutein		
Food Category	**Proposed Food**	**Use levels (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 levels same as GRN 000140.
*Does not include infant formula.

Safety Studies

Background

In GRN 000140, studies on lutein in the literature and on FloraGLO® Crystalline Lutein were presented which supported the safety of crystalline lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of crystalline lutein for the proposed food uses. Because the *Hi Fil*™ Lutein product is substantially equivalent in composition to the crystalline lutein product that was the subject of GRN 000140, these studies can be utilized for safety assessment of the crystalline lutein that is the subject of this notification.

"The safety of FloraGLO® Crystalline Lutein has been established in toxicological studies in rats, mutagenicity studies conducted with *Salmonella typhimurium*, and is further supported by intervention studies conducted with healthy subjects designed to measure metabolic endpoints. The safety of FloraGLO® Crystalline Lutein is also corroborated by additional animal and human studies conducted with other sources of lutein, lutein-rich foods, and lutein supplements. In addition, the safety of lutein and zeaxanthin is well established in the literature based on the historical consumption of eggs and fruits and vegetables where these carotenoids predominate (e.g., green leafy vegetables, such as spinach and kale). Lutein is the major xanthophyll found in human serum, with smaller amounts of zeaxanthin and cryptoxanthin also present (Williams *et al.*, 1998; Boileau *et al.*, 1999). Lutein is also present in breast milk (Gossage *et al.*, 2002), and, together with zeaxanthin, are the only carotenoids found in the macular region of the human retina (Bone et *al.*, 1985, 1988, 1993; Omaye *et al.*, 1997). In general, carotenoids in foods are not known to be toxic, even when ingested in large amounts (e.g., >30 mg carotenoids) (Olson, 1996; Omaye *et al.*, 1997)."

"The safety of lutein and zeaxanthin was addressed by the Institute of Medicine (IOM, 2000) following a review of the available data regarding β-carotene and other carotenoids. The IOM concluded that no adverse effects, other than carotenodermia, have been reported from the consumption of carotenoids, including lutein and zeaxanthin, in foods. Carotenodermia, characterized by a yellowish discoloration of the skin, is a harmless and reversible biological effect of high carotenoid intake (IOM, 2000). No tolerable upper intake levels were established for lutein or zeaxanthin, or for any other carotenoid, (β-carotene, α-carotene, lycopene, and β-cryptoxanthin) considered by the IOM (2000)."

Studies of Absorption and Bioavailability

"As fat-soluble compounds, lutein and zeaxanthin generally follow the same digestion and intestinal absorption pathways as dietary fat (Bendich, 1988; Furr and Clark, 1997; Boileau *et al.*, 1999; van den Berg, 1999). Incorporation into mixed bile salt micelles in the small intestine is required for mucosal uptake in the enterocyte and transport to the lymphatic and/or portal circulation (Furr and Clark, 1997; Boileau *et al.*, 1999; van den Berg, 1999). Dietary factors potentially affecting the degree of absorption of lutein and zeaxanthin following ingestion *(i.e.* factors affecting the bioavailability of lutein and zeaxanthin) include interactions with other carotenoids and/or other nutrients, the association with and digestibility of the food matrix and the isomeric form of the carotenoids *(cis versus trans)*. Absorbed 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). Distribution to extra-hepatic tissue purportedly

occurs *via* the interaction of lipoprotein particles *(e.g.*, HDL, LDL) with receptors, and degradation of lipoproteins by extra- hepatic enzymes *(e.g.*, lipoprotein lipase) (Boileau *et al.*, 1999). A specific xanthophyll-binding protein (XBP) isolated from solubilized carotenoid-rich membrane extracts from human macula has been demonstrated to mediate the uptake of lutein and zeaxanthin from the bloodstream (Yemelyanov *et al.*, 2001)."

"Intervention trials conducted with FloraGLO® Crystalline Lutein have demonstrated that, following its ingestion, lutein and zeaxanthin are absorbed intact in healthy human subjects, as evidenced by increased plasma levels (Kostic *et al.* 1995; Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999; Schalch *et al.*, 2001). In comparison with plant sources of lutein, the bioavailability of lutein from FloraGLO® Crystalline Lutein (incorporated in the diet as a suspension in oil added to salad dressing) is reported to be approximately 30 to 50% greater (Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999). However, considering that lutein from egg yolks is more bioavailable than lutein from vegetable sources (Johnson and Mayer, 2003), the increase in bioavailability of lutein from FloraGLO® Crystalline Lutein compared with all foods *(i.e.* fruits, vegetables, and eggs) may be less than that reported by van het Hof *et al.* (1999) and Castenmiller *et al.* (1999). The mean intake of lutein provided by the intended uses of FloraGLO® Crystalline Lutein is approximately 2.5 times greater than background (assuming background intake of 3 mg lutein/day); however, considering bioavailability differences (Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999; Johnson and Mayer, 2003), systemic exposure may be expected to increase to approximately 4 times above background."

"Metabolic intervention studies have indicated that carotenoid interactions may occur at the metabolic level *(e.g.*, the absorption of both β-carotene and lutein have been demonstrated to be reduced following simultaneous ingestion); however, FloraGLO® Crystalline Lutein is not expected to have a negative impact on total carotenoid balance due to the low intake levels of lutein provided under the intended conditions of use. Taken together, intervention studies indicate that lutein and zeaxanthin are effectively absorbed from FloraGLO® Crystalline Lutein, that lutein is more bioavailable from FloraGLO® Crystalline Lutein as a supplement compared with fruits and vegetables, and that lutein and zeaxanthin from FloraGLO® Crystalline Lutein are not expected to adversely affect carotenoid balance under the intended conditions of use."

As noted above, the absorption and bioavailability of the *Hi Fil*™ Lutein product is considered substantially equivalent in composition to the FloraGLO® Crystalline Lutein product, so the absorption and bioavailability would be the same for both products.

Toxicological Studies

The toxicological evaluation conducted for GRN 000140 is considered directly applicable to *HiFil*™ Lutein due to their nearly identical composition. In GRN 000140, the "Expert Panel reviewed toxicological studies conducted with FloraGLO® Crystalline Lutein, as well as safety studies of lutein and zeaxanthin, in general, from other sources *(e.g.*, marigold extracts). Based on bioavailability studies reporting up to 43% absorption, and measured tissue and/or plasma levels of lutein and/or zeaxanthin, these studies have demonstrated that each of lutein and zeaxanthin (from FloraGLO® Crystalline Lutein or as an extract from marigold) are effectively absorbed from the diet in mice, rats, cats, dogs, and monkeys, supporting the suitability of these experimental animals as appropriate models for the study of lutein and zeaxanthin absorption and safety in humans (Park *et al.*, 1998; Jewell and O'Brien, 1999; Jenkins *et al.*, 2000; Kim *et al.*,

2000 a,b; Kruger *et al.*, 2002; Schierle *et al.*, 2002)." However, of this group of animal models, only primates can concentrate lutein and zeazanthin in the retina.

"In 4-week and 13-week toxicological studies conducted with FloraGLO® Crystalline Lutein, no adverse effects were reported in terms of body weight gain, organ weights, feed intake, clinical chemistry, or histopathology in Wistar rats (Kruger *et al.*, 2002). Animals were exposed to FloraGLO® Crystalline Lutein in the diet at levels up to 773 mg/kg body weight/day (delivering 639 mg lutein plus zeaxanthin/kg body weight/day) for 4 weeks, or up to 260 mg/kg body weight/day (delivering 208 mg lutein plus zeaxanthin/kg body weight/day) for 13 weeks. The no-observed-adverse-effect-levels (NOAELs) for FloraGLO® Crystalline Lutein in Wistar rats corresponded to the highest doses tested in each toxicology study; 773 mg/kg body weight/day following 4 weeks of exposure, and 260 mg/kg body weight/day following 13 weeks of exposure. Additional toxicological studies conducted with FloraGLO® Crystalline Lutein, ranging in length from 8 to 12 weeks, have measured feed intake, body weight, organ weights, and plasma levels of cholesterol and triglycerides (Jenkins *et al.*, 2000; Kim *et al.*, 2000 a,b). No adverse effects were reported in male weanling Fischer 344 rats following exposure to up to 621 mg FloraGLO® Crystalline Lutein/kg body weight/day (31 mg lutein/kg body weight/day) in the diet for 8 weeks. Higher dose levels (1,169 and 2,383 mg FloraGLO® Crystalline Lutein/kg body weight/day) resulted in increased body weights (presumably due to an increase in caloric intake), increased plasma levels of triglycerides and cholesterol, and decreased relative lung and brain weights (2,383 mg FloraGLO® Crystalline Lutein/kg body weight/day dose group only). The reported decreased relative organ weights were considered by the Panel not to be treatment-related effects, and to be consequential of increased body weights. Furthermore, similar changes increased plasma levels of cholesterol and triglycerides, decreased relative organ weights) were not reported in toxicological studies of similar or longer duration conducted with higher doses of lutein from FloraGLO® Crystalline Lutein (Buser *et al.*, 1999; Pfannkuch *et al.*, 2000, 2001; Kruger *et al.*, 2002)."

"The genotoxic potential of FloraGLO® Crystalline Lutein (10% beadlet formulation, and without beadlet) was investigated using the reverse mutation assay (Ames test), conducted with *Salmonella typhimurium* strains TA 1535, TA 97, TA 98, TA 100, and TA 102, with and without metabolic activation (S9 fraction from rat liver) (Kruger *et al.*, 2002). No increase in the number of mutant colonies was observed for any of the five tester strains after treatment with 10% beadlet (15.8 to 5,000 pg/plate) or FloraGLO® Crystalline Lutein (15.8 to 500 pg/plate), demonstrating that neither formulation is mutagenic in S. *typhimurium* strains. The mutagenic potential of lutein from dietary sources (extracts of fruits, vegetables, and marigold) has also been investigated in various studies, indicating that lutein is non-mutagenic in bacterial test systems *in vitro*, and has no clastogenic activity or DNA damaging effects in mammalian test systems *in vivo* (Yoshikawa *et al.*, 1996; Collins *et al.*, 1998; Rauscher *et al.*, 1998)."

"Additional supportive toxicological studies conducted with rodents have investigated the effects of dietary exposure to lutein and zeaxanthin, from other sources (*e.g.*, marigold extract), for 2 to 8 weeks on body weight, feed intake, and organ weights. No changes in body weight gain, feed intake, liver weight, or spleen weight, and no external signs of toxicity were reported in BALB/c mice following 2 to 4 weeks of dietary exposure to up to 803 mg lutein/kg body weight/day and 10.9 mg zeaxanthin/kg body weight/day (Chew *et al.*, 1996; Park *et al.*, 1998, 1999). Similarly, no changes in body weights, feed intake, or organ weights were reported in Wistar rats following 16 days of dietary exposure to up to 45 mg lutein/kg body weight/day (Gradelet *et al.*, 1996;

Jewell and O'Brien, 1999), and 8 weeks of dietary exposure to 500 mg lutein/kg body weight/day was well tolerated in experimental mice models of atherosclerosis (Dwyer *et al.*, 2001)."

"The chronic toxicity of lutein and zeaxanthin (0, 0.2, or 20 mg/kg body weight/day, source not specified) was investigated in Cynomolgus monkeys following daily administration *via* gavage for 52 weeks (Schierle *et al.*, 2002). No clinical or morphological evidence of treatment-related adverse changes were reported, and it was concluded that long-term administration of lutein and zeaxanthin in Cynomolgus monkeys at dose levels up to 20 mg/kg bodyweight/day resulted in no toxic effects, as evidenced by clinical investigation parameters, necropsy, and histopathology (Schierle *et al.*, 2002). Specific to ocular toxicity, the Panel discussed ongoing long-term studies in monkeys administered up to 20 mg lutein and zeaxanthin/kg body weight/day and evaluated abstracts reporting the results of these studies. No evidence of clinical or morphological toxicity to the eyes was reported, and further, no evidence of crystal formation in the eyes of treated monkeys was noted (Schierle *et al.*, 2002; Wolz *et al.*, 2002). The Panel considered these ongoing studies to be corroborative of the overall safety of FloraGLO® Crystalline Lutein, and supportive of the shorter-term clinical studies (see below)."

Human Safety Data

"In addition to published pivotal animal toxicology studies with FloraGLO® Crystalline Lutein, the Expert Panel considered clinical data from safety/tolerance studies with FloraGLO® Crystalline Lutein, as well as with lutein and zeaxanthin from other dietary sources, to corroborate the safety of FloraGLO® Crystalline Lutein. In a 42-day study designed to evaluate plasma response to oral lutein administration (FloraGLO® Crystalline Lutein formulated as beadlets in capsules), healthy volunteers were given capsules delivering either 4 or 20 mg lutein daily (8 subjects/dose group) (Schalch *et al.*, 2001). A total of 12 subjects experienced 26 adverse events, 24 of which were reported not to be related to the study intervention, and 2 of which (mild forms of conjunctivitis and abdominal pain) were unknown in their etiology. In addition, no clinically relevant abnormalities were reported with reference to laboratory values for serum liver enzyme levels, total bilirubin, glucose, creatinine, uric acid, calcium, total cholesterol, triglycerides, sodium, potassium, total protein, prothrombin time, blood urea nitrogen, and blood cell count. Together, these findings demonstrated that exposure to up to 20 mg lutein from FloraGLO® Crystalline Lutein in healthy subjects was not associated with adverse health effects (Schalch *et al.*, 2001)."

In a study designed to examine the effect of the food matrix on carotenoid bioavailability, Castenmiller *et al.* (1999) included a measurement of serum cholesterol and triacylglycerol concentrations. Following 3 weeks of dietary intervention with 6.6 mg lutein/day from FloraGLO® Crystalline Lutein (suspension in vegetable oil with β-carotene), no significant differences were reported compared with control values, demonstrating that lutein from FloraGLO® Crystalline Lutein had no effect on plasma lipid levels in healthy non-smoking, normolipidemic volunteers (Castenmiller *et al.*, 1999). Supporting intervention studies conducted with healthy subjects have demonstrated that daily exposure to 15 mg lutein (from marigold extract) for up to 6 months results in no adverse side effects, and no changes in serum blood lipid levels (individual and total fatty acids, total saturated, monounsaturated, and polyunsaturated, total cholesterol, HDL-cholesterol, and LDL cholesterol), or in hematological or biochemical parameters (Olmedilla *et al.*, 1997, 2001; Wright *et al.*, 1999; Falsini *et al.*, 2003). Similarly, no changes in blood hemoglobin concentrations, white blood cell levels, or

serum electrolyte levels were reported in healthy subjects following 2 weeks of exposure to 11 mg lutein/day from spinach powder (Muller *et al.*, 1999), or in patients with cataract or age-related maculopathy following 13 months of dietary supplementation with capsules containing 12 mg all-trans-lutein, 13 mg of 13/15-cis-lutein and 3.3 mg vitamin E (Olmedilla *et al.*, 2002). In addition, no evidence of ocular toxicity was noted in a single healthy male volunteer receiving 20 mg lutein/day as an oil suspension prepared from marigold flowers for a total of 3 weeks (Khachik *et al.*, 1997). Similarly, clinical studies in patients with retinal degenerative diseases have reported no losses in visual acuity and no significant changes in foveal vision parameters following daily supplementation with up to 20 mg lutein for up to 6 months (Richer, 1999; Aleman *et al.*, 2001; Duncan *et al.*, 2002). Carotenodermia (yellowish discoloration of the skin) has been reported in healthy subjects exposed to 15 mg lutein/day (from mixed ester forms extracted from marigold) for 4 months (Olmedilla *et al.*, 1997, 2002; Granado *et al.*, 1998); however, carotenodermia is considered a harmless and reversible biological effect of high carotenoid intake (IOM, 2000), and 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 to approximately 25 mg lutein/day, 3 times/week for 13 months (Olmedilla *et al.*, 2001). These findings of Khachik *et al.* (1997), Olmedilla *et al.* (1997, 2002), Granado *et al.* (1998), and Muller *et al.* (1999), indicate that oral exposures to up to 20 mg lutein/day do not result in any adverse health effects, including retinal damage *(e.g.*, crystal deposition).

"In addition, studies investigating the relationship of lutein and zeaxanthin to chronic disease have reported no adverse relationships between increased serum levels of lutein and zeaxanthin, or dietary intake of lutein and zeaxanthin, and eye health *(i.e.*, risk of AMD, risk of cataracts) (EDCC Study Group, 1993; Seddon *et al.*, 1994; Mares-Perlman *et al.*, 1995; Brown *et al.*, 1999; Chasan-Taber *et al.*, 1999; Lyle *et al.*, 1999 a,b; Snellen *et al.*, 2002), and no adverse relationships between serum levels of lutein and zeaxanthin and the risk for subsequent myocardial infarction (Street *et al.*, 1994) or the progression of intima-media thickness of the common carotid arteries (Dwyer *et al.*, 2001)." In a further study, ninety patients with atrophic ARMD received 10 mg lutein daily over 12 months. Mean eye macular pigment optical density increased and visual function was improved with lutein alone or lutein together with other nutrients (Richer *et al.*, 2004).

"The Expert Panel considered the results of clinical studies with β-carotene to be of limited relevance to the evaluation of the safety of FloraGLO® Crystalline Lutein. Intervention studies conducted with β-carotene have reported that supplemental β-carotene may enhance lung tumorigenesis in cigarette smokers (ATBC, 1994; Omenn *et al.*, 1996 a,b), potentially via altered retinoid signaling (Paolini *et al.*, 1999; Wang *et al.*, 1999). Unlike β-carotene, lutein is a non-provitamin A carotenoid, and is not expected to directly alter retinoid signaling. Furthermore, the numerous epidemiological studies conducted with lutein have reported protective effects against various forms of cancer (breast, lung, adenocarcinomas of the esophagus, and gastric cardia), and have therefore provided no evidence to suggest that exposure to lutein contributes towards an enhanced risk of cancer in humans. In addition, toxicology studies conducted with FloraGLO® Crystalline Lutein have reported no adverse toxicological effects, and no mutagenic activity of FloraGLO® Crystalline Lutein has been reported *in vitro* or *in vivo* "

Additional Supporting Information on Safety of Lutein

It is well known that lutein esters are readily degraded in the GI tract by esterase enzymes to lutein and constituent compounds such as palmitate. They are absorbed by the intestinal mucosal

enterocytes as lutein. Thus, GRAS notifications on lutein esters are applicable to the safety assessment of lutein as that is the form that is present in systemic exposure. Cognis Corporation had previously submitted a GRAS Notification to FDA on July 17, 2002 (GRN 000110). This contained the unanimous concurrence from a Panel of qualified experts that Xangold® lutein esters are GRAS, based on their expert evaluation of the scientific literature for addition to conventional foods as an ingredient to provide consumers with a supplementary source of lutein in their diets. The Panel of independent qualified experts conducted and extensive review of all generally available scientific literature for safety and toxicity as well as evaluated all available data from dietary consumption of lutein and zeaxanthin. The typical daily consumption level of lutein and zeaxanthin was estimated at 1.35-1.97 mg/day. No toxicity from lutein ester supplement consumption has been reported and human clinical studies indicate that long-term consumption of lutein esters is well tolerated. Seven clinical studies ranging from 84 days to 3 years have reported that consumption of between 18 and 60 mg/day lutein ester equivalents is safe (GRN 000110). The Expert Panel established a conservative acceptable daily intake of Xangold® lutein esters of 40 mg/day.

No evidence of toxicity or safety concerns was noted by the Panel in their review of all available toxicology and clinical studies. The Expert Panel evaluated the proposed use of Xangold® lutein esters at specified levels seen in the following foods: baked goods and baking mixes, soy milk, beverages and beverage substitutes, frozen dairy desserts and mixes, processed fruit and vegetable products, egg products and egg substitutes, breakfast cereals (ready-to-eat), fats and oils, hard candy, fruit snacks and dairy products. They concluded that such use would result in an estimated daily intake that is below 40 mg/day lutein esters When the Expert Panel evaluated the combination of the 90th percentile lutein ester consumption levels from foods supplemented with Xangold® lutein esters, with the 90th percentile current consumption of lutein esters from conventional foods, the estimated daily intake of lutein esters from conventional foods is 22.3 mg/day. In addition, the potential lutein ester consumption from dietary supplements may add an additional maximum intake of 12 mg/day. They also concluded that the maximum potential theoretical lutein ester consumption at the 90th percentile may reach 34.3 mg/day and that this was a conservative estimate since it is highly unlikely that an individual would consume lutein from both conventional foods and dietary supplements at the 90th percentile level. Even this conservative estimate is well within the Acceptable Daily Intake of 40 mg/day established and was deemed to be safe.

The July 17, 2002 GRAS notification included the supporting data on which this conclusion was based. FDA responded to this GRAS notification in a letter dated January 21, 2003 stating "based on the information provided by Cognis, as well as other information available to FDA, the agency has no questions at this time regarding Cognis' conclusion that lutein esters are GRAS under the intended conditions of use."

Safety Assessment of Crystalline Lutein

As noted above, the composition and purity of the *Hi Fil*™ Lutein product is considered substantially equivalent in composition to the FloraGLO® Crystalline Lutein product, so the animal and human studies reviewed herein are applicable to both products. Subchronic studies in rats up to 208 mg/kg/day (Kruger *et al.*, 2002) and chronic studies in Cynomolgus monkeys up to 20 mg/kg/day lutein and zeaxanthin (Schierle *et al.*, 2002) did not result in any adverse effects. Lutein has not shown any genotoxic effects in multiple assays and has shown some

evidence of a protective effect against cancer. With the exception of a reversible carotenodermia seen in two studies, human clinical trials have shown no significant adverse effects in subjects taking lutein and zeaxanthin up to 20-25 mg/day for over a year. Therefore, intake of *Hi Fil*™ Lutein in the diet from specified foods at estimated mean intakes of lutein and zeaxanthin of 7.3 mg/person/day (0.14 mg/kg/day) and 0.7 mg/person/day (0.01 mg/kg/day) respectively, and 90th percentile intakes of lutein and zeaxanthin at 13.4 mg/person/day (0.28 mg/kg/day) and 1.2 mg/person/day (0.03 mg/kg/day), are not considered to pose any safety concerns.

CONCLUSION

Based on a critical evaluation of the pertinent data and information summarized above, the Expert Panel members, whose signatures appear below, have individually and collectively determined by scientific procedures that addition of the Lutein 10 CWD formulated product, meeting the formula for preparation and specifications cited above and manufactured accordance with current good manufacturing practice, is generally recognized as safe (GRAS) under the conditions of intended use in foods and medical foods, as specified herein.

(b)(6)

James W. Barnett, Jr., Ph.D., DABT.

6/27/06
Date

(b)(6)

D. Max Shodderly, Ph.D.

21 June 06
Date

(b)(6)

Stanley M. Tarka, Jr., Ph.D.

6/30/06
Date

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Appendix I

Sub Appendix A

Pesticide Analysis

Industrial
Orgánica

Mar. 23, 06

Mr. Michael J. O'Flaherty
Olsson, Frank & Weeda, P.C.
1400 - 16th St., N.W., Suite 400
Washington, DC 20036-2220
Tel: 202/518-6320
Fax: 202/234-2686
Re. Lutein information

Dear Michael,

We hope you are fine as well as ourselves.

Enclosed find the following information requested for three different lots of our *Hi Fil* ™ Lutein crystals:

- Carotenoid composition
- Pesticides results

Please let us know if we can be of further assistance.

Our best regards,

(b)(6)

Carlos Torres
Industrial Organica S.A. de C.V.

Industrial Orgánica, S.A. de C.V.
Ave. Almazán No. 100 Col. Topo Chico 64260 Apdo. Postal 1654 Monterrey, N.L., México
Tel. (81) 83-52-22-90 01-800 926-7000 Fax (81) 83-76-72-14 e-mail: iosa@att.net.mx

Industrial
Orgánica

CERTIFICATE OF ANALYSIS

Product: *Hi-Fil 85 % LUTEIN crystal*

Lot No: 5 30 S-97HF85 **Date:** September 30, 2005.

Mfg. date: September 2005. **Expiration date:** September 2006.

TEST	SPECIFICATION	RESULTS
Content of total carotenoids (USP) :	Min. 90.0 %	91.3
Content of Lutein (USP) :	Min. 75.0 %	84.5
Content of Zeaxanthin (USP) :	Max. 8.0 %	5.9

(b)(6)

M.Sc. (b) (6)
Quality Control

Industrial Orgánica, S.A. de C.V.
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Microbac Laboratories, Inc.
KENTUCKY TESTING LABORATORY DIVISION
3323 Gilmore Industrial Blvd. Louisville, KY 40213 502.962.6400 Fax: 502.962.6411
Evansville, IN 812.464.9000 • Frankfort, KY 502.803.0254 • Paducah, KY 270.898.3637

Chemical, Biological, Physical, Molecular, and Toxicological Services

CERTIFICATE OF ANALYSIS

0511-00062

INDUSTRIAL ORGANICA
(b) (6)
AVE. ALMAZAN NO. 100
COL. TOPO CHICO 64260 POSTAL
MONTERREY, N.L., MX

LUTEIN CAROTENOID CONCENTRATES

Date Reported	12/14/2005
Date Due	11/23/2005
Date Received	11/02/2005
Date Sampled	11/01/2005
Invoice No.	72249
Customer #	W031
Customer P.O.	CHECK 10191

Analysis	Qualif	Result	Unit	Method	Date	Tech
Sample: 001 HI FIL 85% LUTEIN / 20 GRS / LOT #530S-47HF85 / 10-24-0 Date & Time Sampled: 11/1/2005 @ 0:00						
		SEE BELOW				
Sample: 002 HI FIL 85% LUTEIN / 20 GRS / LOT #5180-94HF851a / 10-27 Date & Time Sampled: 11/1/2005 @ 0:00						
		SEE BELOW				
Sample: 003 HI FIL 85% LUTEIN / 250 GRS / LOT #5305-97HF85 / 10-24- Date & Time Sampled: 11/1/2005 @ 0:00						
[Pesticide Residue - CDFA]						
CDFA, CARBAMATE		SEE BELOW	MG/KG	CDFA MRM HPLC	11/30/2005	COR
CDFA, ORGANOCHLORINE		SEE BELOW	MG/KG	CDFA MRM GC/ELCD	11/30/2005	COR
CDFA, ORGANOPHOSPHATE		SEE BELOW	MG/KG	CDFA MRM GC/FPD	11/30/2005	COR
Sample: 004 HI FIL 85% LUTEIN / 250 GRS / LOT #5180-94HF85 / 10-27- Date & Time Sampled: 11/1/2005 @ 0:00						
[Pesticide Residue - CDFA]						
CDFA, CARBAMATE		SEE BELOW	MG/KG	CDFA MRM HPLC	11/30/2005	COR
CDFA, ORGANOCHLORINE		SEE BELOW	MG/KG	CDFA MRM GC/ELCD	11/30/2005	COR
CDFA, ORGANOPHOSPHATE		SEE BELOW	MG/KG	CDFA MRM GC/FPD	12/07/2005	OST

COR = ANALYSIS SUBCONTRACTED TO MICROBAC CORONA DIVISION
OST = ANALYSIS SUBCONTRACTED TO ENO RIVER LABS

UNLESS OTHERWISE NOTED, SAMPLES ARE ANALYZED AS RECEIVED.

RESPECTFULLY SUBMITTED: (b)(6)

MICROBAC LABORATORIES, INC.

For any feedback concerning our services, please contact Sean Hyde, the Managing Director at 502.962.6400, or Trevor Boyce, President, at tboyce@microbac.com or Robert Morgan, Chief Operating Officer, at rmorgan@microbac.com.

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Microbac Laboratories, Inc.
CORONA DIVISION
280 N SMITH STREET
CORONA CA, 92880
(951) 734-9600 FAX (951)734-2803
www.microbac.com e-mail: corona@microbac.com

FDA #2030513
LA City #10159
DHS #2122

CHEMICAL, BIOLOGICAL PHYSICAL MOLECULAR AND TOXICOLOGICAL ANALYSIS

CERTIFICATE OF ANALYSIS

MICROBAC - KENTUCKY DIVISION
PAM ELLIS
3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

LUTEIN CONC- AZTEC MARIGOLD

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003						
[FDA EXTENDED MRM]					11/10/2005	TRS
[CARBAMATES]					11/10/2005	TRS
ALDICARB SULFONE	<0.050	MG/KG		0.050	11/10/2005	TRS
ALDICARB SULFOXIDE	<0.050	MG/KG		0.050	11/10/2005	TRS
ALDICARB (TEMIK)	<0.050	MG/KG		0.050	11/10/2005	TRS
CARBARYL (SEVIN)	<0.050	MG/KG		0.050	11/10/2005	TRS
CARBOFURAN (FURADAN)	<0.100	MG/KG		0.100	11/10/2005	TRS
METHIOCARB (MESUROL)	<0.026	MG/KG		0.026	11/10/2005	TRS
METHOMYL (LANNATE)	<0.050	MG/KG		0.050	11/10/2005	TRS
OXAMYL (VYDATE)	<0.050	MG/KG		0.050	11/10/2005	TRS
PROPOXUR (BAYGON)	<0.050	MG/KG		0.050	11/10/2005	TRS
3-HYDROXYCARBOFURAN	<0.050	MG/KG		0.050	11/10/2005	TRS
[ORGANOCHLORINES]					11/10/2005	AVY
ALACHLOR (LAZO)	<0.050	MG/KG		0.050	11/10/2005	AVY
ALDRIN (ALDREX)	<0.005	MG/KG		0.005	11/10/2005	AVY
ANILAZINE (DYRENE)	<0.125	MG/KG		0.125	11/10/2005	AVY
ATRAZINE	<0.050	MG/KG		0.050	11/10/2005	AVY
BENEFIN (BALAN/BENFLURALIN)	<0.012	MG/KG		0.012	11/10/2005	AVY
BHC ALPHA ISOMER	<0.005	MG/KG		0.005	11/10/2005	AVY
BHC BETA ISOMER	<0.005	MG/KG		0.005	11/10/2005	AVY
BHC DELTA ISOMER	<0.005	MG/KG		0.005	11/10/2005	AVY
BHC GAMMA ISOMER (LINDANE)	<0.005	MG/KG		0.005	11/10/2005	AVY
BIFENOX (MOWDOWN)	<0.025	MG/KG		0.025	11/10/2005	AVY
BIFENTHRIN	<0.125	MG/KG		0.125	11/10/2005	AVY
BROMACIL	<0.250	MG/KG		0.250	11/10/2005	AVY
BROMOPROPYLATE	<0.025	MG/KG		0.025	11/10/2005	AVY
CAPTAFOL (DIFOLATAN)	<0.125	MG/KG		0.125	11/10/2005	AVY
CAPTAN (ORTHOCIDE)	<0.250	MG/KG		0.250	11/10/2005	AVY
CHLORDANE (OCTACHLOR)	<0.062	MG/KG		0.062	11/10/2005	AVY
CHLORDIMEFORM	<0.100	MG/KG		0.100	11/10/2005	AVY

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CORONA DIVISION
280 N SMITH STREET
CORONA CA, 92880
(951) 734-9600 FAX (951)734-2803
www.microbac.com e-mail: corona@microbac.com

FDA #2030513
LA City #10159
DHS #2122

CHEMICAL, BIOLOGICAL PHYSICAL MOLECULAR AND TOXICOLOGICAL ANALYSIS

CERTIFICATE OF ANALYSIS

MICROBAC - KENTUCKY DIVISION
(b) (6)
3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN CONC- AZTEC MARIGOLD

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001: LUTEIN 0511-062-003 ....continued						
CHLORFENSON (OVEX)	<0.125	MG/KG		0.125	11/10/2005	AVY
CHLOROBENZILATE (AKAR)	<0.500	MG/KG		0.500	11/10/2005	AVY
CHLORONEB (TERRANEB SP)	<0.025	MG/KG		0.025	11/10/2005	AVY
CHLOROPROPYLATE	<0.025	MG/KG		0.025	11/10/2005	AVY
CHLOROTHALONIL (BRAVO)	<0.025	MG/KG		0.025	11/10/2005	AVY
CHLORPROPHAM (CIPC)	<0.125	MG/KG		0.125	11/10/2005	AVY
CYANAZINE (BLADEX)	<0.500	MG/KG		0.500	11/10/2005	AVY
CYFLUTHRIN	<0.025	MG/KG		0.025	11/10/2005	AVY
LAMBDA CYHALOTHRIN	<0.025	MG/KG		0.025	11/10/2005	AVY
CYPERMETHRIN (AMMO)	<0.125	MG/KG		0.125	11/10/2005	AVY
DCPA (DACTHAL)	<0.012	MG/KG		0.012	11/10/2005	AVY
DDD, O,P-ISOMER	<0.012	MG/KG		0.012	11/10/2005	AVY
DDD, P,P-ISOMER	<0.012	MG/KG		0.012	11/10/2005	AVY
DDE, O,P-ISOMER	<0.007	MG/KG		0.007	11/10/2005	AVY
DDE, P,P-ISOMER	<0.012	MG/KG		0.012	11/10/2005	AVY
DDT, O,P-ISOMER	<0.007	MG/KG		0.007	11/10/2005	AVY
DDT, P,P-ISOMER	<0.012	MG/KG		0.012	11/10/2005	AVY
DELTAMETHRIN	<0.125	MG/KG		0.125	11/10/2005	AVY
DICHLOBENIL (CASORON)	<0.012	MG/KG		0.012	11/10/2005	AVY
DICHLONE (PHYGON)	<0.125	MG/KG		0.125	11/10/2005	AVY
DICHLORAN (BOTRAN)	<0.012	MG/KG		0.012	11/10/2005	AVY
DICLOFOP METHYL	<0.125	MG/KG		0.125	11/10/2005	AVY
DICOFOL (KELTHANE)	<0.125	MG/KG		0.125	11/10/2005	AVY
DIELDRIN (OCTALOX)	<0.005	MG/KG		0.005	11/10/2005	AVY
DIMETHACHLOR	<0.025	MG/KG		0.025	11/10/2005	AVY
DIURON	<0.100	MG/KG		0.100	11/10/2005	AVY
ENDOSULFAN I (THIODAN)	<0.025	MG/KG		0.025	11/10/2005	AVY
ENDOSULFAN II	<0.025	MG/KG		0.025	11/10/2005	AVY
ENDOSULFAN III ENDO-SULFATE	<0.025	MG/KG		0.025	11/10/2005	AVY
ENDRIN (ENDREX)	<0.012	MG/KG		0.012	11/10/2005	AVY

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LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN CONC- AZTEC MARIGOLD

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003						
...continued						
ENDRIN ALDEHYDE	<0.025	MG/KG		0.025	11/10/2005	AVY
ETACONAZOLE	<0.125	MG/KG		0.125	11/10/2005	AVY
ETHALFURALIN (SONALAN)	<0.012	MG/KG		0.012	11/10/2005	AVY
ETHYLAN (PERTHANE)	<0.125	MG/KG		0.125	11/10/2005	AVY
FENARIMOL (RUBIGAN)	<0.125	MG/KG		0.125	11/10/2005	AVY
FENVALERATE (PYDRIN)	<0.125	MG/KG		0.125	11/10/2005	AVY
FLAMPROP-ISOPROPYL	<0.250	MG/KG		0.250	11/10/2005	AVY
FLAMPROP-METHYL	<0.250	MG/KG		0.250	11/10/2005	AVY
FLUCHLORALIN (BASALIN)	<0.125	MG/KG		0.125	11/10/2005	AVY
FLUOMETRON	<0.250	MG/KG		0.250	11/10/2005	AVY
FOLPET (PHALTAN)	<0.025	MG/KG		0.025	11/10/2005	AVY
HCB (HEXACHLOROBENZENE)	<0.002	MG/KG		0.002	11/10/2005	AVY
HEPTACHLOR (DRINOX)	<0.002	MG/KG		0.002	11/10/2005	AVY
HEPTACHLOR EPOXIDE	<0.005	MG/KG		0.005	11/10/2005	AVY
IMAZALIL	<0.012	MG/KG		0.012	11/10/2005	AVY
IPRODION (ROVRAL)	<0.500	MG/KG		0.500	11/10/2005	AVY
METHOXYCHLOR (MARLATE)	<0.125	MG/KG		0.125	11/10/2005	AVY
METOLACHLOR	<0.100	MG/KG		0.100	11/10/2005	AVY
MIREX	<0.025	MG/KG		0.025	11/10/2005	AVY
MONOLINURON	<0.300	MG/KG		0.300	11/10/2005	AVY
MYCOBUTANIL (RALLY)	<0.025	MG/KG		0.025	11/10/2005	AVY
NITROFEN (TOK)	<0.025	MG/KG		0.025	11/10/2005	AVY
NORFLURAZON	<0.500	MG/KG		0.500	11/10/2005	AVY
OXADIAZON (RONSTAR)	<0.025	MG/KG		0.025	11/10/2005	AVY
OXYFLUORFEN (GOAL)	<0.025	MG/KG		0.025	11/10/2005	AVY
OXYTHION	<0.125	MG/KG		0.125	11/10/2005	AVY
PCB (1016)	<0.025	MG/KG		0.025	11/10/2005	AVY
PCB (1221)	<0.025	MG/KG		0.025	11/10/2005	AVY
PCB (1242)	<0.025	MG/KG		0.025	11/10/2005	AVY
PCB (1248)	<0.025	MG/KG		0.025	11/10/2005	AVY

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3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN CONC- AZTEC MARIGOLD

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003 continued						
PCB (1254)	<0.025	MG/KG		0.025	11/10/2005	AVY
PCB (1260)	<0.025	MG/KG		0.025	11/10/2005	AVY
PCNB - QUINTOZENE (AVICOL)	<0.010	MG/KG		0.010	11/10/2005	AVY
PCB (1232)	<0.025	MG/KG		0.025	11/10/2005	AVY
PERMETHRIN (AMBUSH, POUNCE)	<0.050	MG/KG		0.050	11/10/2005	AVY
PROCHLORAZ	<0.125	MG/KG		0.125	11/10/2005	AVY
PROFLUORALIN (TOLBAN)	<0.012	MG/KG		0.012	11/10/2005	AVY
PRONAMIDE (KERB)	<0.010	MG/KG		0.010	11/10/2005	AVY
PROPANIL	<0.250	MG/KG		0.250	11/10/2005	AVY
PROPICONAZOLE	<0.125	MG/KG		0.125	11/10/2005	AVY
SIMAZINE	<0.050	MG/KG		0.050	11/10/2005	AVY
SULFALLATE (VEGADEX)	<0.025	MG/KG		0.025	11/10/2005	AVY
TERBACIL	<0.500	MG/KG		0.500	11/10/2005	AVY
TETRADIFON (TEDION)	<0.500	MG/KG		0.500	11/10/2005	AVY
THIOBENCARB (BOLERO)	<0.500	MG/KG		0.500	11/10/2005	AVY
TOLYFLUANID	<0.500	MG/KG		0.500	11/10/2005	AVY
TOXAPHENE (CAMPHECHLOR)	<0.500	MG/KG		0.500	11/10/2005	AVY
TRIADIMEFON (BAYLETON)	<0.075	MG/KG		0.075	11/10/2005	AVY
TRIFLURALIN (TREFLAN)	<0.012	MG/KG		0.012	11/10/2005	AVY
VINCLOZOLIN (RONILAN)	<0.025	MG/KG		0.025	11/10/2005	AVY
[ORGANOPHOSPHATES]					11/20/2005	AVY
ACEPHATE (ORTHENE)	<0.025	MG/KG		0.025	11/20/2005	AVY
AZINPHOS-METHYL (GUTHION)	<0.500	MG/KG		0.500	11/20/2005	AVY
CADUSAFOS (RUGBY)	<0.025	MG/KG		0.025	11/20/2005	AVY
CARBOPHENOTHION (TRITHION)	<0.050	MG/KG		0.050	11/20/2005	AVY
CHLORFENVINPHOS (SUPONA)	<0.050	MG/KG		0.050	11/20/2005	AVY
CHLORPYRIFOS (DURSBAN)	<0.025	MG/KG		0.025	11/20/2005	AVY
CHLORPYRIFOS, METHYL	<0.025	MG/KG		0.006	11/20/2005	AVY
COUMAPHOS (CO-RAL)	<0.250	MG/KG		0.250	11/20/2005	AVY
CROTOXYPHOS (CIODRIN)	<0.250	MG/KG		0.250	11/20/2005	AVY

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Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003 ...continued						
DEMETON I (SYSTOX)	<0.250	MG/KG		0.250	11/20/2005	AVY
DEMETON-S	<0.025	MG/KG		0.025	11/20/2005	AVY
DIAZINON	<0.025	MG/KG		0.025	11/20/2005	AVY
DICHLORVOS-DDVP (VAPONA)	<0.025	MG/KG		0.025	11/20/2005	AVY
DICROTHOPHOS (BIDRIN)	<0.050	MG/KG		0.050	11/20/2005	AVY
DIMETHOATE (CYGON)	<0.025	MG/KG		0.025	11/20/2005	AVY
DIOXATHION (DELNAV)	<0.050	MG/KG		0.050	11/20/2005	AVY
DISULFOTON (DI-SYSTON)	<0.025	MG/KG		0.025	11/20/2005	AVY
EPN	<0.075	MG/KG		0.075	11/20/2005	AVY
ETHION	<0.025	MG/KG		0.025	11/20/2005	AVY
ETHOPROP (MOCAP)	<0.025	MG/KG		0.025	11/20/2005	AVY
FENAMIPHOS (NEMACUR)	<0.075	MG/KG		0.075	11/20/2005	AVY
FENCHLOROVOS (RONNEL)	<0.125	MG/KG		0.125	11/20/2005	AVY
FENITROTHION (SUMITHION)	<0.025	MG/KG		0.025	11/20/2005	AVY
FENSULFOTHION (DASANIT)	<0.025	MG/KG		0.025	11/20/2005	AVY
FENTHION (BAYTEX)	<0.050	MG/KG		0.050	11/20/2005	AVY
FONOPHOS (DYFONATE)	<0.025	MG/KG		0.025	11/20/2005	AVY
IMIDAN (PHOSMET)	<0.250	MG/KG		0.250	11/20/2005	AVY
ISAZOPHOS (TRIUMPH)	<0.005	MG/KG		0.005	11/20/2005	AVY
ISOFENPHOS (OFTANOL)	<0.050	MG/KG		0.050	11/20/2005	AVY
MALATHION (CYTHION)	<0.025	MG/KG		0.025	11/20/2005	AVY
MERPHOS (TRIBUFOS)	<0.125	MG/KG		0.125	11/20/2005	AVY
METASYSTOX R	<0.125	MG/KG		0.125	11/20/2005	AVY
METHIDATHION (SUPRACIDE)	<0.050	MG/KG		0.050	11/20/2005	AVY
METHAMIDOPHOS (MONITOR)	<0.025	MG/KG		0.025	11/20/2005	AVY
MEVINPHOS (PHOSDRIN)	<0.025	MG/KG		0.025	11/20/2005	AVY
MONOCROTOPHOS	<0.500	MG/KG		0.500	11/20/2005	AVY
NALED (DIBROM)	<0.125	MG/KG		0.125	11/20/2005	AVY
OMETHOATE (FOLIMAT)	<0.025	MG/KG		0.025	11/20/2005	AVY
PARATHION (ETHYL)	<0.025	MG/KG		0.025	11/20/2005	AVY

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CORONA DIVISION
280 N SMITH STREET
CORONA CA, 92880
(951) 734-9600 FAX (951)734-2803
www.microbac.com e-mail: corona@microbac.com

FDA #2030513
LA City #10159
DHS #2122

CHEMICAL, BIOLOGICAL PHYSICAL MOLECULAR AND TOXICOLOGICAL ANALYSIS

CERTIFICATE OF ANALYSIS

MICROBAC - KENTUCKY DIVISION
(b) (6)
3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN CONC- AZTEC MARIGOLD

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003 ...continued						
PARATHION-METHYL (METACIDE)	<0.025	MG/KG		0.025	11/20/2005	AVY
PHORATE (THIMET)	<0.025	MG/KG		0.025	11/20/2005	AVY
PHOSALONE (ZOLONE)	<0.100	MG/KG		0.100	11/20/2005	AVY
PHOSPHAMIDON (DIMECRON)	<0.125	MG/KG		0.125	11/20/2005	AVY
PIRIMIPHOS	<0.025	MG/KG		0.025	11/20/2005	AVY
PIRIMIPHOS, METHYL	<0.025	MG/KG		0.025	11/20/2005	AVY
PROFENOPHOS (CURACRON)	<0.125	MG/KG		0.125	11/20/2005	AVY
PROPETAMPHOS (SAFROTIN)	<0.025	MG/KG		0.025	11/20/2005	AVY
SULFOTEPP	<0.025	MG/KG		0.025	11/20/2005	AVY
SULPROFOS (BOLSTAR)	<0.050	MG/KG		0.050	11/20/2005	AVY
TERBUFOS (COUNTER)	<0.050	MG/KG		0.050	11/20/2005	AVY
TETRACHLORVINPHOS (GARDONA)	<0.100	MG/KG		0.100	11/20/2005	AVY
THIONAZIN (ZINOPHOS)	<0.012	MG/KG		0.012	11/20/2005	AVY
TOKUTHION	<0.025	MG/KG		0.025	11/20/2005	AVY
TRIAZAPHOS (HOSTATHION)	<0.075	MG/KG		0.075	11/20/2005	AVY
TRICHLORONATE	<0.050	MG/KG		0.050	11/20/2005	AVY
[ORGANONITROGEN]					11/20/2005	AVY
AMETRYNE	<0.2	MG/KG		0.2	11/20/2005	AVY
BITERTANOL	<1.0	MG/KG		1.0	11/20/2005	AVY
DIPHENYLAMINE (DPA)	<0.5	MG/KG		0.5	11/20/2005	AVY
FENPROPATHRIN	<0.5	MG/KG		0.5	11/20/2005	AVY
HEXAZINON	<1.0	MG/KG		1.0	11/20/2005	AVY
LENACIL	<2.0	MG/KG		2.0	11/20/2005	AVY
LINURON	<2.0	MG/KG		2.0	11/20/2005	AVY
METALAXYL	<1.0	MG/KG		1.0	11/20/2005	AVY
METRIBUZIN (SENCOR)	<1.0	MG/KG		1.0	11/20/2005	AVY
MOLINATE	<1.0	MG/KG		1.0	11/20/2005	AVY
PENDIMETHALIN (PROWL)	<0.5	MG/KG		0.5	11/20/2005	AVY
PROMETON	<1.0	MG/KG		1.0	11/20/2005	AVY
PROPHAM	<1.0	MG/KG		1.0	11/20/2005	AVY

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502-962-6400 FAX: 502-962-6411

LUTEIN CONC- AZTEC MARIGOLD

Date Reported	11/30/2005
Date Received	11/3/2005
Order Number	0511-00049
Invoice No.	29161
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 LUTEIN 0511-062-003						
....continued						
QUINOMETHIONATE	<1.0	MG/KG		1.0	11/20/2005	AVY
LUKE PREP	COMPLETE				11/9/2005	TRS

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Product: *Hi-Fil 85 % LUTEIN crystal*

Lot No: 5 26 N-93HF85 **Date:** November 26, 2006.

Mfg. date: November 2005. **Expiration date:** November 2006.

TEST	SPECIFICATION	RESULTS
Content of total carotenoids (USP) :	Min. 90.0 %	92.0
Content of Lutein (USP) :	Min. 75.0 %	84.7
Content of Zeaxanthin (USP) :	Max. 8.0 %	7.1

(b)(6)

M.Sc. (b) (6)

Quality Control

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Industrial Orgánica, S.A. de C.V.
Ave. Almazán No. 100 Col. Topo Chico 64260 Apdo. Postal 1654 Monterrey, N.L., México
Tel. (81) 83-52-22-90 01-800 926-7000 Fax (81) 83-76-72-14 e-mail: iosa@att.net.mx

Industrial
Orgánica

CERTIFICATE OF ANALYSIS

Product: *Hi-Fil 85 % LUTEIN crystal*

Lot No: 5 15 D-92HF85 **Date:** December 15, 2006.

Mfg. date: December 2005. **Expiration date:** December 2006.

TEST	SPECIFICATION	RESULTS
Content of total carotenoids (USP) :	Min. 90.0 %	93.5
Content of Lutein (USP) :	Min. 75.0 %	86.8
Content of Zeaxanthin (USP) :	Max. 8.0 %	6.5

(b)(6)

M.Sc. (b) (6)

Quality Control

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KENTUCKY TESTING LABORATORY DIVISION
3323 Gilmore Industrial Blvd. Louisville, KY 40[illegible]3 502.962.6400 Fax: 502.962.6411
Evansville, IN 812.464.9000 • Frankfort, KY 502.[illegible]3.0254 • Paducah, KY 270.898.3637

Chemical, Biological, Physical, Molecular, and Toxicological Services

CERTIFICATE OF ANALYSIS

0601-01063

INDUSTRIAL ORGANICA
(b) (6)
AVE. ALMAZAN NO. 100
COL. TOPO CHICO 64260 POSTAL
MONTERREY, N.L., MX

LUTEIN CAROTENOID CONCENTRATES

Date Reported	02/16/2006
Date Due	02/03/2006
Date Received	01/25/2006
Date Sampled	01/20/2006
Invoice No.	74919
Customer #	W031
Customer P.O.	CHECK # 10216

Analysis	Qualif	Result	Unit	Method	Date	Tech
Sample: 001 HI FIL 85% LUTEIN / 20 GRS / LOT #526N-93HF85		SEE BELOW		01-24-0 Date & Time Sampled: [redacted] 1/24/2006 @ 0:00		
Sample: 002 HI FIL 85% LUTEIN / 20 GRS / LOT #515D-92HF85		SEE BELOW		01-24-0 Date & Time Sampled: [redacted] 1/24/2006 @ 0:00		
Sample: 003 HI FIL 85% LUTEIN / 250 GRS / LOT #526N-93HF[illegible] / 01-24- Date & Time Sampled: [redacted] 1/24/2006 @ 0:00						
[Pesticide Residue - CDFA]						
CDFA, CARBAMATE		SEE ATTACHED	MG/KG	C A MRM HPLC	01/30/2006	OST
CDFA, ORGANOCHLORINE		SEE ATTACHED	MG/KG	C A MRM GC/ELCD	01/30/2006	OST
CDFA, ORGANOPHOSPHATE		SEE ATTACHED	MG/KG	C A MRM GC/FPD	01/30/2006	OST
Sample: 004 HI FIL 85% LUTEIN / 250 GRS / LOT #515D-92HF[illegible] / 01-24- Date & Time Sampled: [redacted] 1/24/2006 @ 0:00						
[Pesticide Residue - CDFA]						
CDFA, CARBAMATE		SEE ATTACHED	MG/KG	C A MRM HPLC	01/30/2006	OST
CDFA, ORGANOCHLORINE		SEE ATTACHED	MG/KG	C A MRM GC/ELCD	01/30/2006	OST
CDFA, ORGANOPHOSPHATE		SEE ATTACHED	MG/KG	C A MRM GC/FPD	01/30/2006	OST

COR = ANALYSIS SUBCONTRACTED TO MICROBAC CORONA DIVISION
OST = ANALYSIS SUBCONTRACTED TO ENO RIVER LABS

UNLESS OTHERWISE NOTED, SAMPLES ARE ANALYZED AS RECEIVED.

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KENTUCKY TESTING LABORATORY DIVISION
3323 Gilmore Industrial Blvd. Louisville, KY 40213 502.962.6400 Fax: 502.962.6411
Evansville, IN 812.464.9000 • Frankfort, KY 502.803.0254 • Paducah, KY 270.898.3637

Chemical, Biological, Physical, Molecular, and Toxicological Services

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(b) (6)

0601-01063

Date Reported	02/16/2006
Date Received	01/25/2006
Date Sampled	01/20/2006

LUTEIN CAROTENOID CONCENTRATES

Analysis	Qualif	Result	Unit	Method	Date	Tech

RESPECTFULLY SUBMITTED: (b)(6)

MICROBAC LABORATORIES, INC.

For any feedback concerning our services, please contact Sean Hyde, the Managing Director at 502.962.6400, or Trevor Boyce, President, at tboyce@microbac.com or Robert Morgan, Chief Operating Officer, at rmorgan@microbac.com.

QUALIFIER DEFINITIONS:

AR	Results reported on an as received basis.
B1	Analyte value in the method blank above control limit.
B2	Analyte value in the method blank is between the method detection limit and the reporting detection limit.
C1	Continuing calibration verification (CCV) above upper control limit, analyte(s) not detected
DJ	Surrogate recoveries not calculated due to necessary sample dilution
DW	Results reported on a dry weight basis.
E1	Elevated reporting or detection limit(s) due to sample matrix interference and sample dilution.
E2	Elevated reporting or detection limit(s) due to high analyte concentration and sample dilution
E3	Elevated reporting or detection limit(s) due to insufficient sample volume
H1	Sample received outside of holding time for these analytes.
H2	Analyte was prepared and/or analyzed outside of the analytical method holding time.
J1	The analyte was positively identified, analyte was detected between the reporting limit and method detection limit and the result is an estimated value
J2	The analyte was positively identified;the result is above the quantitation range and is an estimated value.
L1	Lab control sample (LCS) recovery below lower control limit, all other batch QC acceptable.
L2	Lab control sample (LCS) recovery above upper control limit, all other batch QC acceptable
L3	Lab control sample (LCS) recovery above upper control limit, analyte not detected.
M1	Matrix spike recovery outside control limits due to sample matrix interference or high analyte concentration
MC	Miscellaneous (see conclusion statement)
N	The analysis indicates the presence of an analyte for which there is presumptive evidence to make a "tentative identification"
ND	Not detected at or below the reporting limit (or method detection limit, if listed).
NJ	The analysis indicates the presence of an analyte that has been "tentatively identified" and is an estimated value
OUT	The above value, over the specification limit, was verified by a second analysis.
P1	Sample received was improperly preserved for these analytes
P2	Sample pH greater than method limit of 2
R	The sample results are rejected due to serious deficiencies in the ability to analyze the sample and meet quality control criteria. the presence or absence of the analyte cannot be verified
R1	Relative percent difference (RPD) of matrix spike outside of control limit
R2	Relative percent difference (RPD) of LCS outside of control limit.
R3	Relative percent difference (RPD) of sample duplicate matrix spike outside of control limit
S1	One or more surrogates outside control limits, no target analytes detected
S2	One or more surrogates outside control limits due to matrix interference.
S3	One or more surrogates outside control limits the data was accepted based on the valid recovery of remaining surrogate(s).
SUB	Analysis subcontracted.
U	Analyte was analyzed for, but was not detected above the reported sample quantitation limit.
UJ	Analyte was not detected above the reporting limit, however, the reporting limit is approximate & may or may not represent the actual limit of quantitation necessary to accurately & precisely measure the analyte in the sample
V	Analyte concentration estimated due to sample matrix interference and/or high analyte concentration interference.

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3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

Date Reported	2/6/2006
Date Received	1/26/2006
Order Number	0601-00386
Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
[FDA EXTENDED MRM]					1/30/2006	AVY
[CARBAMATES]					1/30/2006	AVY
ALDICARB SULFONE	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDICARB SULFOXIDE	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDICARB (TEMIK)	<0.050	MG/KG		0.050	1/30/2006	AVY
CARBARYL (SEVIN)	<0.050	MG/KG		0.050	1/30/2006	AVY
CARBOFURAN (FURADAN)	<0.100	MG/KG		0.100	1/30/2006	AVY
METHIOCARB (MESUROL)	<0.026	MG/KG		0.026	1/30/2006	AVY
METHOMYL (LANNATE)	<0.050	MG/KG		0.050	1/30/2006	AVY
OXAMYL (VYDATE)	<0.050	MG/KG		0.050	1/30/2006	AVY
PROPOXUR (BAYGON)	<0.050	MG/KG		0.050	1/30/2006	AVY
3-HYDROXYCARBOFURAN	<0.050	MG/KG		0.050	1/30/2006	AVY
[ORGANOCHLORINES]					1/30/2006	AVY
ALACHLOR (LAZO)	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDRIN (ALDREX)	<0.005	MG/KG		0.005	1/30/2006	AVY
ANILAZINE (DYRENE)	<0.125	MG/KG		0.125	1/30/2006	AVY
ATRAZINE	<0.050	MG/KG		0.050	1/30/2006	AVY
BENEFIN (BALAN/BENFLURALIN)	<0.012	MG/KG		0.012	1/30/2006	AVY
BHC ALPHA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY
BHC BETA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY
BHC DELTA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY
BHC GAMMA ISOMER (LINDANE)	<0.005	MG/KG		0.005	1/30/2006	AVY
BIFENOX (MOWDOWN)	<0.025	MG/KG		0.025	1/30/2006	AVY
BIFENTHRIN	<0.125	MG/KG		0.125	1/30/2006	AVY
BROMACIL	<0.250	MG/KG		0.250	1/30/2006	AVY
BROMOPROPYLATE	<0.025	MG/KG		0.025	1/30/2006	AVY
CAPTAFOL (DIFOLATAN)	<0.125	MG/KG		0.125	1/30/2006	AVY
CAPTAN (ORTHOCIDE)	<0.250	MG/KG		0.250	1/30/2006	AVY
CHLORDANE (OCTACHLOR)	<0.062	MG/KG		0.062	1/30/2006	AVY
CHLORDIMEFORM	<0.100	MG/KG		0.100	1/30/2006	AVY

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Date Reported	2/6/2006
Date Received	1/26/2006
Order Number	0601-00386
Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
CHLORFENSON (OVEX)	<0.125	MG/KG		0.125	1/30/2006	AVY
CHLOROBENZILATE (AKAR)	<0.500	MG/KG		0.500	1/30/2006	AVY
CHLORONEB (TERRANEB SP)	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLOROPROPYLATE	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLOROTHALONIL (BRAVO)	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLORPROPHAM (CIPC)	<0.125	MG/KG		0.125	1/30/2006	AVY
CYANAZINE (BLADEX)	<0.500	MG/KG		0.500	1/30/2006	AVY
CYFLUTHRIN	<0.025	MG/KG		0.025	1/30/2006	AVY
LAMBDA CYHALOTHRIN	<0.025	MG/KG		0.025	1/30/2006	AVY
CYPERMETHRIN (AMMO)	<0.125	MG/KG		0.125	1/30/2006	AVY
DCPA (DACTHAL)	<0.012	MG/KG		0.012	1/30/2006	AVY
DDD, O,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDD, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDE, O,P-ISOMER	<0.007	MG/KG		0.007	1/30/2006	AVY
DDE, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDT, O,P-ISOMER	<0.007	MG/KG		0.007	1/30/2006	AVY
DDT, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DELTAMETHRIN	<0.125	MG/KG		0.125	1/30/2006	AVY
DICHLOBENIL (CASORON)	<0.012	MG/KG		0.012	1/30/2006	AVY
DICHLONE (PHYGON)	<0.125	MG/KG		0.125	1/30/2006	AVY
DICHLORAN (BOTRAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
DICLOFOP METHYL	<0.125	MG/KG		0.125	1/30/2006	AVY
DICOFOL (KELTHANE)	<0.125	MG/KG		0.125	1/30/2006	AVY
DIELDRIN (OCTALOX)	<0.005	MG/KG		0.005	1/30/2006	AVY
DIMETHACHLOR	<0.025	MG/KG		0.025	1/30/2006	AVY
DIURON	<0.100	MG/KG		0.100	1/30/2006	AVY
ENDOSULFAN I (THIODAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
ENDOSULFAN II	<0.025	MG/KG		0.025	1/30/2006	AVY
ENDOSULFAN III ENDO-SULFATE	<0.025	MG/KG		0.025	1/30/2006	AVY

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LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
ENDRIN (ENDREX)	<0.012	MG/KG		0.012	1/30/2006	AVY
ENDRIN ALDEHYDE	<0.025	MG/KG		0.025	1/30/2006	AVY
ETACONAZOLE	<0.125	MG/KG		0.125	1/30/2006	AVY
ETHALFURALIN (SONALAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
ETHYLAN (PERTHANE)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENARIMOL (RUBIGAN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENVALERATE (PYDRIN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FLAMPROP-ISOPROPYL	<0.250	MG/KG		0.250	1/30/2006	AVY
FLAMPROP-METHYL	<0.250	MG/KG		0.250	1/30/2006	AVY
FLUCHLORALIN (BASALIN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FLUOMETRON	<0.250	MG/KG		0.250	1/30/2006	AVY
FOLPET (PHALTAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
HCB (HEXACHLOROBENZENE)	<0.002	MG/KG		0.002	1/30/2006	AVY
HEPTACHLOR (DRINOX)	<0.002	MG/KG		0.002	1/30/2006	AVY
HEPTACHLOR EPOXIDE	<0.005	MG/KG		0.005	1/30/2006	AVY
IMAZALIL	<0.012	MG/KG		0.012	1/30/2006	AVY
IPRODION (ROVRAL)	<0.500	MG/KG		0.500	1/30/2006	AVY
METHOXYCHLOR (MARLATE)	<0.125	MG/KG		0.125	1/30/2006	AVY
METOLACHLOR	<0.100	MG/KG		0.100	1/30/2006	AVY
MIREX	<0.025	MG/KG		0.025	1/30/2006	AVY
MONOLINURON	<0.300	MG/KG		0.300	1/30/2006	AVY
MYCOBUTANIL (RALLY)	<0.025	MG/KG		0.025	1/30/2006	AVY
NITROFEN (TOK)	<0.025	MG/KG		0.025	1/30/2006	AVY
NORFLURAZON	<0.500	MG/KG		0.500	1/30/2006	AVY
OXADIAZON (RONSTAR)	<0.025	MG/KG		0.025	1/30/2006	AVY
OXYFLUORFEN (GOAL)	<0.025	MG/KG		0.025	1/30/2006	AVY
OXYTHION	<0.125	MG/KG		0.125	1/30/2006	AVY
PCB (1016)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1221)	<0.025	MG/KG		0.025	1/30/2006	AVY

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CORONA DIVISION
280 N SMITH STREET
CORONA CA, 92880
(951) 734-9600 FAX (951)734-2803
www.microbac.com e-mail: corona@microbac.com

FDA #2030513
LA City #10159
DHS #2122

CHEMICAL, BIOLOGICAL PHYSICAL MOLECULAR AND TOXICOLOGICAL ANALYSIS

CERTIFICATE OF ANALYSIS

MICROBAC - KENTUCKY DIVISION
(b) (6)
3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN

Date Reported	2/6/2006
Date Received	1/26/2006
Order Number	0601-00386
Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
PCB (1242)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1248)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1254)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1260)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCNB - QUINTOZENE (AVICOL)	<0.010	MG/KG		0.010	1/30/2006	AVY
PCB (1232)	<0.025	MG/KG		0.025	1/30/2006	AVY
PERMETHRIN (AMBUSH, POUNCE)	<0.050	MG/KG		0.050	1/30/2006	AVY
PROCHLORAZ	<0.125	MG/KG		0.125	1/30/2006	AVY
PROFLUORALIN (TOLBAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
PRONAMIDE (KERB)	<0.010	MG/KG		0.010	1/30/2006	AVY
PROPANIL	<0.250	MG/KG		0.250	1/30/2006	AVY
PROPICONAZOLE	<0.125	MG/KG		0.125	1/30/2006	AVY
SIMAZINE	<0.050	MG/KG		0.050	1/30/2006	AVY
SULFALLATE (VEGADEX)	<0.025	MG/KG		0.025	1/30/2006	AVY
TERBACIL	<0.500	MG/KG		0.500	1/30/2006	AVY
TETRADIFON (TEDION)	<0.500	MG/KG		0.500	1/30/2006	AVY
THIOBENCARB (BOLERO)	<0.500	MG/KG		0.500	1/30/2006	AVY
TOLYFLUANID	<0.500	MG/KG		0.500	1/30/2006	AVY
TOXAPHENE (CAMPHECHLOR)	<0.500	MG/KG		0.500	1/30/2006	AVY
TRIADIMEFON (BAYLETON)	<0.075	MG/KG		0.075	1/30/2006	AVY
TRIFLURALIN (TREFLAN)	<0.012.	MG/KG		0.012	1/30/2006	AVY
VINCLOZOLIN (RONILAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
[ORGANOPHOSPHATES]					1/30/2006	AVY
ACEPHATE (ORTHENE)	<0.025	MG/KG		0.025	1/30/2006	AVY
AZINPHOS-METHYL (GUTHION)	<0.500	MG/KG		0.500	1/30/2006	AVY
CADUSAFOS (RUGBY)	<0.025	MG/KG		0.025	1/30/2006	AVY
CARBOPHENOTHION (TRITHION)	<0.050	MG/KG		0.050	1/30/2006	AVY
CHLORFENVINPHOS (SUPONA)	<0.050	MG/KG		0.050	1/30/2006	AVY
CHLORPYRIFOS (DURSBAN)	<0.025	MG/KG		0.025	1/30/2006	AVY

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Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
CHLORPYRIFOS, METHYL	<0.025	MG/KG		0.006	1/30/2006	AVY
COUMAPHOS (CO-RAL)	<0.250	MG/KG		0.250	1/30/2006	AVY
CROTOXYPHOS (CIODRIN)	<0.250	MG/KG		0.250	1/30/2006	AVY
DEMETON I (SYSTOX)	<0.250	MG/KG		0.250	1/30/2006	AVY
DEMETON-S	<0.025	MG/KG		0.025	1/30/2006	AVY
DIAZINON	<0.025	MG/KG		0.025	1/30/2006	AVY
DICHLORVOS-DDVP (VAPONA)	<0.025	MG/KG		0.025	1/30/2006	AVY
DICROTHOPHOS (BIDRIN)	<0.050	MG/KG		0.050	1/30/2006	AVY
DIMETHOATE (CYGON)	<0.025	MG/KG		0.025	1/30/2006	AVY
DIOXATHION (DELNAV)	<0.050	MG/KG		0.050	1/30/2006	AVY
DISULFOTON (DI-SYSTON)	<0.025	MG/KG		0.025	1/30/2006	AVY
EPN	<0.075	MG/KG		0.075	1/30/2006	AVY
ETHION	<0.025	MG/KG		0.025	1/30/2006	AVY
ETHOPROP (MOCAP)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENAMIPHOS (NEMACUR)	<0.075	MG/KG		0.075	1/30/2006	AVY
FENCHLOROVOS (RONNEL)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENITROTHION (SUMITHION)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENSULFOTHION (DASANIT)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENTHION (BAYTEX)	<0.050	MG/KG		0.050	1/30/2006	AVY
FONOPHOS (DYFONATE)	<0.025	MG/KG		0.025	1/30/2006	AVY
IMIDAN (PHOSMET)	<0.250	MG/KG		0.250	1/30/2006	AVY
ISAZOPHOS (TRIUMPH)	<0.005	MG/KG		0.005	1/30/2006	AVY
ISOFENPHOS (OFTANOL)	<0.050	MG/KG		0.050	1/30/2006	AVY
MALATHION (CYTHION)	<0.025	MG/KG		0.025	1/30/2006	AVY
MERPHOS (TRIBUFOS)	<0.125	MG/KG		0.125	1/30/2006	AVY
METASYSTOX R	<0.125	MG/KG		0.125	1/30/2006	AVY
METHIDATHION (SUPRACIDE)	<0.050	MG/KG		0.050	1/30/2006	AVY
METHAMIDOPHOS (MONITOR)	<0.025	MG/KG		0.025	1/30/2006	AVY
MEVINPHOS (PHOSDRIN)	<0.025	MG/KG		0.025	1/30/2006	AVY

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LUTEIN

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Date Received	1/26/2006
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Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
MONOCROTOPHOS	<0.500	MG/KG		0.500	1/30/2006	AVY
NALED (DIBROM)	<0.125	MG/KG		0.125	1/30/2006	AVY
OMETHOATE (FOLIMAT)	<0.025	MG/KG		0.025	1/30/2006	AVY
PARATHION (ETHYL)	<0.025	MG/KG		0.025	1/30/2006	AVY
PARATHION-METHYL (METACIDE)	<0.025	MG/KG		0.025	1/30/2006	AVY
PHORATE (THIMET)	<0.025	MG/KG		0.025	1/30/2006	AVY
PHOSALONE (ZOLONE)	<0.100	MG/KG		0.100	1/30/2006	AVY
PHOSPHAMIDON (DIMECRON)	<0.125	MG/KG		0.125	1/30/2006	AVY
PIRIMIPHOS	<0.025	MG/KG		0.025	1/30/2006	AVY
PIRIMIPHOS, METHYL	<0.025	MG/KG		0.025	1/30/2006	AVY
PROFENOPHOS (CURACRON)	<0.125	MG/KG		0.125	1/30/2006	AVY
PROPETAMPHOS (SAFROTIN)	<0.025	MG/KG		0.025	1/30/2006	AVY
SULFOTEPP	<0.025	MG/KG		0.025	1/30/2006	AVY
SULPROFOS (BOLSTAR)	<0.050	MG/KG		0.050	1/30/2006	AVY
TERBUFOS (COUNTER)	<0.050	MG/KG		0.050	1/30/2006	AVY
TETRACHLORVINPHOS (GARDONA)	<0.100	MG/KG		0.100	1/30/2006	AVY
THIONAZIN (ZINOPHOS)	<0.012	MG/KG		0.012	1/30/2006	AVY
TOKUTHION	<0.025	MG/KG		0.025	1/30/2006	AVY
TRIAZAPHOS (HOSTATHION)	<0.075	MG/KG		0.075	1/30/2006	AVY
TRICHLORONATE	<0.050	MG/KG		0.050	1/30/2006	AVY
[ORGANONITROGEN]					1/30/2006	AVY
AMETRYNE	<0.2	MG/KG		0.2	1/30/2006	AVY
BITERTANOL	<1.0	MG/KG		1.0	1/30/2006	AVY
DIPHENYLAMINE (DPA)	<0.5	MG/KG		0.5	1/30/2006	AVY
FENPROPATHRIN	<0.5	MG/KG		0.5	1/30/2006	AVY
HEXAZINON	<1.0	MG/KG		1.0	1/30/2006	AVY
LENACIL	<2.0	MG/KG		2.0	1/30/2006	AVY
LINURON	<2.0	MG/KG		2.0	1/30/2006	AVY
METALAXYL	<1.0	MG/KG		1.0	1/30/2006	AVY

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LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
001 **LUTEIN 0601-1063-003 01/24/06**						
.....continued						
METRIBUZIN (SENCOR)	<1.0	MG/KG		1.0	1/30/2006	AVY
MOLINATE	<1.0	MG/KG		1.0	1/30/2006	AVY
PENDIMETHALIN (PROWL)	<0.5	MG/KG		0.5	1/30/2006	AVY
PROMETON	<1.0	MG/KG		1.0	1/30/2006	AVY
PROPHAM	<1.0	MG/KG		1.0	1/30/2006	AVY
QUINOMETHIONATE	<1.0	MG/KG		1.0	1/30/2006	AVY
LUKE PREP	COMPLETE				1/27/2006	TRS
002 **LUTEIN 0601-1063-004 01/24/06**						
[FDA EXTENDED MRM]					1/30/2006	AVY
[CARBAMATES]					1/30/2006	AVY
ALDICARB SULFONE	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDICARB SULFOXIDE	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDICARB (TEMIK)	<0.050	MG/KG		0.050	1/30/2006	AVY
CARBARYL (SEVIN)	<0.050	MG/KG		0.050	1/30/2006	AVY
CARBOFURAN (FURADAN)	<0.100	MG/KG		0.100	1/30/2006	AVY
METHIOCARB (MESUROL)	<0.026	MG/KG		0.026	1/30/2006	AVY
METHOMYL (LANNATE)	<0.050	MG/KG		0.050	1/30/2006	AVY
OXAMYL (VYDATE)	<0.050	MG/KG		0.050	1/30/2006	AVY
PROPOXUR (BAYGON)	<0.050	MG/KG		0.050	1/30/2006	AVY
3-HYDROXYCARBOFURAN	<0.050	MG/KG		0.050	1/30/2006	AVY
[ORGANOCHLORINES]					1/30/2006	AVY
ALACHLOR (LAZO)	<0.050	MG/KG		0.050	1/30/2006	AVY
ALDRIN (ALDREX)	<0.005	MG/KG		0.005	1/30/2006	AVY
ANILAZINE (DYRENE)	<0.125	MG/KG		0.125	1/30/2006	AVY
ATRAZINE	<0.050	MG/KG		0.050	1/30/2006	AVY
BENEFIN (BALAN/BENFLURALIN)	<0.012	MG/KG		0.012	1/30/2006	AVY
BHC ALPHA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY
BHC BETA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY

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LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
BHC DELTA ISOMER	<0.005	MG/KG		0.005	1/30/2006	AVY
BHC GAMMA ISOMER (LINDANE)	<0.005	MG/KG		0.005	1/30/2006	AVY
BIFENOX (MOWDOWN)	<0.025	MG/KG		0.025	1/30/2006	AVY
BIFENTHRIN	<0.125	MG/KG		0.125	1/30/2006	AVY
BROMACIL	<0.250	MG/KG		0.250	1/30/2006	AVY
BROMOPROPYLATE	<0.025	MG/KG		0.025	1/30/2006	AVY
CAPTAFOL (DIFOLATAN)	<0.125	MG/KG		0.125	1/30/2006	AVY
CAPTAN (ORTHOCIDE)	<0.250	MG/KG		0.250	1/30/2006	AVY
CHLORDANE (OCTACHLOR)	<0.062	MG/KG		0.062	1/30/2006	AVY
CHLORDIMEFORM	<0.100	MG/KG		0.100	1/30/2006	AVY
CHLORFENSON (OVEX)	<0.125	MG/KG		0.125	1/30/2006	AVY
CHLOROBENZILATE (AKAR)	<0.500	MG/KG		0.500	1/30/2006	AVY
CHLORONEB (TERRANEB SP)	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLOROPROPYLATE	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLOROTHALONIL (BRAVO)	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLORPROPHAM (CIPC)	<0.125	MG/KG		0.125	1/30/2006	AVY
CYANAZINE (BLADEX)	<0.500	MG/KG		0.500	1/30/2006	AVY
CYFLUTHRIN	<0.025	MG/KG		0.025	1/30/2006	AVY
LAMBDA CYHALOTHRIN	<0.025	MG/KG		0.025	1/30/2006	AVY
CYPERMETHRIN (AMMO)	<0.125	MG/KG		0.125	1/30/2006	AVY
DCPA (DACTHAL)	<0.012	MG/KG		0.012	1/30/2006	AVY
DDD, O,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDD, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDE, O,P-ISOMER	<0.007	MG/KG		0.007	1/30/2006	AVY
DDE, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DDT, O,P-ISOMER	<0.007	MG/KG		0.007	1/30/2006	AVY
DDT, P,P-ISOMER	<0.012	MG/KG		0.012	1/30/2006	AVY
DELTAMETHRIN	<0.125	MG/KG		0.125	1/30/2006	AVY
DICHLOBENIL (CASORON)	<0.012	MG/KG		0.012	1/30/2006	AVY

000112

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LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
DICHLONE (PHYGON)	<0.125	MG/KG		0.125	1/30/2006	AVY
DICHLORAN (BOTRAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
DICLOFOP METHYL	<0.125	MG/KG		0.125	1/30/2006	AVY
DICOFOL (KELTHANE)	<0.125	MG/KG		0.125	1/30/2006	AVY
DIELDRIN (OCTALOX)	<0.005	MG/KG		0.005	1/30/2006	AVY
DIMETHACHLOR	<0.025	MG/KG		0.025	1/30/2006	AVY
DIURON	<0.100	MG/KG		0.100	1/30/2006	AVY
ENDOSULFAN I (THIODAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
ENDOSULFAN II	<0.025	MG/KG		0.025	1/30/2006	AVY
ENDOSULFAN III ENDO-SULFATE	<0.025	MG/KG		0.025	1/30/2006	AVY
ENDRIN (ENDREX)	<0.012	MG/KG		0.012	1/30/2006	AVY
ENDRIN ALDEHYDE	<0.025	MG/KG		0.025	1/30/2006	AVY
ETACONAZOLE	<0.125	MG/KG		0.125	1/30/2006	AVY
ETHALFURALIN (SONALAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
ETHYLAN (PERTHANE)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENARIMOL (RUBIGAN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENVALERATE (PYDRIN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FLAMPROP-ISOPROPYL	<0.250	MG/KG		0.250	1/30/2006	AVY
FLAMPROP-METHYL	<0.250	MG/KG		0.250	1/30/2006	AVY
FLUCHLORALIN (BASALIN)	<0.125	MG/KG		0.125	1/30/2006	AVY
FLUOMETRON	<0.250	MG/KG		0.250	1/30/2006	AVY
FOLPET (PHALTAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
HCB (HEXACHLOROBENZENE)	<0.002	MG/KG		0.002	1/30/2006	AVY
HEPTACHLOR (DRINOX)	<0.002	MG/KG		0.002	1/30/2006	AVY
HEPTACHLOR EPOXIDE	<0.005	MG/KG		0.005	1/30/2006	AVY
IMAZALIL	<0.012	MG/KG		0.012	1/30/2006	AVY
IPRODION (ROVRAL)	<0.500	MG/KG		0.500	1/30/2006	AVY
METHOXYCHLOR (MARLATE)	<0.125	MG/KG		0.125	1/30/2006	AVY
METOLACHLOR	<0.100	MG/KG		0.100	1/30/2006	AVY

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CORONA DIVISION
280 N SMITH STREET
CORONA CA, 92880
(951) 734-9600 FAX (951)734-2803
www.microbac.com e-mail: corona@microbac.com

FDA #2030513
LA City #10159
DHS #2122

CHEMICAL, BIOLOGICAL PHYSICAL MOLECULAR AND TOXICOLOGICAL ANALYSIS

CERTIFICATE OF ANALYSIS

MICROBAC - KENTUCKY DIVISION
(b) (6)
3323 GILMORE INDUSTRIAL BLVD.
LOUISVILLE,KY 40213
502-962-6400 FAX: 502-962-6411

LUTEIN

Date Reported	2/6/2006
Date Received	1/26/2006
Order Number	0601-00386
Invoice No.	30128
Cust #	9005
Permit Number	
Customer P.O.	

Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
MIREX	<0.025	MG/KG		0.025	1/30/2006	AVY
MONOLINURON	<0.300	MG/KG		0.300	1/30/2006	AVY
MYCOBUTANIL (RALLY)	<0.025	MG/KG		0.025	1/30/2006	AVY
NITROFEN (TOK)	<0.025	MG/KG		0.025	1/30/2006	AVY
NORFLURAZON	<0.500	MG/KG		0.500	1/30/2006	AVY
OXADIAZON (RONSTAR)	<0.025	MG/KG		0.025	1/30/2006	AVY
OXYFLUORFEN (GOAL)	<0.025	MG/KG		0.025	1/30/2006	AVY
OXYTHION	<0.125	MG/KG		0.125	1/30/2006	AVY
PCB (1016)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1221)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1242)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1248)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1254)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCB (1260)	<0.025	MG/KG		0.025	1/30/2006	AVY
PCNB - QUINTOZENE (AVICOL)	<0.010	MG/KG		0.010	1/30/2006	AVY
PCB (1232)	<0.025	MG/KG		0.025	1/30/2006	AVY
PERMETHRIN (AMBUSH, POUNCE)	<0.050	MG/KG		0.050	1/30/2006	AVY
PROCHLORAZ	<0.125	MG/KG		0.125	1/30/2006	AVY
PROFLUORALIN (TOLBAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
PRONAMIDE (KERB)	<0.010	MG/KG		0.010	1/30/2006	AVY
PROPANIL	<0.250	MG/KG		0.250	1/30/2006	AVY
PROPICONAZOLE	<0.125	MG/KG		0.125	1/30/2006	AVY
SIMAZINE	<0.050	MG/KG		0.050	1/30/2006	AVY
SULFALLATE (VEGADEX)	<0.025	MG/KG		0.025	1/30/2006	AVY
TERBACIL	<0.500	MG/KG		0.500	1/30/2006	AVY
TETRADIFON (TEDION)	<0.500	MG/KG		0.500	1/30/2006	AVY
THIOBENCARB (BOLERO)	<0.500	MG/KG		0.500	1/30/2006	AVY
TOLYFLUANID	<0.500	MG/KG		0.500	1/30/2006	AVY
TOXAPHENE (CAMPHECHLOR)	<0.500	MG/KG		0.500	1/30/2006	AVY

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Customer P.O.	

LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
TRIADIMEFON (BAYLETON)	<0.075	MG/KG		0.075	1/30/2006	AVY
TRIFLURALIN (TREFLAN)	<0.012	MG/KG		0.012	1/30/2006	AVY
VINCLOZOLIN (RONILAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
[ORGANOPHOSPHATES]					1/30/2006	AVY
ACEPHATE (ORTHENE)	<0.025	MG/KG		0.025	1/30/2006	AVY
AZINPHOS-METHYL (GUTHION)	<0.500	MG/KG		0.500	1/30/2006	AVY
CADUSAFOS (RUGBY)	<0.025	MG/KG		0.025	1/30/2006	AVY
CARBOPHENOTHION (TRITHION)	<0.050	MG/KG		0.050	1/30/2006	AVY
CHLORFENVINPHOS (SUPONA)	<0.050	MG/KG		0.050	1/30/2006	AVY
CHLORPYRIFOS (DURSBAN)	<0.025	MG/KG		0.025	1/30/2006	AVY
CHLORPYRIFOS, METHYL	<0.025	MG/KG		0.006	1/30/2006	AVY
COUMAPHOS (CO-RAL)	<0.250	MG/KG		0.250	1/30/2006	AVY
CROTOXYPHOS (CIODRIN)	<0.250	MG/KG		0.250	1/30/2006	AVY
DEMETON I (SYSTOX)	<0.250	MG/KG		0.250	1/30/2006	AVY
DEMETON-S	<0.025	MG/KG		0.025	1/30/2006	AVY
DIAZINON	<0.025	MG/KG		0.025	1/30/2006	AVY
DICHLORVOS-DDVP (VAPONA)	<0.025	MG/KG		0.025	1/30/2006	AVY
DICROTHOPHOS (BIDRIN)	<0.050	MG/KG		0.050	1/30/2006	AVY
DIMETHOATE (CYGON)	<0.025	MG/KG		0.025	1/30/2006	AVY
DIOXATHION (DELNAV)	<0.050	MG/KG		0.050	1/30/2006	AVY
DISULFOTON (DI-SYSTON)	<0.025	MG/KG		0.025	1/30/2006	AVY
EPN	<0.075	MG/KG		0.075	1/30/2006	AVY
ETHION	<0.025	MG/KG		0.025	1/30/2006	AVY
ETHOPROP (MOCAP)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENAMIPHOS (NEMACUR)	<0.075	MG/KG		0.075	1/30/2006	AVY
FENCHLOROVOS (RONNEL)	<0.125	MG/KG		0.125	1/30/2006	AVY
FENITROTHION (SUMITHION)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENSULFOTHION (DASANIT)	<0.025	MG/KG		0.025	1/30/2006	AVY
FENTHION (BAYTEX)	<0.050	MG/KG		0.050	1/30/2006	AVY

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Permit Number	
Customer P.O.	

LUTEIN

Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
FONOPHOS (DYFONATE)	<0.025	MG/KG		0.025	1/30/2006	AVY
IMIDAN (PHOSMET)	<0.250	MG/KG		0.250	1/30/2006	AVY
ISAZOPHOS (TRIUMPH)	<0.005	MG/KG		0.005	1/30/2006	AVY
ISOFENPHOS (OFTANOL)	<0.050	MG/KG		0.050	1/30/2006	AVY
MALATHION (CYTHION)	<0.025	MG/KG		0.025	1/30/2006	AVY
MERPHOS (TRIBUFOS)	<0.125	MG/KG		0.125	1/30/2006	AVY
METASYSTOX R	<0.125	MG/KG		0.125	1/30/2006	AVY
METHIOATHION (SUPRACIDE)	<0.050	MG/KG		0.050	1/30/2006	AVY
METHAMIDOPHOS (MONITOR)	<0.025	MG/KG		0.025	1/30/2006	AVY
MEVINPHOS (PHOSDRIN)	<0.025	MG/KG		0.025	1/30/2006	AVY
MONOCROTOPHOS	<0.500	MG/KG		0.500	1/30/2006	AVY
NALED (DIBROM)	<0.125	MG/KG		0.125	1/30/2006	AVY
OMETHOATE (FOLIMAT)	<0.025	MG/KG		0.025	1/30/2006	AVY
PARATHION (ETHYL)	<0.025	MG/KG		0.025	1/30/2006	AVY
PARATHION-METHYL (METACIDE)	<0.025	MG/KG		0.025	1/30/2006	AVY
PHORATE (THIMET)	<0.025	MG/KG		0.025	1/30/2006	AVY
PHOSALONE (ZOLONE)	<0.100	MG/KG		0.100	1/30/2006	AVY
PHOSPHAMIDON (DIMECRON)	<0.125	MG/KG		0.125	1/30/2006	AVY
PIRIMIPHOS	<0.025	MG/KG		0.025	1/30/2006	AVY
PIRIMIPHOS, METHYL	<0.025	MG/KG		0.025	1/30/2006	AVY
PROFENOPHOS (CURACRON)	<0.125	MG/KG		0.125	1/30/2006	AVY
PROPETAMPHOS (SAFROTIN)	<0.025	MG/KG		0.025	1/30/2006	AVY
SULFOTEPP	<0.025	MG/KG		0.025	1/30/2006	AVY
SULPROFOS (BOLSTAR)	<0.050	MG/KG		0.050	1/30/2006	AVY
TERBUFOS (COUNTER)	<0.050	MG/KG		0.050	1/30/2006	AVY
TETRACHLORVINPHOS (GARDONA)	<0.100	MG/KG		0.100	1/30/2006	AVY
THIONAZIN (ZINOPHOS)	<0.012	MG/KG		0.012	1/30/2006	AVY
TOKUTHION	<0.025	MG/KG		0.025	1/30/2006	AVY
TRIAZAPHOS (HOSTATHION)	<0.075	MG/KG		0.075	1/30/2006	AVY

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LUTEIN

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Invoice No.	30128
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Analysis	Result	Units	Method	DLR	Date	Tech
002 **LUTEIN 0601-1063-004 01/24/06**						
.....continued						
TRICHLORONATE	<0.050	MG/KG		0.050	1/30/2006	AVY
[ORGANONITROGEN]					1/30/2006	AVY
AMETRYNE	<0.2	MG/KG		0.2	1/30/2006	AVY
BITERTANOL	<1.0	MG/KG		1.0	1/30/2006	AVY
DIPHENYLAMINE (DPA)	<0.5	MG/KG		0.5	1/30/2006	AVY
FENPROPATHRIN	<0.5	MG/KG		0.5	1/30/2006	AVY
HEXAZINON	<1.0	MG/KG		1.0	1/30/2006	AVY
LENACIL	<2.0	MG/KG		2.0	1/30/2006	AVY
LINURON	<2.0	MG/KG		2.0	1/30/2006	AVY
METALAXYL	<1.0	MG/KG		1.0	1/30/2006	AVY
METRIBUZIN (SENCOR)	<1.0	MG/KG		1.0	1/30/2006	AVY
MOLINATE	<1.0	MG/KG		1.0	1/30/2006	AVY
PENDIMETHALIN (PROWL)	<0.5	MG/KG		0.5	1/30/2006	AVY
PROMETON	<1.0	MG/KG		1.0	1/30/2006	AVY
PROPHAM	<1.0	MG/KG		1.0	1/30/2006	AVY
QUINOMETHIONATE	<1.0	MG/KG		1.0	1/30/2006	AVY
LUKE PREP	COMPLETE				1/27/2006	TRS

NOTE: A 1:250 DILUTION WAS MADE. ALL DLR SHOULD BE MULTIPLIED BY 250.

Respectfully Submitted: (b)(6)

(b) (6)
Laboratory Director

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Appendix II

Review of safety studies of lutein (2006 – present) for GRAS update

EAS CONSULTING GROUP

Specializing in FDA Regulatory Matters

October 24, 2008

Mr. Mark L. Itzkoff
Olsson Frank Weeda PC
1400 - 16th St., N.W., Suite 400
Washington, DC 20036-2220

Tel: 202/518-6327
fax: 202/234-2686

RE: Review of safety studies of lutein (2006-present) for GRAS update

Executive Summary

In response to a request from Olsson Frank Weeda PC for one of their clients Industrial Organica S.A de C.V. (IOSA), this report summarizes the safety studies of lutein that have appeared in the scientific literature since the last database search performed in February 2006 for the Generally Recognized As Safe (GRAS) determination of lutein (*Hi Fil*™ crystalline lutein). In 2006, a panel of experts determined that IOSA's *Hi Fil*™ crystalline lutein was GRAS and safe for use in specified foods with user intakes determined in 90th percentile all-user intakes of lutein and zeaxanthin of 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.

Since the last database searches for the previous GRAS determination performed in February 2006, over 350 articles have appeared on lutein in these databases. Of these, about 20 articles, related to the safety of lutein, were selected for further review. In two cross-sectional studies from Australia by one group, a positive association between the intake of lutein/zeaxanthin and ω-3 fatty acids with progression of age-related macular degeneration (AMD) was reported, while another independent report did not find such an association. A critical review of the other published studies did not reveal any significant safety-related concern that will affect the previous GRAS determination. An independent panel of recognized experts reviewed the studies published over the last two years and concluded that the recent publications do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous GRAS determination of lutein by IOSA.

1. Background

Recently, Industrial Organica S.A de C.V. (IOSA) gained self affirmed Generally Recognized As Safe (GRAS) status for use of *Hi Fil*™ crystalline lutein in a variety of selected foods. IOSA now proposes to submit the self affirmed GRAS determination of lutein to the Food and Drug Administration in the form of a GRAS Notification. As the self- affirmed GRAS

assessment was conducted in 2006, IOSA asked EAS Consulting Group to update the available safety-related information on lutein that has appeared since the GRAS determination. IOSA assured EAS Consulting Group that the food categories, use levels and resulting exposure identified in the self-affirmed GRAS will remain the same. A comprehensive search of the scientific literature related to safety and toxicity of lutein was conducted since January 2006. The database searches revealed over 350 articles on lutein, of which about 20 articles related to the safety of lutein were selected for further review. These articles were obtained, reviewed and a summary of all safety-related findings from the relevant articles is presented in the following section.

2. Safety Studies

2.1. Animal studies

Khachik et al. (2006b) investigated the effects of lutein, zeaxanthin, or a combination of the two, on changes in plasma levels of these carotenoids as well as any effects on ocular and renal toxicity. 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 12-month period. 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 kidney 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.

2.2. Mutagenicity and carcinogenicity studies

Wang et al. (2006) investigated the mutagenic and clastogenic potentials of lutein from marigold flowers. The mutagenicity of lutein (334, 668 and 1335 μg/plate) was examined 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.

Santocomo 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 contrast to the *in vitro* findings reported above, 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.

2.3. 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 any dose level 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.

Following the publication of the above described study, 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 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 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) suggested 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. Thus, the totality of evidence from available animal and clinical studies suggest that supplementation with lutein is safe at 10 mg/day.

In a double-blind, randomized 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 and 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; 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.

In a double-blind 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.

2.4. Review articles

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 no-observed adverse effect level (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.

3. Discussion and Conclusion

This supplemental and updated report assesses the safety of lutein from studies published since 2006. A critical review of the available publications on lutein revealed:

- 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). From this study, the equivalent dose for an average human individual weighing 60 kg would be 60 mg/day.
- The results of a recent genotoxicity study suggest that lutein is neither mutagenic or 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 heptaocarcinogenesis, 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), a positive 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 measurement of 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 summary, a comprehensive review of the totality of available scientific data published since 2006 again revealed no significant reproducible safety-related adverse effects of lutein at use levels previously evaluated. Accordingly, these studies published over the last two years also do not affect the previous GRAS determination of the safety of lutein for use in food categories specified in the previous self affirmed GRAS determination of lutein by IOSA. The food categories and use levels specified in previous IOSA GRAS determination were identical to GRN 000140. In the previous GRAS determination, the 90th percentile all-user intakes of lutein and zeaxanthin from its intended uses was estimated as 13.4 mg/person/day and 1.2 mg/person/day, respectively, which is lower than the safe levels.

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John Thomas, Ph.D. F.A.T.S., D.A.T.S. — 11/14/08 Date

(b)(6)

Stanley M. Tarka, Jr., Ph.D. — 17 Nov 2008 Date

(b)(6)

Madhusudan G. Soni, Ph.D., FACN — 18 Nov. 2008 Date

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