GR

ORIGINAL SUBMISSION

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November 14, 2003

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

Re: GRAS Notification

Dear Sir or Madam:

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register (62 FR 18939-18964), I am submitting in triplicate, as the notifier, Kemin Foods, L.C., 600 E. Court Avenue, Suite A, Des Moines, IA 50309-2021, a GRAS notification of FloraGLO® Crystalline Lutein for use as a food ingredient to provide a dietary source of lutein and zeaxanthin, in certain conventional foods and for use as an ingredient in medical foods intended as the sole item of the diet, a GRAS panel report setting forth the basis for the GRAS determination, and *curricula vitae* of the members of the GRAS panel for review by the agency.

Sincerely,

Zoraida DeFreitas, Ph.D
Director, Research and Development

Enclosures

 03-11-18P02:07 RCVD

GENERALLY RECOGNIZED AS SAFE (GRAS) EXEMPTION CLAIM for FLORAGLO® CRYSTALLINE LUTEIN

I GRAS Exemption Claim

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

FloraGLO® Crystalline Lutein has been determined to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in foods and medical foods, among experts qualified by scientific training and expertise. Therefore, the use of FloraGLO® Crystalline Lutein in foods and medical foods as described below is exempt from the requirement of premarket approval.

Signed,

11/14/03

Zoraida DeFreitas, Ph.D.
Kemin Foods, L.C.
600 E. Court Avenue, Suite A
Des Moines, IA
50309-2021

Date

I.B Name and Address of Notifier

Zoraida DeFreitas, Ph.D.
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600 E. Court Avenue, Suite A
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I.C Common Name of the Notified Substance

FloraGLO® Crystalline Lutein

I.D Conditions of Intended Use in Food

FloraGLO® Crystalline Lutein is intended for use 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 1.

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Table 1 Summary of the Individual Proposed Food-Uses for FloraGLO® Crystalline Lutein and Corresponding Use-Levels for Lutein in the U.S.

Food Category	Proposed Food-Use for FloraGLO® Crystalline Lutein	Use-Levels for Lutein (mg/RACC[1])	Use-Levels for Lutein (%)[2]
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.0	5.0×10^{-3}
	Crackers and Crispbreads	2.0	6.7×10^{-3}
Beverages and Beverage Bases	Bottled Water	0.5	2.1×10^{-4}
	Carbonated Beverages	2.0	8.3×10^{-4}
	Meal Replacements	2.0	8.3×10^{-4}
	Tea, Ready-to-Drink	0.6	2.6×10^{-4}
Breakfast Cereals	Instant and Regular Hot Cereals	2.0	8.3×10^{-4}
	Ready-to-Eat Cereals	2.0	3.6×10^{-3} - 1.3×10^{-2}
Chewing Gum	Chewing Gum	1.0	3.3×10^{-2}
Dairy Product Analogs	Imitation Milks	2.0	8.3×10^{-4}
	Soy Milks	1.5	6.3×10^{-4}
Egg Products	Liquid, Frozen, or Dried Egg Substitutes	2.0	4.0×10^{-3}
Fats and Oils	Margarine-like Spreads	1.5	1.0×10^{-2}
	Salad Dressings	1.5	5.0×10^{-3} - 1.0×10^{-2}
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.0	8.3×10^{-4}
Gravies and Sauces	Tomato-Based Sauces	0.3	2.6×10^{-4}
Hard Candy	Hard Candy	1.0	6.7×10^{-3}
Infant and Toddler Foods*	Junior, Strained, and Toddler Type Baby Foods	1.0	5.9×10^{-4} - 1.4×10^{-2}
Milk Products	Dry Milk	3.0	1.3×10^{-3}
	Fermented Milk Beverages	0.6	2.6×10^{-4}
	Flavored Milk and Milk Drinks	3.0	1.3×10^{-3}
	Milk-Based Meal Replacements	3.0	1.3×10^{-3}
	Yogurt	3.0	1.3×10^{-3}
Processed Fruits and Fruit Juices	Energy, Sport, and Isotonic Drinks	2.0	8.3×10^{-4}
	Fruit-Flavored Drinks	2.0	8.3×10^{-4}
	Fruit Juice	2.0	8.3×10^{-4}
	Nectars	2.0	8.3×10^{-4}
	Vegetable Juice	2.0	8.3×10^{-4}
Soft Candy	Chewy and Nougat Candy	1.0	2.5×10^{-3}
	Fruit Snacks	1.0	2.5×10^{-3}
Soups and Soup Mixes	Canned Soups	0.6	2.6×10^{-4}

[1] RACC – Reference amounts customarily consumed per eating occasion (21 CFR §101.12).
[2] 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.
*Does not include infant formula.

FloraGLO® Crystalline Lutein may also be used as an ingredient in medical foods intended as the sole item of the diet, at levels not to exceed 20 mg lutein/day.
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.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, FloraGLO® Crystalline Lutein has been determined to be GRAS on the basis of scientific procedures. This determination is based on the opinion of experts who are qualified by scientific training and experience to evaluate the safety of FloraGLO® Crystalline Lutein as a component of foods and medical foods [see Appendix I – Expert Panel Opinion Regarding the Generally Recognized as Safe (GRAS) Status of FloraGLO® Crystalline Lutein]. The safety of FloraGLO® Crystalline Lutein is supported by a number of 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).

I.F Availability of Information

The data and information that serve as the basis for this GRAS determination will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for FDA review and copying at reasonable times at the offices of:

Beth Thompson
Kemin Foods, L.C.
600 E. Court Avenue, Suite A
Des Moines, IA
50309-2021

Should the FDA have any questions or additional information requests regarding this notification, Kemin Foods, L.C. will supply these data and information.

II Detailed Information About the Identity of the Substance

II.A Identity

FloraGLO® Crystalline Lutein, of common or usual name, lutein, is a purified crystalline extract from the marigold (*Tagetes erecta*) flower. FloraGLO® Crystalline Lutein typically contains approximately 83% by weight total carotenoids, with about 90% as lutein. The chemical and physical characteristics of lutein and zeaxanthin are detailed in Table 2. The composition of FloraGLO® Crystalline Lutein is detailed in Table 3.

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; Bo-Xan; all-*trans*-(+)-xanthophyll; all-trans-lutein	β, β-carotene-3,3'-diol; all-*trans*-β-carotene-3,3'-diol; (3*R*,3'*R*)-dihydroxy-β-carotene; zeaxanthol; anchovyxanthin
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 (@25°C)	Insoluble	Insoluble

Structural Formula

The structural formulae of lutein and zeaxanthin are presented in Figures 1 and 2, below.

Figure 1 Structural Formula of Lutein

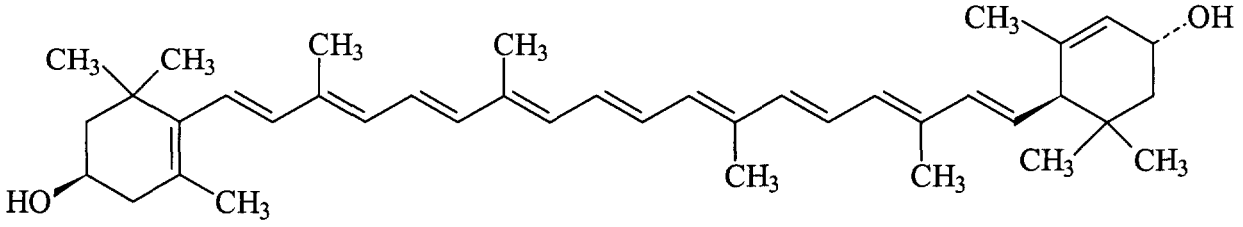
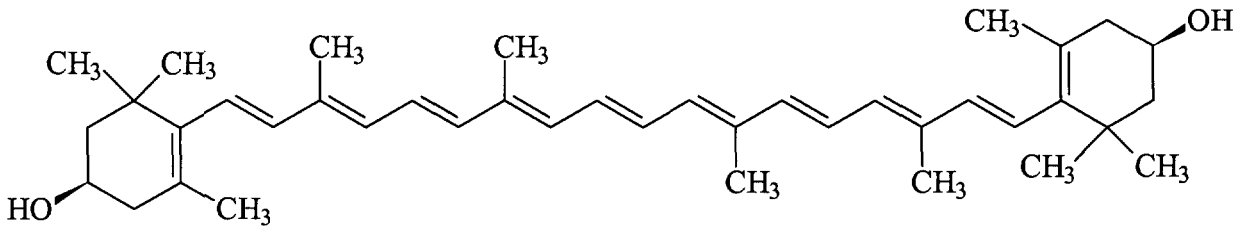


Figure 2 Structural Formula of Zeaxanthin



Composition and Formulation

FloraGLO® Crystalline Lutein is composed of the ingredients listed in Table 3, with their levels respective levels indicated.

Table 3 Composition of FloraGLO® Crystalline Lutein

Principal Constituent	Mean Amount (% dry weight)
Total Carotenoids[1]	≥ 83.0
Waxes	≤ 14.0
Water	≤ 1.0
Ash	≤ 1.0
Fatty Acids	≤ 1.0
Total	100

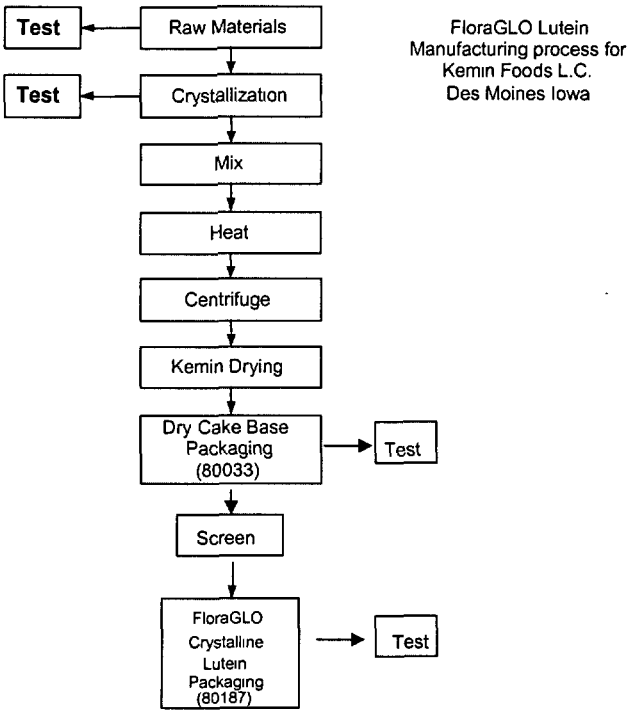
[1] Total Carotenoids include lutein, zeaxanthin and other carotenoids by HPLC.

II.B Method of Manufacture

FloraGLO® Crystalline Lutein is produced in accordance with current good manufacturing practice, using marigold oleoresin as a starting raw material. The marigold oleoresin is derived from the hexane extraction of dried marigold flower petals (*Tagetes erecta*). The final product is packaged under appropriate food grade conditions. The manufacturing process is further described below and is illustrated in Figure 3.

The lutein and zeaxanthin extract is generated using a proprietary technology in a 3-step process that does not include chemical synthesis, but rather extracts the lutein from the natural source material. During the crystallization step, the marigold oleoresin is subjected to conditions to remove the fatty acids from lutein esters and zeaxanthin esters. This process employs potassium hydroxide (potash), propylene glycol, and water with mixing and heat. During this process crystals of lutein and zeaxanthin are formed. The reaction mixture from the crystallization step is then diluted with water and the lutein and zeaxanthin crystals are collected by centrifugation and washed with additional water. The water used is purified by reverse osmosis. The lutein and zeaxanthin crystals are dried by the removal of the residual water, which results in dry cake base (80033). Dry cake base is additionally screened to become FloraGLO® Crystalline Lutein (80187) (Figure 3), of typical composition provided in Table 3. Dry cake base and FloraGLO® Crystalline Lutein are packaged directly into drums and purged with nitrogen gas.

Figure 3 FloraGLO® Crystalline Lutein Manufacturing Process



II.C Specifications for Food Grade Material

Specifications for FloraGLO® Crystalline Lutein meet appropriate food grade specifications, and are presented in Table 4.

Table 4 Specifications for Food-Grade FloraGLO® Crystalline Lutein

Principal Constituents		
Total Carotenoids (including lutein and zeaxanthin)		≥ 80.0% wt
Lutein	≥ 74.0% wt	
Zeaxanthin	≥ 2.0% wt ≤ 9.0% wt	
Waxes		≤ 14.0 % wt
Moisture		≤ 1.0% wt
Ash		≤ 1.0% wt
Pesticides and Related Potential Contaminants		≤ 0.1% (total of other constituents)
Protein	<100 ppm	
Propylene glycol	<1,000 ppm	
Hexane	<50 ppm	
Thiophenes	<300 ppm	
Chlorinated and Organo-Phosphate Pesticides	Not Detected	
Metals		
Lead (Pb)	<0.65 ppm	

Table 4 Specifications for Food-Grade FloraGLO® Crystalline Lutein		
Cadmium (Cd)	<1.25 ppm	
Arsenic (As)	<2.5 ppm	
Mercury (Hg)	<0.15 ppm	
Microbiological Assays		
Aerobic plate count	≤100 cfu/g	
E. coli enrichment	Negative/10g	
Listeria monocytogenes	Negative/25g	
Salmonella	Negative/10g	
Staph enrichment	Negative/10g	
Coliform enrichment	Negative/25g	
Yeast count	≤100 cfu/g	
Mold count	≤100 cfu/g	

IV Basis for GRAS Determination

The determination that FloraGLO® Crystalline Lutein is GRAS is on the basis of scientific procedures [see Appendix I – Expert Panel Opinion Regarding the Generally Recognized as Safe (GRAS) Status of FloraGLO® Crystalline Lutein].

The safety of FloraGLO® Crystalline Lutein is supported by a number of 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 (Buser *et al.*, 1999; Castenmiller *et al.*, 1999; Jenkins *et al.*, 2000; Kim *et al.*, 2000a,b; Pfannkuch *et al.*, 2000, 2001; Schalch *et al.*, 2001; Kruger *et al.*, 2002). The safety of FloraGLO® Crystalline Lutein is also corroborated by additional animal, human and mutagenicity studies conducted with other sources of lutein, lutein-rich foods, and lutein supplements (Chew *et al.*, 1996; Gradelet *et al.*, 1996; Yoshikawa *et al.*, 1996; Khachik *et al.*, 1997; Olmedilla *et al.*, 1997, 2001; Collins *et al.*, 1998; Park *et al.*, 1998, 1999; Rauscher *et al.*, 1998; Jewell and O'Brien, 1999; Müller *et al.*, 1999; Richer, 1999; Wright *et al.*, 1999; Aleman *et al.*, 2001; Dwyer *et al.*, 2001; Duncan *et al.*, 2002; Schierle *et al.*, 2002; Wolz *et al.*, 2002; Falsini *et al.*, 2003). 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). Together, these data support the safety of lutein and zeaxanthin.

In assessing the safety and formulating their opinion, the panel further considered data pertaining to lutein exposure, carotenodermia and potential ocular toxicity. Carotenodermia, a yellow discoloration of the skin following high intake levels of carotenoids, is considered by the Institute of Medicine (IOM) to be harmless and readily reversible when the high levels of carotene ingestion are discontinued (IOM, 2000).

Data from the U.S. Third National Health and Nutrition Examination Survey 1988-1994 (NHANES 111, 1988-1994) indicates that the background serum lutein concentration for all individuals is 0.36μmol/L, while the IOM has reported that human serum lutein concentrations

range from 0.1 to 1.23 μmol/L. Supplementation studies, up to 20 mg/day, over a period of 3 to 6 weeks increased the plasma concentration to a level of 1.72 μmol/L with steady state being reached within approximately 40 days (Khachik *et al.*, 1995, 1997; Schalch *et al.*, 2001). Therefore, an approximate 7-fold increase in lutein intake (up to 20 mg/day) for 6 weeks, results in an approximate 5- to 10-fold increase in background plasma lutein levels.

None of the dietary supplementation intervention studies conducted with lutein and zeaxanthin, resulting in increased plasma levels have demonstrated any potential for human ocular toxicity. Eye examinations following 3 weeks of lutein supplementation (20 mg/day) and a 13-month intervention study (25 mg/day) revealed no evidence of accumulation in the retina, increased molecule pigment density or toxicity (Khachik *et al.*, 1997; Olmedilla *et al.*, 1997, 2001, 2002; Granado *et al.*, 1998; Müller *et al.*, 1999; Schalch *et al.*, 2001). The lack of ocular toxicity has further been corroborated in a 52-week study (2 or 20 mg/kg body weight/day of lutein and zeaxanthin by gavage), conducted in Cynomologus monkeys (Schierle *et al.*, 2002). Dose dependent increases in plasma and retina levels revealed no evidence of crystal formation in the monkey's eyes or toxicity following a comprehensive examination including ophthalmoscopy and biomicroscopy examinations, fundus photography, and electroretinography.

Likewise, the majority of lutein human intervention studies have failed to identify any evidence of carotenodermia in subjects exposed to levels up to 25 mg lutein/day 3 times a week for 13 months (Khachik *et al.*, 1995, 1997; Olmedilla *et al.*, 2001; Schalch *et al.*, 2001). These results are supported in GRAS notice (GRN 000110) for lutein esters, where it was noted that carotenodermia was not observed in a study in which subjects consumed supplements containing up to 40 mg/person/day of lutein esters (theoretically estimated to be equivalent to 20 mg lutein) for more than 90 days (Agency response letter GRAS Notice No. GRN 000110). However, in a multi-centre study where the subjects were supplemented with15 mg/day of mixed esters extracted from marigolds, it was observed that 40% of the Spanish cohort experienced carotenodermia (evaluated by visual examination of the palms of the hands). Carotenodermia was not observed in any of the other cohorts (France, Northern Ireland, Republic of Ireland, and the Netherlands). The results from this subjective evaluation were therefore called into question given the lack of analytical quantification and the fact that this effect was seen only within the Spanish cohort (Olmedilla *et al.*, 2002).

Overall, lutein intervention studies have indicated a dose dependent increase in plasma levels. The maximum plasma concentration recorded (1.72 μmol/L) following a 20 mg/day intake for 6 weeks, resulted in an approximate 2-fold higher plasma concentration than the highest background level reported by the IOM (1.23 μmol/L). An increase in plasma lutein levels of this magnitude is well tolerated and results in no accumulation in the retina or any ocular toxicity. Lutein intervention studies have also failed to detect carotenodermia [a harmless and reversible effect (IOM, 2000)] at dosage levels greater than those anticipated from the intended use level of FloraGLO® Crystalline Lutein. Furthermore, epidemiological studies have failed to identify any adverse effects and therefore the consumption of FloraGLO® Crystalline Lutein is not anticipated to have any deleterious health effects.

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EXPERT PANEL OPINION REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF FLORAGLO® CRYSTALLINE LUTEIN

INTRODUCTION

At the request of Kemin Foods L.C. (Kemin), an Expert Panel (hereinafter referred to as the Panel) of independent scientists, qualified by relevant national and international experience and scientific training to evaluate the safety of food ingredients, was convened to evaluate the safety of FloraGLO® Crystalline Lutein, a purified extract from the marigold (*Tagetes erecta*) flower that contains approximately 83% by weight of the carotenoids lutein and zeaxanthin (76 and 7%, respectively), and to determine the Generally Recognized as Safe (GRAS) status of FloraGLO® Crystalline Lutein for use in foods. The Expert Panel included Dr. Robert Russell (Tufts University), Professor Ron Walker (University of Surrey), and Dr. Ian Munro (CANTOX Health Sciences International). *Curricula vitae* evidencing the qualifications of the Panel for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel was requested to evaluate the safety of FloraGLO® Crystalline Lutein under the conditions of intended use as a food ingredient, and as an ingredient in medical foods intended for use as the sole item of the diet. The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources. The data evaluated by the Panel comprised information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, safety studies conducted with FloraGLO® Crystalline Lutein, and other relevant data on the safety of lutein and zeaxanthin obtained from the literature.

Following independent critical evaluation of the data and information, the Panel met on June 2, 2003. At this meeting, the Panel concluded that FloraGLO® Crystalline Lutein meets appropriate food grade specifications, is manufactured in compliance with current Good Manufacturing Practices (cGMP), and is GRAS based on scientific procedures for use under the conditions of use proposed by Kemin. A summary of the basis for our conclusion is set forth below.

MANUFACTURING AND SPECIFICATIONS

FloraGLO® Crystalline Lutein is produced in accordance with current good manufacturing practice *via* solvent extraction using food grade material, from dried marigold flower petals (*Tagetes erecta*), followed by crystallization, centrifugation, and drying steps. The final product is packaged under appropriate food-grade conditions. Specifications for FloraGLO® Crystalline Lutein meet acceptable food grade specifications, and are presented in Table 1. The panel reviewed the results of analysis of several non-consecutive representative lots of FloraGLO® Crystalline Lutein and concluded the product complied with final product specifications.

Table 1 Specifications for Food-Grade FloraGLO® Crystalline Lutein

Principal Constituents		
Total Carotenoids (including lutein and zeaxanthin)		≥ 80.0% wt
Lutein	≥ 74.0% wt	
Zeaxanthin	≥ 2.0% wt ≤ 9.0% wt	
Waxes		≤ 14.0 % wt
Moisture		≤ 1.0% wt
Ash		≤ 1.0% wt
Pesticides and Related Potential Contaminants		≤ 0.1% (total of other constituents)
Protein	<100 ppm	
Propylene glycol	<1,000 ppm	
Hexane	<50 ppm	
Thiophenes	<300 ppm	
Chlorinated and Organo-Phosphate Pesticides	Not Detected	
Metals		
Lead (Pb)	<0.65 ppm	
Cadmium (Cd)	<1.25 ppm	
Arsenic (As)	<2.5 ppm	
Mercury (Hg)	<0.15 ppm	
Microbiological Assays		
Aerobic plate count	≤100 cfu/g	
E. coli enrichment	Negative/10g	
Listeria monocytogenes	Negative/25g	
Salmonella	Negative/10g	
Staph enrichment	Negative/10g	
Coliform enrichment	Negative/25g	
Yeast count	≤100 cfu/g	
Mold count	≤100 cfu/g	

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INTENDED USE AND ESTIMATED INTAKE

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.

FloraGLO® Crystalline Lutein is intended for use 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 2.

Table 2 Summary of the Individual Proposed Food-Uses for FloraGLO® Crystalline Lutein and Corresponding Use-Levels for Lutein in the U.S.

Food Category	Proposed Food-Use	Use-Levels for Lutein (mg/RACC[1])	Use-Levels for Lutein (%)[2]
Baked Goods and Baking Mixes	Cereal and Energy Bars	2.0	5.0×10^{-3}
	Crackers and Crispbreads	2.0	6.7×10^{-3}
Beverages and Beverage Bases	Bottled Water	0.5	2.1×10^{-4}
	Carbonated Beverages	2.0	8.3×10^{-4}
	Meal Replacements	2.0	8.3×10^{-4}
	Tea, Ready-to-Drink	0.6	2.6×10^{-4}
Breakfast Cereals	Instant and Regular Hot Cereals	2.0	8.3×10^{-4}
	Ready-to-Eat Cereals	2.0	3.6×10^{-3} - 1.3×10^{-2}
Chewing Gum	Chewing Gum	1.0	3.3×10^{-2}
Dairy Product Analogs	Imitation Milks	2.0	8.3×10^{-4}
	Soy Milks	1.5	6.3×10^{-4}
Egg Products	Liquid, Frozen, or Dried Egg Substitutes	2.0	4.0×10^{-3}
Fats and Oils	Margarine-like Spreads	1.5	1.0×10^{-2}
	Salad Dressings	1.5	5.0×10^{-3} - 1.0×10^{-2}
Frozen Dairy Desserts and Mixes	Frozen Yogurt	1.0	8.3×10^{-4}
Gravies and Sauces	Tomato-Based Sauces	0.3	2.6×10^{-4}

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Table 2	Summary of the Individual Proposed Food-Uses for FloraGLO® Crystalline Lutein and Corresponding Use-Levels for Lutein in the U.S.		
Food Category	**Proposed Food-Use**	**Use-Levels for Lutein (mg/RACC[1])**	**Use-Levels for Lutein (%)[2]**
Hard Candy	Hard Candy	1.0	6.7×10^{-3}
Infant and Toddler Foods*	Junior, Strained, and Toddler Type Baby Foods	1.0	5.9×10^{-4} -1.4×10^{-2}
Milk Products	Dry Milk	3.0	1.3×10^{-3}
	Fermented Milk Beverages	0.6	2.6×10^{-4}
	Flavored Milk and Milk Drinks	3.0	1.3×10^{-3}
	Milk-Based Meal Replacements	3.0	1.3×10^{-3}
	Yogurt	3.0	1.3×10^{-3}
Processed Fruits and Fruit Juices	Energy, Sport, and Isotonic Drinks	2.0	8.3×10^{-4}
	Fruit-Flavored Drinks	2.0	8.3×10^{-4}
	Fruit Juice	2.0	8.3×10^{-4}
	Nectars	2.0	8.3×10^{-4}
	Vegetable Juice	2.0	8.3×10^{-4}
Soft Candy	Chewy and Nougat Candy	1.0	2.5×10^{-3}
	Fruit Snacks	1.0	2.5×10^{-3}
Soups and Soup Mixes	Canned Soups	0.6	2.6×10^{-4}

[1]RACC – Reference amounts customarily consumed per eating occasion (21 CFR §101.12).
[2]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.
*Does not include infant formula.

FloraGLO® Crystalline Lutein is also proposed for use as an ingredient in medical foods intended as the sole item of the diet, at levels not to exceed 20 mg lutein/day.

The consumption of lutein 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 lutein by the total U.S. population from all proposed food-uses of FloraGLO® were estimated to be 7.3 mg/person/day (0.14 mg/kg body weight/day) and 13.4 mg/person/day (0.28 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 FloraGLO® Crystalline Lutein and zeaxanthin from the consumption of proposed food-uses were 9.6 mg/person/day (0.18 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 FloraGLO® Crystalline Lutein and zeaxanthin were 17.6 mg/person/day (0.37 mg/kg body weight/day) and 1.2 mg/person/day (0.03 mg/kg body weight/day), respectively.

DATA PERTAINING TO SAFETY

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 carotenoid) (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 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.

Toxicological Studies

The 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.*, 2000a,b; Kruger *et al.*, 2002; Schierle *et al.*, 2002).

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-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.*, 2000a,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 (158 to 5,000 μg/plate) or FloraGLO® Crystalline Lutein (15.8 to 500 μg/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 body/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, 2000; 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 (Müller *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.*, 2001). 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 Müller *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.*, 1999a,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).

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.*, 1996a,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*.

SUMMARY

Overall, when viewed in its entirety, the scientific data summarized herein support the safety of FloraGLO® Crystalline Lutein under the conditions of intended use as a food ingredient, and for use as an ingredient in medical foods intended as the sole item of the diet. Experimental animals studies and clinical trials reveal no potential for toxicity of FloraGLO® Crystalline Lutein under the conditions of intended use.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the available pertinent scientific evidence, and conclude that FloraGLO® Crystalline Lutein, meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS), based on scientific procedures, under the conditions of intended use in foods and medical foods, as specified herein.

6/26/03
Date

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CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the available pertinent scientific evidence, and conclude that FloraGLO® Crystalline Lutein, meeting appropriate food grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS), based on scientific procedures, under the conditions of intended use in foods and medical foods, as specified herein.

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