ORIGINAL SUBMISSION

ABBOTT NUTRITION • ABBOTT LABORATORIES

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GRAS Notice Review

July 18, 2011

Mary Ditto, Ph.D.
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 Exemption Claim for FloraGLO® Lutein 20% in Safflower Oil for Use in Formulas Intended for Premature Infants

Dear Dr. Ditto:

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 18938 (17 April 1997)], I am submitting 3 copies of a GRAS notification for FloraGLO® Lutein 20% in Safflower Oil. Abbott Nutrition has determined through scientific procedures that this ingredient is GRAS for use in formulas intended for premature infants. Based on this GRAS determination, FloraGLO® Lutein 20% in Safflower Oil is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*.

I trust that the enclosed notification is acceptable. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Leslie Lake Curry
Director, Global Innovation Regulatory Affairs
Abbott Nutrition

Encl.

GRAS Exemption Claim for FloraGLO® Lutein 20% in Safflower Oil for Use in Formulas Intended for Premature Infants

Submitted to:

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

Submitted by:

Abbott Nutrition
3300 Stelzer Road
Columbus, OH
43219
USA

July 18, 2011

GRAS Exemption Claim for FloraGLO® Lutein 20% in Safflower Oil for Use in Formulas Intended for Premature Infants

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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) (U.S. FDA, 1997)]

FloraGLO® Lutein 20% in Safflower Oil has been determined to be Generally Recognized as Safe (GRAS) by Abbott Nutrition, a division of Abbott Laboratories, for use in formulas intended for premature infants. All preterm formulas marketed by Abbott Nutrition are categorized as exempt formulas under section 412 of the Federal Food Drug and Cosmetic Act (FDCA). An exempt infant formula is designed for use by infants who have inborn errors of metabolism, low birth weight, or other unusual medical or dietary problems.

As a GRAS ingredient for this use, FloraGLO® Lutein 20% in Safflower Oil is not a food additive under section 201(s) of the FDCA, and, accordingly, is exempt from premarket notification. This determination is based on scientific procedures as described in the following sections.

Signed,

(b) (6) [redacted]

July 18, 2011
Date

I.B Name and Address of Notifier

Leslie Lake Curry
Abbott Nutrition
3300 Stelzer Road
Columbus, OH
43219
USA

I.C Common Name of the Notified Substance

The common name of the notified substance is lutein.

I.D Conditions of Intended Use in Food

I.D.1 Intended Use of FloraGLO® Lutein 20% in Safflower Oil and Level of Use

Abbott Nutrition intends to use FloraGLO® Lutein 20% in Safflower Oil as an ingredient in formulas designed for premature infants. FloraGLO® Lutein 20% in Safflower Oil will be added at a level up to 210 µg/L to powder and liquid formulas intended for premature infants.

I.D.2 Estimated Consumption of FloraGLO® Lutein 20% in Safflower Oil Based Upon Intended Use

Abbott Nutrition conducted a clinical trial with preterm infants fed formulas supplemented with lutein in the range of the proposed use-level (Rubin *et al.*, 2011). In this study, preterm infants were fed formulas supplemented with lutein [FloraGLO® Lutein, Kemin Health, LC (Kemin Health)], with the mean lutein concentration in the preterm in-hospital and post-discharge formulas being 211 µg/L and 68.7 µg/L, respectively. This resulted in estimated mean lutein intakes of 24, 32, 15, and 13 µg/kg body weight/day during the in-hospital phase, and at 40, 44, and 50 weeks PMA[1], respectively.

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2011), FloraGLO® Lutein 20% in Safflower Oil has been determined to be GRAS for the addition to formulas intended for premature infants on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of lutein, as discussed herein, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of the FloraGLO® Lutein 20% in Safflower Oil as an ingredient in formulas intended for premature infants [see Appendix A, entitled "Expert Panel Consensus Statement Concerning the Generally Recognized As Safe (GRAS) Status of FloraGLO® Lutein 20% in Safflower Oil in Formulas Intended for Premature Infants"].

At the request of Abbott Nutrition, an Expert Panel ("the Expert Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients in preterm infant formulas, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended use of FloraGLO® Lutein 20% in Safflower Oil in

[1] Postmenstrual age is the sum of gestational age (time between last normal menstrual period and day of delivery) and chronological age (time after delivery).

formulas intended for premature infants is safe and suitable and is GRAS based on scientific procedures.

The Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Dr. Ronald E. Kleinman, MD (Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital), and Dr. Robert M. Russell, MD (Friedman School of Nutrition Science, Tufts University).

The Expert Panel convened independently and collectively to critically evaluate the data and information summarized in the following sections. The Expert Panel concluded that the intended use in formulas intended for premature infants described herein for FloraGLO® Lutein 20% in Safflower Oil, meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), is safe and suitable and GRAS based on scientific procedures. It also is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion.

FloraGLO® Lutein 20% in Safflower Oil is GRAS based on scientific procedures for the intended use as a food ingredient; therefore, it is excluded from the definition of a food additive, and thus may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

I.F Availability of Information

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

Abbott Nutrition
3300 Stelzer Road
Columbus, OH
43219
USA

Should FDA have any questions or additional information requests regarding this notification, Abbott Nutrition will supply these data and information.

II. DETAILED INFORMATION ABOUT THE SOURCE AND IDENTITY OF THE SUBSTANCE

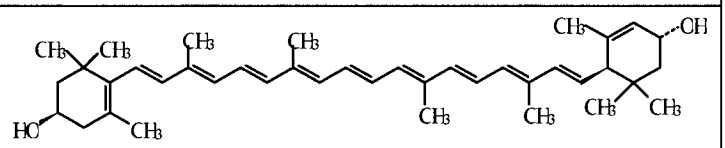
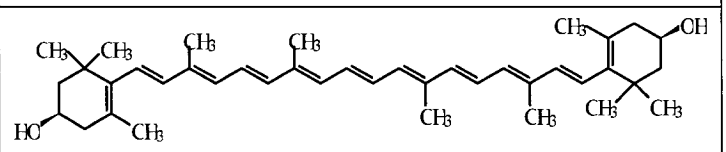
II.A Source and Identity

The crystalline lutein portion of FloraGLO® Lutein 20% in Safflower Oil is a purified extract from the marigold (*Tagetes erecta*) flower that contains approximately 83% by weight of the carotenoids lutein and zeaxanthin, manufactured under cGMP by Kemin Health, to meet appropriate food grade specifications. Crystalline lutein (FloraGLO® crystalline lutein) is suspended in food-grade safflower oil to form FloraGLO® Lutein 20% in Safflower Oil that contains approximately 20% lutein and 1% zeaxanthin by weight.

Lutein and its isomer zeaxanthin are oxycarotenoids that contain 2 cyclic end groups (one β- and one ε-ionone ring) and the basic C_{40} isoprenoid structure common to all carotenoids. In principal, each of the polyene chain double bonds present in lutein and zeaxanthin could exist in a *cis* or *trans* conformation, giving rise to a large number of possible mono-*cis* and poly-*cis* isomers; however, in practice, the vast majority of carotenoids are in the all-*trans* configurations (Rice-Evans *et al.*, 1997; IOM, 2000). Small amounts of *cis* isomers of lutein and zeaxanthin, accompanied by their respective all-*trans* configurations, have been isolated and purified from extracts of marigold flowers, fresh raw kale, corn meal, spinach, and human plasma (Krinsky *et al.*, 1990; Khachik *et al.*, 1999).

The common names, chemical names, Chemical Abstracts Services (CAS) numbers, and structural formulae for lutein and zeaxanthin are presented in Table II.A-1.

Table II.A-1 Descriptions of Lutein and Zeaxanthin

Common Name	Chemical Names	CAS No.	Structural Formula
Lutein	xanthophyll; β,ε-carotene-3,3'-diol; vegetable lutein; vegetable luteol; Bo-Xan; all-*trans*-(+)-xanthophyll; all-trans-lutein	127-40-2	
Zeaxanthin	β, β-carotene-3,3'-diol; all-*trans*-β-carotene-3,3'-diol; (3*R*,3'*R*)-dihydroxy-β-carotene; zeaxanthol; anchovyxanthin	144-68-3	

II.B Method of Manufacture

FloraGLO® crystalline lutein is manufactured by Kemin Health *via* a 3-step process wherein lutein and zeaxanthin are extracted from dried marigold flower petals (*Tagetes erecta*). Marigold oleoresin is first extracted from the dried flower petals with hexane. Using potassium hydroxide, propylene glycol, and water in the presence of heat and mixing, the fatty acids are removed from the oleoresin, and crystals of lutein and zeaxanthin are formed. The resulting product is diluted with water, centrifuged, washed with additional water, and dried to form a dry cake base. The dry cake base is then screened, and sifted to form the final product, FloraGLO® crystalline lutein. Kemin Health's FloraGLO® crystalline lutein is then suspended in food-grade safflower oil to produce FloraGLO® Lutein 20% in Safflower Oil.

II.C Ingredient Specifications

The ingredient specifications for FloraGLO® Lutein 20% in Safflower Oil are presented in Tables II.C-1 and II.C-2, respectively.

Table II.C-1 Ingredient Specifications for FloraGLO® Crystalline Lutein

Parameter	Specification	Method
Appearance	Free flowing powder, free of foreign matter	KHM-005-916
Color	Orange-Red	KHM-005-916
Odor	Bland	KHM-005-916
Moisture (%)	≤1.0	KHM-005-913
Total Carotenoids (%)	≥80.0	KHM-005-904
Lutein (%)	≥74.0	KHM-005-904
Zeaxanthin (%)	4.0 to 9.0	KHM-005-904
Heavy Metals		
Lead (ppm)	≤0.65	AOAC 993.14
Cadmium (ppm)	≤1.25	AOAC 993.14
Mercury (ppm)	≤0.15	Analyst 86.608, Analytical Chem. 40:2085
Arsenic (ppm)	≤2.5	AOAC 993.14
Microbiological Contaminants		
Aerobic Plate Count (CFU/g)	≤100	AOAC966.23
Escherichia coli enrichment	Negative/10 g	BAM-FDA, 7th Ed.
Listeria monocytogenes	Negative/25 g	CMMEF 4th Ed.
Salmonella	Negative/10 g	USP 29/NF 24, 2006
Staphylococcus enrichment	Negative/10 g	USP 29/NF 24, 2006
Coliform Enrichment (MPN)	Negative/25 g	USP 29/NF 24, 2006
Yeast and Mold Count	≤100	AOAC 999.06

Table II.C-1 Ingredient Specifications for FloraGLO® Crystalline Lutein		
Parameter	**Specification**	**Method**
(CFU/g)		
Solvent Residues		
Hexane	≤50 ppm	KHM-005-023
Propylene Glycol	≤1,000 ppm	KHM-005-033

AOAC = Association of Official Analytical Chemists; BAM-FDA = Bacteriological Analytical Manual – Food and Drug Administration; cfu = colony forming units; CMMEF = Compendium of Methods for the Microbiological Examination of Foods; KHM = Kemin Health, LC Method; MPN = most probable number; NF = National Formulary; USP = U.S. Pharmacopeia

Table II.C-2 Ingredient Specifications for FloraGLO® Lutein 20% in Safflower Oil		
Specification Parameter	**Specification**	**Method**
Appearance	Viscous oil	Visual inspection
Color	Brown-orange to brown-red	Visual inspection
VIS-absorption in ethanol	444 to 448 nm	UV absorption spectroscopy
Moisture	≤1.0%	Karl Fischer titration
Residue on ignition	≤1.0%	USP <281> *Residue on Ignition*
Lutein	≥20.0%	HPLC
Zeaxanthin	≥1.0 %	HPLC

HPLC = high-performance liquid chromatography; USP = United States Pharmacopeia; UV = ultraviolet

An Expert Panel of independent scientists, qualified by their relevant national and international experience and scientific training, reviewed the results of analysis of several non-consecutive representative lots of FloraGLO® crystalline lutein and FloraGLO® Lutein 20% in Safflower Oil, and concluded that the final products comply with the specifications.

IV. BASIS FOR GRAS DETERMINATION

IV.A Documentation to Support the Safety of FloraGLO® Lutein 20% in Safflower Oil

The determination that FloraGLO® Lutein 20% in Safflower Oil is GRAS for the addition to formulas intended for premature infants is based on scientific procedures, and the information supporting the general recognition of the safety of the ingredient includes:

- data pertaining to the identity, intended use, and estimated intake of lutein;
- the natural occurrence of lutein and zeaxanthin in food;
- the metabolic fate of lutein; and
- preclinical and human studies (including infant) conducted with lutein.

These studies were reviewed by the Expert Panel, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the intended use of FloraGLO® Lutein 20% in Safflower Oil is safe and suitable, and is GRAS based on scientific procedures [see Appendix A, entitled "Expert Panel Consensus Statement Concerning the Generally Recognized As Safe (GRAS) Status of FloraGLO® Lutein 20% in Safflower Oil in Formulas Intended for Premature Infants"]. A summary of these data is presented below.

IV.B Current Regulatory Status of FloraGLO® Lutein 20% in Safflower Oil and Other Forms of Lutein

IV.B.1 United States

FloraGLO® Lutein 20% in Safflower Oil is GRAS for use as an ingredient in term infant formula at a maximum level of 250 µg/L (GRN Notice No. 221 – U.S. FDA, 2007). In addition, FloraGLO® crystalline lutein is determined as GRAS for use as an ingredient in a variety of food and beverage products, including baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy products analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula, at levels up to 1 mg/serving), milk products, processed fruit and vegetable products, soft candy, soups, and soup mixes up to 3 mg/serving. FloraGLO® crystalline lutein also is GRAS for use as an ingredient in medical foods intended as the sole item of the diet at levels not to exceed 20 mg/day (GRN Notice No. 140 – U.S. FDA, 2004). A mixture of carotenoid xanthophyll esters derived from marigold flowers (*Tagetes erecta*) has been determined to be GRAS for use in a variety of foods and in medical foods intended as the sole item of the diet (GRN Notice No. 110 – U.S. FDA, 2003).

IV.B.2 Europe

In 2008, the European Food Safety Authority (EFSA, 2008) released a scientific opinion on the suitability of lutein in infant formula and follow-on formula; in the opinion, EFSA stated that the proposed use of 250 µg lutein/L in infant formula products raised no safety concerns. In 2010, EFSA re-evaluated lutein as a food additive and derived an acceptable daily intake (ADI) of 1 mg/kg body weight/day, based on a no-observed-adverse-effect level (NOAEL) of 200 mg/kg body weight/day in a 90-day study in rats, and a 200-fold safety factor (EFSA, 2010).

IV.B.3 Australia and New Zealand

In July 2008, the Food Standards Australia New Zealand (FSANZ) approved the addition of up to 250 µg lutein/L in infant formula products. In March 2009, FSANZ completed a First Review in which they recommended that use of up to 250 µg lutein/L be reduced to a level of up to 143 µg lutein/L (5 µg/100 kJ) in infant formula products (FSANZ, 2009a). FSANZ (2009a) indicated that the evidence submitted to support the higher proposed levels of use in the original application did not demonstrate that lutein bioavailability from breast milk is higher than that from infant formula products. FSANZ (2009a) adopted a conservative approach and reduced the permitted use level of lutein in infant formula to reflect concentrations within the range present in breast milk. In 2009, FSANZ also recommended amending standards to permit the voluntary addition of lutein as a nutritive substance to formulated supplementary foods for young children up to a potential maximum concentration of 100 µg lutein/serving, or 500 µg lutein/L (FSANZ, 2009b).

IV.B.4 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA established a group Acceptable Daily Intake (ADI) for lutein from *Tagetes erecta* L. and synthetic zeaxanthin of up to 2 mg/kg body weight/day (JECFA, 2006).

IV.C Background Exposure

IV.C.1 Occurrence of Lutein and Zeaxanthin in the Diet

Lutein and zeaxanthin are among the most prevalent carotenoids in the North American diet (IOM, 2000). Both lutein and zeaxanthin are present at high concentrations in green leafy vegetables, such as spinach and kale (Khachik *et al.*, 1995; Omaye *et al.*, 1997; Cena *et al.*, 2009; Maiani *et al.,* 2009), as well as herbs, including basil and coriander (Daly *et al.,* 2010). Both carotenoids also are present in chicken egg yolks, which contain 292 and 213 µg/egg yolk of lutein and zeaxanthin, respectively (Handelman *et al.*, 1999). Lutein is abundant in various vegetables, including broccoli, brussel sprouts, cabbage, and green beans (Khachik *et al.*, 1995), whereas zeaxanthin is only present at modest concentrations in corn (Khachik *et al.*,

1995). In the U.S., the major dietary source of lutein is green leafy vegetables, while the primary sources of dietary zeaxanthin are eggs and corn products (Johnson *et al.*, 2010). In addition to their natural occurrence in various foods, lutein and zeaxanthin are available in dietary supplements (IOM, 2000).

In conjunction with dietary intake data from the U.S. Third National Health and Nutrition Examination Survey, 1988-1994 (NHANES III), the Institute of Medicine (IOM) used an expanded carotenoid database for foods reported in NHANES III, to estimate the usual consumption of lutein and zeaxanthin by the total U.S. population greater than 2 months of age (29,015 individuals) (IOM, 2001). The estimated mean and 90th percentile consumption of total lutein and zeaxanthin by the surveyed sample were 1.7 and 3 mg/person/day, respectively. Slightly higher mean intakes of 2.2 and 1.9 mg/person/day of total lutein and zeaxanthin, for men and women, respectively, were estimated from food frequency data obtained from 8,341 adults in the 1992 National Health and Interview Survey using the USDA National Cancer Institute carotenoid food composition database (Nebeling *et al.*, 1997).

Kruger *et al.* (2002) estimated the intake of lutein and zeaxanthin using the dietary records for only those respondents to NHANES III who met their recommended daily intake of vegetables, as described in the Dietary Guidelines for Americans, and the carotenoid database employed by the IOM. The mean and 90th percentile for total lutein and zeaxanthin intakes for these individuals (5,708 participants, approximately 25% of the total surveyed sample) were determined to be 3.83 and 7.29 mg/person/day, respectively. Therefore, estimated intakes of lutein and zeaxanthin based on recommended levels of vegetable consumption appear to be greater than twice the estimated actual intakes for the total U.S. population (Kruger *et al.*, 2002).

More recently, Johnson *et al.* (2010) estimated the individual intakes of lutein and zeaxanthin using lutein and zeaxanthin values in major dietary sources, applied to data from the NHANES 2003-2004 survey. Lutein intakes were reported to be significantly greater than zeaxanthin intakes among all age groups (zeaxanthin to lutein ratios ranging from 0.32 to 0.42) and all ethnicities (zeaxanthin to lutein ratios ranging from 0.29 to 0.50) for both sexes.

IV.C.2 Exposure to Lutein during Gestation

During gestation, the amount of lutein that crosses the placenta is dependent on maternal diet. Several studies have reported cord blood concentrations of lutein, which reflect *in utero* nutrient exposure. Significant positive correlations between maternal and cord blood concentrations have been reported for lutein and zeaxanthin and cord blood concentrations are consistently lower than maternal plasma (Ostrea *et al.*, 1986; Oostenbrug *et al.*, 1998; Yeum *et al.*, 1998; Kiely *et al.*, 1999; Jewell *et al.*, 2001; Herrera *et al.*, 2004). Cord blood lutein concentrations in infants (Table IV.C.2-1) are in the range of plasma lutein concentrations in preterm infants fed formulas supplemented with 211 μg lutein/L (Table IV.D.1-1).

Table IV.C.2-1 Infant Cord Blood Lutein Concentrations

Study	Lutein (μg/L)
Jewell *et al.* 2000[1]	33 (23-52)
Jewell *et al.* 2001[1]	26 (9-58)
Kiely *et al.* 1999[2]	74 ± 66
Oostenbrug *et al.* 1998[3]	85 ± 10
Yeum *et al.* 1998[3]	28 ± 6

[1]Lutein concentration is reported as median (interquartile range).
[2]Lutein concentration is reported as mean ± SD
[3]Lutein concentration is reported as mean ± SEM

IV.C.3 Exposure to Lutein from Human Milk

The American Academy of Pediatrics (AAP) recommends exclusive use of human milk during the first 6 months of life, and in combination with solid foods from about six months through the first year of life and beyond as determined by mother and child (AAP, 2005). The AAP also encourages hospitals and physicians to recommend human milk feedings for premature and other high risk infants, and supports use of human milk fortifiers for many low birth weight infants (AAP, 2005).

Human milk is a complex substance, containing thousands of constituents including not only vitamins and minerals, but hormones, immune factors, and live cells (Picciano, 2001). Human milk contains more than 30 carotenoids, including geometrical isomers and metabolites (Khachik *et al.*, 1997). Lycopene, lutein, and *beta*-carotene are among the predominant carotenoids in both serum and human milk (Giuliano *et al.*, 1994; Khachik *et al.*, 1997). Mean lutein concentrations of human milk have been reported to range from 3 to 232 μg/L (Canfield *et al.*, 1997, 2001, 2003; Lietz *et al.*, 2006; Cena *et al.,* 2009; Bettler *et al.*, 2010; Jackson *et al.,* 1998; Gossage *et al.,* 2002; Jewell *et al.*, 2004; Schweigert *et al.*, 2004; Sommerburg *et al.*, 2000; Jackson and Zimmer, 2007), and can vary with stage of lactation. The mechanisms underlying the apparent wide variation in milk composition existing among and between women are not yet fully understood (Picciano, 2001); however, just as dietary intake of carotenoid-rich foods is associated with increased serum carotenoid concentrations (Campbell *et al.*, 1994; Al-Delaimy *et al.*, 2005), dietary intake of carotenoids appears to have an impact on breast milk carotenoid levels. Consumption of carotenoid-rich vegetables or supplements by lactating women has been reported to increase breast milk levels of these nutrients (Canfield *et al.*, 1997; Haftel *et al.*, 2001; Allen *et al.*, 2002).

Canfield *et al.* (2003) conducted the largest survey of breast milk carotenoids levels across 9 countries [*i.e.,* Australia, Canada, Chile, China, Japan, Philippines, United Kingdom (UK), Mexico, and the U.S.]. The mean concentration of combined lutein and zeaxanthin across the

different countries ranged from 15 to 43 µg/L. The average lutein and zeaxanthin concentration across all of the sites was 25 ± 19 µg/L.

IV.C.4 Estimated Lutein Exposure from Current Infant Formula Products

Premature infants may be exposed to lutein in formulas in two ways; from lutein inherent to ingredients used in the manufacture of infant formula, or when lutein is intentionally added. Results from an analysis of carotenoid concentrations in six formulas available in Ireland indicated that the median concentration of lutein ranged from 100 to 243 µg/L in preterm formulas, and 0 to 158 µg/L in term formulas (Jewell *et al.*, 2004). The presence of lutein was attributed to the use of egg lipids in the European formulas. It should be noted that egg lipids are not an approved ingredient in infant formulas in the U.S.

As noted above in Section IV.B.1, FloraGLO® Lutein 20% in Safflower Oil is presently GRAS for use as an ingredient in term infant formula at a maximum level of 250 µg/L (GRN Notice No. 221 – U.S. FDA, 2007). Formulas supplemented with up to 250 µg lutein/L were estimated to provide lutein exposures of approximately 44 µg/kg body weight/day, and total lutein exposures from formula and other dietary sources of up to 230 µg/kg body weight/day.

IV.C.5 Estimated Lutein Exposure from Abbott Nutrition Infant Formula Products

Abbott Nutrition conducted a double-blind, randomized, controlled, parallel multicenter clinical trial in preterm infants fed formulas with added lutein (FloraGLO® Lutein, Kemin Health), synthetic lycopene (Lycovit®, BASF), and synthetic β-carotene (Lucarotin®, BASF) (Rubin *et al.*, 2011). The mean concentration of lutein in the in-hospital preterm infant formula was 211 µg/L, and the mean concentration in the post-discharge preterm formula was 68.7 µg/L. The population in this study was a representative sample of infants who are fed preterm infant formulas in the U.S. Formula intake was monitored daily during the in-hospital phase, and at 40, 44, and 50 weeks post-menstrual age (PMA) during the post-discharge phase (based on 3-day infant dietary intake diaries). Based on these intake data from the evaluable study population (*i.e.,* the subpopulation of infants who consumed exclusively or nearly exclusively the assigned study formula), the mean intakes of lutein were estimated to be 24, 32, 15, and 13 µg/kg body weight/day during the in-hospital phase, and at 40, 44, and 50 weeks PMA, respectively.

Abbott Nutrition has also conducted a clinical study with term infants to measure infant plasma response to feeding formulas supplemented with lutein. In this study, term infants were fed formulas containing different concentrations of lutein ranging from 33 µg/L in low-carotenoid formula to 117 µg/L in high-carotenoid formula for a period of 8 weeks. Based on the three-day formula intake estimates collected during the clinical trial, the highest absolute intake of total

lutein per day occurred on study Day 56 and was 138 µg; the highest intake based on body weight occurred on study Day 28, at 23 µg/kg body weight/day.

IV.D Metabolic Fate

IV.D.1 Absorption and Bioavailability of Lutein and Zeaxanthin

Lutein is a lipid-soluble carotenoid that generally follows the same digestion and intestinal absorption pathways as dietary fat (Bendich, 1988; Furr and Clark, 1997; Boileau *et al.*, 1999; van den Berg, 1999; Yeum and Russell, 2002). After consumption, carotenoids are released from the food matrix and solubilized into the lipid phase. Subsequently, the carotenoids are incorporated into lipid micelles in the small intestine for mucosal uptake and transport to lymphatic and/or portal circulation (Erdman *et al.*, 1993; Parker, 1996). The non-polar carotenoids tend to migrate to the core of the lipid emulsion particles, while more polar xanthophylls, like lutein, likely remain at the surface. Uptake of carotenoids from mixed lipid micelles into mucosal cells of the duodenum appears to occur by passive diffusion (Erdman *et al.*, 1993; Parker, 1996), although facilitated uptake also has been observed (During *et al.*, 2005; Moussa *et al.*, 2008). Carotenoids that are taken up by the mucosal cells, but not incorporated into chylomicrons, are sloughed off when the enterocytes turn over, and are excreted in the lumen of the gastrointestinal tract (Williams *et al.*, 1998; Boileau *et al.*, 1999).

The overall bioavailability of lutein and zeaxanthin may be affected by factors that may interfere with the rate and/or completeness of any of the steps involved in carotenoid absorption, such as micellar incorporation, intestinal uptake, lymphatic transport (Furr and Clark, 1997). Gastric pH level, altered hormone status, exposure to tobacco smoke, liver and kidney diseases, malabsorption diseases, intestinal parasites, poor iron, zinc and protein status, and hyperthyroidism are examples of non-dietary factors that may affect bioavailability of ingested lutein (Albanes *et al.*, 1997; Williams *et al.*, 1998; Patrick, 2000; Alberg, 2002).

Evidence for the absorption of lutein and zeaxanthin following the ingestion of products containing lutein by healthy human subjects, including infants, are indicated by increased plasma levels reported in a number of studies (Kostic *et al.*, 1995; Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999; Schalch *et al.*, 2001; Mackey *et al.*, 2006, 2008; Romagnoli *et al.*, 2010; Rubin *et al.*, 2011; Price *et al.*, 2011). The bioavailability of lutein in crystalline form has been reported to be 30 to 50% greater than that from food, such as mixed vegetables and spinach products (Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999). FloraGLO® Lutein 20% in Safflower Oil is absorbed from infant formula; however, it is approximately four times less bioavailable than lutein from human milk (Bettler *et al.*, 2010).

Abbott Nutrition conducted a double-blind, randomized, controlled, parallel multicenter clinical trial in which preterm infants were fed formulas with added lutein, synthetic lycopene, and synthetic β-carotene (Rubin *et al.*, 2011). The mean concentration of lutein in the in-hospital preterm infant formulas was 211 µg/L, and the mean concentration in the post-discharge preterm formula was 68.7 µg/L. In the evaluable group (*i.e.*, the subpopulation of infants who consumed exclusively or nearly exclusively the assigned study formula), plasma lutein concentrations were significantly higher in the carotenoid formula group compared to the control group. Similarly, at hospital discharge and at 40 and 50 weeks PMA, plasma concentrations of lutein was higher in infants fed the carotenoid formulas compared to infants fed the control formulas. As shown in Table IV.D.1-1, the mean plasma concentration of lutein in preterm infants consuming the carotenoid formulas at 50 weeks PMA (approximately equivalent to a 10 week old term infant) was in the range of plasma lutein concentrations in a reference group of breastfed term infants (10 to 13 weeks of age) (Mackey *et al.*, 2006, 2008). Consistent with the findings of Bettler *et al.* (2010), the results of this study support that the relative bioavailability of lutein added to formula is lower compared to human milk.

Table IV.D.1-1 Plasma Concentrations of Lutein at Study Day 1 and 50 Weeks PMA

Formula Product	Study Time Point	Lutein Concentrations (µg/L)	
		Mean ± SEM (Range)	n
Control Formula	Study Day 1	17 ± 1.4 (3 - 44)	42
	50 weeks PMA	18 ± 4 (2 - 118)	28
Carotenoid Formula	Study Day 1	24 ± 1.9 (6 - 54)	37
	50 weeks PMA	77 ± 5.8 (8 - 155)	26
Human Milk (Reference group)[a]	Study Day 56 (10-13 weeks of age)	59 ± 7.7 (5 - 201)	26

PMA = post-menstrual age; SEM = standard error of the mean
[a] Adapted from Mackey *et al.*, 2008. Plasma lutein concentration was measured at 10 to 13 weeks of age.

IV.D.2 Distribution, Metabolism, and Excretion of Lutein and Zeaxanthin

Carotenoids are incorporated into chylomicrons for transportation to the blood stream *via* the lymphatics. Once in the blood stream, chylomicrons are degraded to chylomicron remnants through the action of lipoprotein lipase and circulating plasma lipoproteins. The polarity of the carotenoid and therefore their position in the chylomicron will affect the disposition of the carotenoids following chylomicron degradation. Depending on their polarity, and their position in the chylomicron, apolar carotenoids either remain in the chylomicron remnants, which are taken up by the liver for storage or re-release into the blood stream, while polar carotenoids are

exchanged with other lipoprotein particles in the circulation (Bierer *et al.*, 1995; van den Berg, 1999). The delivery of carotenoids to extra-hepatic tissue is reported to occur *via* degradation of lipoproteins by extra-hepatic enzymes (*e.g.*, lipoprotein lipase) and the interaction of lipoprotein particles (*e.g.*, HDL, LDL) with receptors (Boileau *et al.*, 1999). Lutein and zeaxanthin are approximately evenly distributed between HDL and LDL in the circulation (Olson, 1996; Furr and Clark, 1997; Goulinet and Chapman, 1997).

In men and women fed a controlled low-carotenoid diet for approximately 10 and 13 weeks (Rock *et al.*, 1992; Burri *et al.*, 2001), it was reported that in females, the half-lives (t½) for lutein and zeaxanthin were 76 and 38 days, respectively, with a decline in serum concentrations of all carotenoids (including lutein and zeaxanthin) following apparent first-order kinetics (Burri *et al.*, 2001). In males, significant decreases in carotenoid levels (including lutein and zeaxanthin) between Days 2 and 3, and between Days 14 and 15, were possibly indicative of 2 separate pools of carotenoids in the body (*i.e.*, one that is rapidly responsive to changes in carotenoid intake, and one that is more resistant). The mean plasma t½ for lutein and zeaxanthin (combined) in male subjects was calculated to be between 33 and 61 days, respectively (Rock *et al.*, 1992).

Lutein, amongst other carotenoids, is reported to undergo appreciable biliary secretion, based on a study carried out in patients with various hepatobiliary conditions (Leo *et al.*, 1995); however, interference with biliary secretion was not reported to result in plasma retention of carotenoids.

IV.E Toxicological Studies

IV.E.1 Acute Toxicity Studies

The results of acute oral toxicity studies have demonstrated that lutein is not acutely toxic. The oral LD_{50} for a purified plant extract containing 70 to 85% lutein was determined to be >2,000 mg/kg body weight in male and female Hanlbm:WIST rats (JECFA, 2006). In an acute toxicity study, the oral LD_{50} of lutein and lutein esters isolated from marigold was determined to be >4,000 mg/kg body weight in female Wistar rats (Harikumar *et al.*, 2008).

IV.E.2 Repeated-Dose Studies

Repeated-dose studies on lutein from various sources has previously been reviewed to establish the safety of FloraGLO® crystalline lutein for addition to various food products, including infant and toddler foods, as part of the GRAS notification for FloraGLO® crystalline lutein (GRN Notice No. 140 – U.S. FDA, 2004). In studies ranging from 2 weeks to 12 months in duration, the administration of FloraGLO® crystalline lutein *via* the diet or by gavage was not associated with toxicologically-significant adverse effects in mice, rats, dogs, and monkeys at

doses ranging from 9.5 to 773 mg/kg body weight/day (Jenkins *et al.*, 2000; Kim *et al.*, 2000; Pfannkuch *et al.*, 2000, 2001; Dwyer *et al.*, 2001; Kruger *et al.*, 2002; Khachik *et al.*, 2006).

IV.E.3 Reproductive and Developmental Toxicity Studies

In a developmental toxicity study, female Sprague-Dawley rats were orally administered doses of up to 1,118 mg lutein/kg body weight/day on gestation days 6 to 20 with no maternal toxicity or embryotoxicity/teratogenicity reported (Edwards *et al.*, 2002).

IV.E.4 Mutagenicity and Genotoxicity Studies

FloraGLO® crystalline lutein (in beadlet and non-beadlet form) was investigated for potential mutagenicity in the reverse mutation assay (Ames test), conducted with *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102, with and without metabolic activation (Kruger *et al.*, 2002). The dose ranges evaluated were 158 to 5,000 µg/plate for the 10% beadlet formulation, and 15.8 to 500 µg/plate for the non-beadlet tests. No increase in the number of mutant colonies was observed for any of the 5 tester strains after treatment with the beadlet or non-beadlet form of FloraGLO® crystalline lutein, demonstrating that neither formulation is mutagenic in *S. typhimurium* strains. In addition, the lack of mutagenicity/clastogenicity is supported by the results of a number of *in vitro* and *in vivo* studies evaluating other sources of lutein (Yoshikawa *et al.*, 1996; Rauscher *et al.*, 1998; Collins *et al.*, 1998).

IV.F Safety Data in Infants

IV.F.1 Premature Infants

The plasma response in preterm infants (<33 weeks gestational age) fed formulas with added lutein (FloraGLO® lutein, Kemin Health), synthetic lycopene (Lycovit®, BASF), and synthetic β-carotene (Lucarotin®, BASF) was investigated by Abbott Nutrition in a double-blind, randomized, controlled, parallel multicenter clinical trial (Rubin *et al.*, 2011). This study was conducted in accordance with the protocol, Good Clinical Practice (GCP), and the Declaration of Helsinki. Stratified by birth weight (500 to 1,250 g, 1,251 to 1,800 g), gender, and mother's intention to feed human milk, infants were randomized to consume two different carotenoid-supplemented formulas or the corresponding control formulas, depending on the age of the infant.

A preterm infant formula (in-hospital formula) was provided to infants from time of feeding initiation until hospital discharge (between 30 and 35 days of age), and a post-discharge preterm infant formula was provided from hospital discharge until 50 weeks PMA, equivalent to 10 weeks corrected age (between 95 and 100 days of age). The mean concentration of lutein in the in-hospital preterm infant formulas was 211 µg/L, and the mean concentration in the post-discharge preterm formula was 68.7 µg/L. The levels of carotenoids added to the formulas were

selected by Abbott Nutrition based on global human milk carotenoid concentrations (Khachik *et al.*, 1997; Canfield *et al.*, 1997, 2001, 2003; Sommerburg *et al.*, 2000; Gossage *et al.*, 2002) and adjusted for low volume intakes of preterm infants. Infant formulas designed for preterm infants are energy and nutrient dense. These formulas deliver maximal nutrition in a small volume. Nutrient concentrations in these formulas are typically in the range of 2-3 times the concentrations in formulas for term infants. The parameters evaluated in the trial included infant growth (*i.e.*, body weight gain, length gain, and head circumference gain), formula intake, formula tolerance, stool characteristics (*i.e.,* number of stools per day, mean rank stool consistency, predominant stool consistency, and stool color), electroretinography, and adverse events.

In the evaluable group (*i.e.*, the subpopulation of infants who consumed exclusively or nearly exclusively the assigned study formula), plasma lutein concentrations were higher in the carotenoid formula group compared to the control group ($p<0.01$). Similarly, at hospital discharge and at 40 and 50 weeks PMA, plasma concentrations of lutein were higher ($p<0.0001$) in infants fed the carotenoid formulas as compared to infants fed the control formulas. The mean plasma concentrations of lutein in preterm infants consuming the carotenoid formulas at 50 weeks PMA was 77 ± 5.8 µg/L, which is in the range of plasma lutein concentrations of a breast fed reference group (see Table IV.D.1-1).

No significant differences between preterm infants fed the carotenoid formulas or control formulas were observed in infant growth, formula intake, formula tolerance, or stool characteristics, indicating that the carotenoid supplemented preterm formulas were well-tolerated from the time of initial enteral feedings through 50 weeks PMA.

Additionally, the overall incidence of adverse and serious adverse events was similar between the control and carotenoid formula groups and did not differ significantly when adjusted for birth weight. A majority of the most commonly reported AEs were related to the gastrointestinal system (constipation, diarrhea, flatulence, spitting up, or vomiting). Adverse and serious events that were possibly or probably related to the study formulas did not differ between study groups. Study physicians classified all of the serious adverse events as probably not related to the study formula. The incidence of confirmed necrotizing enterocolitis (carotenoid formula 8% and control 5%) or sepsis (carotenoid formula 13% and control 9%) did not differ between groups (Rubin *et al.*, 2011). The authors concluded that preterm infant formula supplemented with carotenoids was safe and well tolerated.

IV.F.2 Term Infants

The safety of FloraGLO® Lutein 20% in Safflower Oil for use in formulas intended for premature infants is further corroborated by several studies conducted in term infants (Mackey *et al.*, 2006,

2008; U.S. FDA, 2007; Bettler *et al.*, 2010; Capeding *et al.*, 2010; Perrone *et al.*, 2010; Romagnoli *et al.*, 2010; Price *et al.*, 2011). The results of these studies are described below.

A multi-center clinical trial to investigate the effects of feeding formulas supplemented with carotenoids was conducted in term infants (Mackey *et al.*, 2006, 2008; Price *et al.*, 2011). The study consisted of 2 phases, the first of which was a double-blind, controlled, parallel-arm study, while the second was a non-randomized, unblinded, controlled study. Infants were 14 to 35 days of age at study enrolment in both phases of the study. The study formulas contained lutein (FloraGLO® Lutein 5% in Corn Oil, Kemin Health), synthetic lycopene (LycoVit® 10%, BASF), and synthetic β-carotene (Lucarotin®, BASF). Parameters evaluated in both phases included measures of infant growth (*i.e.,* body weight, length, and head circumference), formula intake, formula tolerance, stool characteristics (*i.e.,* number of stools per day, mean rank stool consistency, predominant stool consistency, and stool color), and adverse events. The concentrations of lutein in the low-, mid-, and high-dose formulas were 33, 53, and 117 µg/L, respectively.

Formula intake was comparable among the study groups, as were measures of spit-up or vomit associated with feedings and within one hour of feedings, number of stools, stool consistency, and stool colors. Formulas fed in both phases of the study supported normal growth, and there were no significant differences in adverse and serious adverse events observed among the formula groups.

Capeding *et al.* (2010) conducted a prospective, randomized, controlled, double-blind clinical trial in healthy term infants to evaluate infant growth, safety, and blood chemistry in relation to the consumption of infant formula with added lutein (FloraGLO® Lutein 20% in Safflower Oil). Two hundred thirty infants (0 to 14 days of age) were randomized to receive a control formula or a formula with 200 µg lutein/L for a period of 16 weeks. Measures of tolerance, growth, and safety as adverse events were collected over the course of the study period. No differences between groups were found in growth, tolerance or adverse events.

FloraGLO® Lutein 20% in Safflower Oil also was evaluated for safety and tolerance in a prospective, randomized, double-blind clinical trial conducted with healthy term infants (n=63) that were randomized to a control formula (baseline of 20 µg lutein/L), formula with an additional 25 µg lutein/L (low dose) or formula with an additional 200 µg lutein/L (high dose) (U.S. FDA, 2007). Lutein-fortified formula, administered *ad libitum* for 5 weeks, was fortified to account for manufacturing and shelf-life loss, such that the formula with 25 µg lutein/L was fortified with 38 µg lutein/L, and the formula with 200 µg lutein/L was fortified with 300 µg lutein/L. The final lutein content of each test formula was tested by laboratory analysis and was reported to be 20, 47, and 289 µg lutein/L for the 3 formulas, respectively. Infant body weight and weight gain, length and length increase, and head circumference increase were comparable between the

treatment and control groups at the end of the 5-week study period. While a small number of infants were withdrawn from the study, the withdrawals were not considered to be treatment-related. Nineteen adverse events occurred in 15 infants: 10 in the control group, 6 in the low-dose group, and 3 in the high-dose group. None of the adverse events was considered to be serious, all resolved during the study, and none were the cause of discontinuation of formula consumption or removal of the infant from the study.

Additional supportive studies evaluating physiological effects of dietary lutein in preterm and term infants were identified in the literature (Bettler *et al.*, 2010; Perrone *et al.*, 2010; Romagnoli *et al.*, 2010). In a randomized, double-blind, placebo-controlled study, newborn infants were given a supplement that provided 0.28 mg lutein 12 and 36 hours after birth with no adverse effects reported (Perrone *et al.*, 2010). Supplementation with a single dose of 0.5 mg lutein/kg body weight was reported to be well-tolerated in newborn preterm infants, with no gastrointestinal or feeding complications reported (Romagnoli *et al.*, 2010). Similarly, no treatment-related adverse effects were reported in healthy term infants fed a formula fortified with 225 µg lutein/L (FloraGLO® Lutein 20% in Safflower Oil) for a period of 12 weeks (Bettler *et al.*, 2010).

IV.G Summary and Basis for GRAS Conclusion

The GRAS determination for the use of FloraGLO® Lutein 20% in Safflower Oil up to 210 µg /L in formulas designed for premature infants is based on scientific procedures. Data summarized in this notification from lutein intervention studies, dietary intake assessments, and metabolic and toxicological studies provide clear evidence of the safety and suitability of FloraGLO® Lutein 20% in Safflower Oil for use in formulas intended for premature infants.

FloraGLO® Lutein 20% in Safflower Oil is manufactured consistent with cGMP to meet food-grade specifications. Lot samples are routinely assayed to verify compliance with specifications. The manufacturing process of FloraGLO® Lutein 20% in Safflower Oil involves a 3-step process that includes extraction from the marigold plant, followed by purification steps to obtain FloraGLO® crystalline lutein, which is subsequently suspended in food-grade safflower oil to form FloraGLO® Lutein 20% in Safflower Oil.

FloraGLO® crystalline lutein is currently GRAS for use as a food ingredient in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (not including infant formula), milk products, processed fruits and fruit juices, soft candy, and in soups and soup mixes at levels ranging from 0.3 to 3.0 mg/serving (U.S. FDA, 2004). FloraGLO® crystalline lutein is also GRAS for use in medical foods intended as the sole item of the diet at levels less than or equal to 20 mg/day (U.S. FDA,

2004), while FloraGLO® Lutein 20% in Safflower Oil is considered GRAS for use in term infant formula containing 250 µg lutein/L formula (U.S. FDA, 2007).

Data from an intervention study conducted by Abbott Nutrition demonstrated that mean lutein concentrations of 211 µg/L in preterm in-hospital formula and 68.7 µg/L in post-discharge formula resulted in intakes of 32 and 13 µg/kg body weight/day (consumed over a period of 40 to 50 weeks PMA), respectively. These lutein intakes yielded plasma lutein concentrations similar to those observed in term breast fed infants and are consistent with the concentrations reported in cord blood.

The Expert Panel convened independently and collectively to critically evaluate the data and information summarized above and concluded that the intended use of FloraGLO® Lutein 20% in Safflower Oil in formulas intended for premature infants up to 210 µg/L, produced consistently with cGMP and meeting appropriate food-grade specifications, is safe and suitable. Furthermore, the Expert Panel unanimously concluded that the intended use of FloraGLO® Lutein 20% in Safflower Oil is GRAS based on scientific procedures. It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusion.

FloraGLO® Lutein 20% in Safflower Oil is GRAS under the intended conditions of use on the basis of scientific procedures and therefore excluded from the definition of a food additive.

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APPENDIX A

Appendix A

Expert Panel Consensus Statement Concerning the Generally Recognized As Safe (GRAS) Status of FloraGLO® Lutein 20% in Safflower Oil in Formulas Intended for Premature Infants

Expert Panel Report on the Generally Recognized as Safe (GRAS) Status of the Intended use in Formulas Intended for Premature Infants of FloraGLO® Lutein 20% in Safflower Oil

June 17, 2011

INTRODUCTION

At the request of Abbott Nutrition – a division of Abbott Laboratories (Abbott Nutrition), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a food ingredient in formulas for preterm infants (born <37 weeks gestation), FloraGLO® Lutein 20% in Safflower Oil would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Panel consisted of: Joseph F. Borzelleca (Ph.D., Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine), Ronald E. Kleinman (M.D., Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital), and Robert M. Russell (M.D., Friedman School of Nutrition Science, Tufts University).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources. This information was presented in a dossier [Documentation Supporting the Evaluation of FloraGLO® Lutein 20% in Safflower Oil as Generally Recognized as Safe (GRAS) for Use in Formulas Intended for Premature Infants] to the Panel. The Panel additionally evaluated other information deemed appropriate or necessary, including data and information provided by Abbott Nutrition. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use-levels, intake estimates, and a comprehensive assessment of the available scientific literature pertaining to the safety of lutein consumption by infants.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in preterm infant formula described herein, FloraGLO® Lutein 20% in Safflower Oil, meeting appropriate food-grade specifications, and manufactured consistent with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY AND BASIS FOR GRAS

Abbott Nutrition intends to add FloraGLO® Lutein 20% in Safflower Oil to formulas designed for preterm infants. Abbott Nutrition proposes to fortify powder and liquid formulas with FloraGLO® Lutein 20% in Safflower Oil to provide lutein at a maximum level of 210 µg/L. The achieved plasma level concentrations of lutein in preterm infants fed the Abbott formula containing FloraGLO® crystalline lutein, was found to be in the range of those measured in term breastfed infants.

The manufacturing process of FloraGLO® Lutein 20% in Safflower Oil involves a 3-step procedure including extraction from the marigold plant, followed by crystallization and purification steps to obtain FloraGLO® crystalline lutein. FloraGLO® crystalline lutein is subsequently suspended in safflower oil to form FloraGLO® Lutein 20% in Safflower Oil that contains approximately 20% lutein and 1% zeaxanthin by weight. FloraGLO® Lutein 20% in Safflower Oil is manufactured consistent with current Good Manufacturing Practice (cGMP) by Kemin Health, L.C. and DSM to meet appropriate food-grade specifications. Lot samples are routinely assayed to verify compliance with specifications. The starting materials and processing aids used in the manufacturing of FloraGLO® Lutein 20% in Safflower Oil are removed during the purification steps and demonstrated through analytical data to be absent from the final product. The product specifications for FloraGLO® crystalline lutein and FloraGLO® Lutein 20% in Safflower Oil are provided in Appendix A.

FloraGLO® crystalline lutein is currently GRAS for use as a food ingredient in a number of food and beverage products including 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 (not including infant formula), milk products, processed fruits and fruit juices, soft candy, and in soups and soup mixes at levels ranging from 0.3 to 3.0 mg/serving, and for use in medical foods intended as the sole item of the diet at levels less than or equal to 20 mg/day (GRN No. 140 - U.S. FDA, 2004). In addition, FloraGLO® Lutein 20% in Safflower Oil is GRAS for use in term infant formula at levels up to 250 µg lutein/L formula (GRN No. 221 - U.S. FDA, 2007).

Overall, results from the acute or repeated-dose animal studies provide evidence that lutein is of a low order of toxicity. The administration of lutein *via* the diet or by gavage was not associated with toxicologically-significant adverse effects in mice, rats, dogs, and monkeys (Jenkins *et al.*, 2000; Kim *et al.*, 2000; Pfannkuch *et al.*, 2000, 2001; Dwyer *et al.*, 2001; Kruger *et al.*, 2002; Khachik *et al.*, 2006). Lutein was not associated with any reproductive toxicity in a study conducted with rats at a dose of up to 1,000 mg/kg body weight/day in the diet (Edwards *et al.*, 2002). *In vitro* assays with lutein conducted in bacterial and mammalian cells indicated no mutagenic or genotoxic activity (Yoshikawa *et al.*, 1996; Rauscher *et al.*, 1998; Collins *et al.*, 1998; Kruger *et al.*, 2002).

The results of numerous intervention studies indicate that lutein and zeaxanthin are effectively absorbed when added to fortified formulas, and that lutein and zeaxanthin from FloraGLO® Lutein 20% in Safflower Oil do not adversely affect carotenoid balance under the intended conditions of use. To determine the effects of consuming a formula containing lutein on growth and tolerability, Abbott Nutrition conducted 2 human studies in preterm (Rubin *et al.*, 2011) and term (Mackey *et al.*, 2008, Price *et al.*, 2011) infants with formulas supplemented with lutein and other carotenoids (lycopene and β-carotene) at levels similar to the proposed use-levels. Data from the intervention studies conducted by Abbott Nutrition demonstrated that mean lutein concentrations of 211 µg/L in preterm in-hospital formula (Similac Special Care) and 69 µg/L in post-discharge formula (Similac NeoSure), resulted in intakes of 32 and 13 µg/kg body weight/day (consumed over a period of 40 to 50 weeks post-menstrual age), respectively, and were well-tolerated with resultant plasma lutein concentrations similar to those observed in human milk-fed term infants (Rubin *et al.*, 2011). Oral exposure to lutein at levels up to 117 µg/L in term infant formula, for up to 56 days resulted in a mean intake of 138 µg lutein/day, was well-tolerated, and did not cause in any adverse health effects in healthy term infants (Mackey *et al.*, 2008, Price *et al.*, 2011). Results from these intervention studies showed no treatment-related adverse effects from feeding formulas supplemented with lutein on infant growth parameters, formula intake, formula tolerance, or stool characteristics in preterm and term infants. The safety of lutein from FloraGLO® Lutein 20% in Safflower Oil for use in preterm infant formulas is further substantiated by other corroborative safety and efficacy studies conducted in healthy term infants (Bettler *et al.*, 2010; Capeding *et al.*, 2010; Perrone *et al.*, 2010; Romagnoli *et al.*, 2010).

The safety of lutein from FloraGLO® Lutein 20% in Safflower Oil is based on: a) historical consumption of lutein as a common component of the typical human diet, b) the absorption, distribution, metabolism and excretion of lutein, c) published safety studies in animals models, and d) studies conducted in preterm infants, demonstrating that consumption of lutein-supplemented formulas by preterm infants resulted in plasma lutein concentrations similar to those in infants consuming human milk exclusively.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that the intended use of FloraGLO® Lutein 20% in Safflower Oil in preterm infant formulas, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of FloraGLO® Lutein 20% in Safflower Oil as Generally Recognized as Safe (GRAS) for Use in Formulas Intended for Premature Infants], is safe and suitable.

We further conclude that the intended use of FloraGLO® Lutein 20% in Safflower Oil in preterm infant formulas, manufactured consistent with cGMP and meeting appropriate food-grade specifications presented in the supporting dossier, is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

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