ORIGINAL SUBMISSION

NutraSource, Inc.
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OFFICE OF
FOOD ADDITIVE SAFETY

GRN 000542

September 5, 2014

Dr. Antonia Mattia
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5 100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS notice for Lutein

Dear Dr. Antonia Mattia:

On behalf of INNOBIO® Limited, we are submitting for FDA review a GRAS notification for lutein. The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance. We believe that this determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

We enclose an original and two copies of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

9/5/2014

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for InnoBio

enclosure

GRAS EXEMPTION CLAIM for INNOBIO® LUTEIN

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336;
Susanscho1@yahoo.com

GRAS EXEMPTION CLAIM

A. Lutein: Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 5 170.36(1)

INNOBIO® Limited (INNOBIO®; the notifier) has determined that its lutein (crystalline, oil suspension, powder, and beadlet forms) are 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 described in the following sections. Since these procedures specify the conditions of its intended use in food, the use of lutein is exempt from the requirement of premarket approval.

Signed

Susan Cho Date:
Agent for
INNOBIO® Limited (INNOBIO®)

B. Notifier's Name and Address

Notifier's name: INNOBIO® Limited (INNOBIO®)
Phone number: 86-411-87406671
Fax number: 86-411-87406801
Physical address: No.49, DDA, Dalian, Liaoning, 116600, China
E mail address: sunx@INNOBIO.cn

C. Name of GRAS Substance

The common name of the notified substance is Lutein. The trade names are:
INNOBIO® Crystalline Lutein,
INNOBIO® Lutein 20% Oil Suspension,
INNOBIO® Lutein 20% Cold Water Dispersible (CWD) Powder,
INNOBIO® Lutein 10% CWD Powder,
INNOBIO® Lutein 10% Beadlets,
INNOBIO® Lutein 5% Beadlets, and
INNOBIO® Lutein 5% Cold Water Soluble (CWS) Powder

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9/4/2014

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INNOBIO® Lutein 5% Cold Water Soluble (CWS) Powder

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D. Product Description

Lutein and zeaxanthin belong to the class of plant compounds called carotenoids, and are present in a wide variety of fruits and vegetables. Interestingly, while other tissues contain a mixture of other carotenoids, the macular pigment in the human retina contains only lutein and zeaxanthin, often referred as xanthophylls or macular pigment. Scientific evidence shows that supplementing diets with lutein and zeaxanthin daily keeps eyes healthy, improves visual performance, and helps reduce the risk of certain eye conditions. The human body does not produce the dietary zeaxanthin and lutein it needs daily, so it is essential to get these nutrients through diet and/or supplementation. Unlike β-carotene, these two carotenoids do not have vitamin A activity. While there is no RDA for lutein and zeaxanthin, intakes of approximately 6-10 mg/day have been associated with a decreased risk of age related macular degeneration (AMD).

The current intakes of lutein and zeaxanthin among adults 50 years of age falls well below this level, with an intake range of lutein at 0.6-2.4 mg/day. Only men in the 99th percentile of lutein/zeaxanthin intake and women in the 95th percentile meet the dietary intakes that have been associated with decreased risk of AMD. Most Americans consume only 10 percent of the zeaxanthin and lutein needed each day to protect their eyes and keep them healthy.

E. Applicable Conditions for Use of the Notified Substance

E.1. Current Regulatory Status and Background Dietary Intake of Lutein

The FDA has received seven GRAS Notices related to food uses of lutein and lutein esters: 3 separate GRAS notifications on crystalline lutein to the FDA for general food applications (GRN 140; FDA, 2004; GRN 291, FDA, 2009; GRN 385, FDA, 2011), 2 notices for lutein esters for general food applications (lutein dipalmitate-GRN 110, FDA, 2003; lutein diacetate- GRN 432, FDA, 2012) and 2 notices on suspended lutein in oil for infant formula applications (GRN 221, FDA, 2007; GRN 390, FDA, 2012). In these GRAS notices, toxicity-related studies on lutein from the literature were presented which support the safety in use of lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of lutein for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that lutein intake up to 20-22 mg/person/day is safe. In addition, The Panel on Food Additives and Nutrient Sources added to Food of the European Food Safety Authority (EFSA, 2010) has established an ADI of 1 mg/kg body weight (BW)/day or 60 mg/person/day for a 60 kg adult, while Joint FAO/WHO Expert Committee on Food Additives (JECFA) established ADI for lutein from *Tagetes erecta* L. and synthetic zeaxanthin) of up to 2 mg/kg BW/day or up to 120 mg/person/day based on animal and human studies (JECFA, 2006).

Intake from regular diets is expected to be less than 2.4 mg/day.

E.2. Intended Use of Lutein and Levels of Use in Foods

As INNOBIO® crystalline lutein represents the concentrated form with ≥70% lutein and ≥4.0% zeaxanthin, Table 1 represents the quantity of lutein when used as such, or the quantity present in a standardized diluted form using food grade/GRAS ingredients.

The intended use of INNOBIO® Lutein (crystalline, suspended oil, powder, and beadlets) is expanded from food products listed in the GRN 000140 by adding protein bars, low sodium tortillas, and vitamin water. INNOBIO® Lutein is intended for use as a food ingredient (a nutrient) in the following food categories: baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute 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 at use levels of 0.3 to 3 mg lutein/serving (reference amounts customarily consumed, 21CFR 101.12). INNOBIO® Lutein will not be added to food categories that come under USDA jurisdiction.

E.3. Estimated Dietary Intakes (EDIs) of Lutein Based upon Intended Food Uses

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2009-2010), estimates of 2-day average intakes of lutein were calculated from the food code list and the survey database of diet recalls. The mean intakes of lutein under the intended use were estimated to be 8.7 mg/person/day for all population and 8.9 mg/person/day for all-users, while the 90th percentile intakes were determined as 16.5 and 16.7 mg/person/day (or 0.289 and 0.251 mg/kg BW/day), respectively. These EDIs are based on the assumption that 100% of the products listed in GRN 140 and newly added food categories (1.6 mg/person/day) will be used at the maximum intended use levels. Intake from regular diets is expected to be less than 2.4 mg/day. Estimated cumulative exposure (intakes from regular diets, and expanded list of food categories) is estimated to be no more than 19.1 mg/person/day.

As reported in GRN 140, the current 90th percentile intake of zeaxanthin from specified foods was estimated to be 1.2 mg/person/day. The contribution of newly added food categories is estimated to be 0.079 mg/person/day at the 90the percentile of all-users. Even if 100% of the products listed in GRN 140 (1.2 mg/person/day) and newly added food categories are used at the maximum intended use levels, the 90th percentile intake of zeaxanthin from all GRAS-proposed use categories by users of one or more foods are 1.3 mg/person/day for all-users.

Table 1. Food Categories and Intended Use Levels of Lutein in the United States.

Food Category	Proposed Food	Use Levels				
		Lutein, mg/RACC[1,2]	INNOBIO® Crystalline Lutein (≥70%)	20% lutein, mg/RACC	10% lutein, mg/RACC	5% lutein, mg/RACC
Baked Goods and Baking Mixes	**Protein bars,** cereal bars, and energy bars	2.0	2.86	10.0	20.0	40.0
	Crackers and crisp-breads	2.0	2.86	10.0	20.0	40.0
	Tortillas, low sodium	2.0	2.86	10.0	20.0	40.0
Beverages and Beverage Bases	**Vitamin water**	2.0	2.86	10.0	20.0	40.0
	Bottled water	0.5	0.72	2.5	5.0	10.0
	Carbonated beverages; Meal replacements	2.0	2.86	10.0	20.0	40.0
	Tea, ready-to-drink	0.6	0.86	3.0	6.0	12.0
Breakfast Cereals	Instant and regular hot cereals	2.0	2.86	10.0	20.0	40.0
	Ready-to-eat cereals	2.0	2.86	10.0	20.0	40.0
Chewing Gum	Chewing gum	1.0	1.43	5.0	10.0	20.0
Dairy Product Analogs	Imitation milks	2.0	2.86	10.0	20.0	40.0
	Soy milks	1.5	2.14	7.5	15.0	
Egg Products	Liquid, frozen or dried egg substitutes	2.0	2.86	10.0	20.0	40.0
Fats and Oils	Margarine-like spreads, salad dressings	1.5	2.14	7.5	15.0	30.0
Frozen Dairy Desserts and Mixes	Frozen yogurt	1.0	1.43	5.0	10.0	20.0
Gravies and Sauces	Tomato based sauces	0.3	0.43	1.5	3.0	6.0
Hard Candy	Hard candy	1.0	1.43	5.0	10.0	20.0
Infant and Toddler Foods	Junior, strained and toddler-type baby foods	1.0	1.43	5.0	10.0	20.0
Milk Products	Dry milk flavored milk and milk drinks	3.0	4.29	15.0	30.0	60.0
	Fermented milk beverages	0.6	0.86	3.0	6.0	12.0
	Yogurt	3.0	4.29	15.0	30.0	60.0
	Milk based meal replacement	2.0	2.86	10.0	20.0	40.0
Processed Fruits and Fruit Juices	Energy, sport and isotonic drinks	2.0	2.86	10.0	20.0	40.0
	Fruit-flavored drinks, fruit juice, nectars	2.0	2.86	10.0	20.0	40.0
	Vegetable Juice	2.0	2.86	10.0	20.0	40.0
Soft Candy	Chewy and nougat candy; Fruit snacks	1.0	1.43	5.0	10.0	20.0
Soup and soup mixes	Canned soup	0.6	0.86	3.0	6.0	12.0

[1]Use levels are the same as those described in GRN 140 except 3 newly added items. [2]RACC Reference amounts customarily consumed per eating occasion (21 CFR § 101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC.

E.4. Basis for the GRAS Determination
As noted above, the FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. For each of these notices, after the FDA's review, the agency issued letters indicating that it had no questions concerning the safety of lutein and its esters. As the lutein in this GRAS determination is similar in specifications compared to the other luteins in the FDA GRAS Notices, it is recognized that the information and data in the other GRAS Notices is pertinent to the safety of the lutein in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 110, 140, 221, 291, 385, 390, and 432. Additionally, this notice discusses additional studies that have been published since the FDA's last reviews of 2011-2012. The subject of present GRAS assessment is lutein.

Pursuant to 21 CFR 9170.35, INNOBIO® Lutein (crystalline, suspended oil, powder, and beadlets) have been determined to be GRAS on the basis of scientific procedures. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of Lutein as a component of food. Expert Panel members, Susan S. Cho, Ph.D., Robert L. Martin, Ph.D., Madhusudan Soni, Ph.D., have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that lutein, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications described herein, is safe under its intended conditions of use. The Expert Panel further unanimously concludes that these uses of lutein are GRAS based on scientific procedures, and that other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

F. Availability of Information
The detailed data and information that serve as a basis for this GRAS determination will be provided to the U. S. FDA upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of NutraSource:
6309 Morning Dew Ct., Clarksville, MD 21029, USA.

G. Basis of GRAS determination: Through scientific procedures.

H. Review and copying statement: The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of notifier, or will be sent to FDA upon request.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF INNOBIO® LUTEIN AS A FOOD INGREDIENT

Prepared for INNOBIO®

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
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1. INTRODUCTION

The undersigned, an independent panel of recognized experts (herein after referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by NutraSource, Inc., at the request of INNOBIO® Limited (INNOBIO®), to determine the Generally Recognized As Safe (GRAS) status of its lutein as a food ingredient (a nutrient) [21CFR 170.3(o)(20)] in selected food products (baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute products; fats and oils; frozen dairy desserts and mixes; gravies and sauces; hard candy; infant and toddler foods; milk products; processed fruits and fruit juices; soft candy; soups and soup mixes) at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on lutein was conducted and made available to the Expert Panel members. The Expert Panel members independently and critically evaluated materials submitted by INNOBIO® and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel unanimously agreed to the decision described herein.

II. INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Common or Trade Name: Lutein produced by INNOBIO®
The trade names are:
INNOBIO® Crystalline Lutein,
INNOBIO® Lutein 20% Oil Suspension,
INNOBIO® Lutein 20% Cold Water Dispersible (CWD) Powder,
INNOBIO® Lutein 10% CWD Powder,
INNOBIO® Lutein 10% Beadlets,
INNOBIO® Lutein 5% Beadlets, and
INNOBIO® Lutein 5% Cold Water Soluble (CWS) Powder

II.B. Background

Lutein and zeaxanthin belong to the class of plant compounds called carotenoids, and are present in a wide variety of fruits and vegetables (Sommerberg et al., 1998). Interestingly, while other tissues contain a mixture of other carotenoids, the macular pigment in the human retina contains only lutein and zeaxanthin, often referred as xanthophylls or macular pigment (MP; Sommerberg et al., 1998). The lutein and zeaxanthin concentration in the eye can be 500-fold higher than in other tissues (Rasmussen and Johnson, 2013).

Lutein is an important nutrient with a recognized role in providing protection of the macula from photo-oxidative stress and evidence indicates that lutein protects the retina from the onset of age related macular degeneration (AMD; Bone and Landrum, 2010; Gao et al., 2011; Landrum and Bone, 2001; Mares et al., 2011). There is accumulating evidence to suggest that lutein and zeaxanthin intakes are positively related to macular pigment optical density (MPOD)—an increased concentration of retinal carotenoids in theMP. In fact, elevated MPOD has been directly associated with foveal function in patients with maculopathy, to preserved visual sensitivity in healthy elderly individuals and to a decreased risk of AMD (Mares-Perlman et al., 2002). AMD is the leading cause of irreversible vision loss in the elderly population.

The human body does not produce the dietary zeaxanthin and lutein it needs daily, so it is essential to get these nutrients through diet and/or supplementation. Unlike β-carotene, these two carotenoids do not have vitamin A activity (IOM, 2001). Of the 20–30 carotenoids found in human blood and tissues, only lutein and zeaxanthin are found in the lens and retina (Yeum et al., 1999). Lutein and zeaxanthin are concentrated in the macula or central region of the retina, and are referred to as macular pigment. In addition to their role as antioxidants, lutein and zeaxanthin are believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching reactive oxygen species (Krinksy, 2002). While there is no RDA for lutein and zeaxanthin, intakes of approximately 6-10 mg/day have been associated with a decreased risk of AMD (Seddon et al., 1994).

The current intakes of lutein and zeaxanthin among adults 50 years of age falls well below this level, with an intake range of lutein at 0.6-2.4 mg/day. Only men in the 99th percentile of lutein/zeaxanthin intake and women in the 95th percentile meet the dietary intakes that have been associated with decreased risk of AMD (Rasmussen and Johnson, 2013). Most Americans consume only 10 percent of the zeaxanthin and lutein needed each day to protect their eyes and keep them healthy.

Zeaxanthin, like lutein, acts as a protective antioxidant in the eye and is proven to promote eye health (Rasmussen and Johnson, 2013). Scientific evidence shows that supplementing the diets with lutein and zeaxanthin daily keeps eyes healthy, improves visual performance, and helps reduce the risk of certain eye conditions.

II.C. Chemistry, Physico-chemical Properties, and Structure

Table 1. Chemical and Physical Characteristics of Lutein and Zeaxanthin (free form)

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical Names	Xanthophyll; beta, e-carotene-3,3'-diol (3*R*,3′*R*,6′*R*); *all-trans*-(+)-xanthophyll; alltrans-lutein	Beta 3, 6-carotene-3,3'-diol; all-trans-I3-carotene-3,3'-diol; (3R,3' R)-dihydroxy-beta-carotene;
Empirical formula	$C_{40}H_{56}0_2$	$C_{40}H_{56}0_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting Point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

*Based on information from INNOBIO® and other publicly available sources

Figure 1. Chemical Structure of Lutein

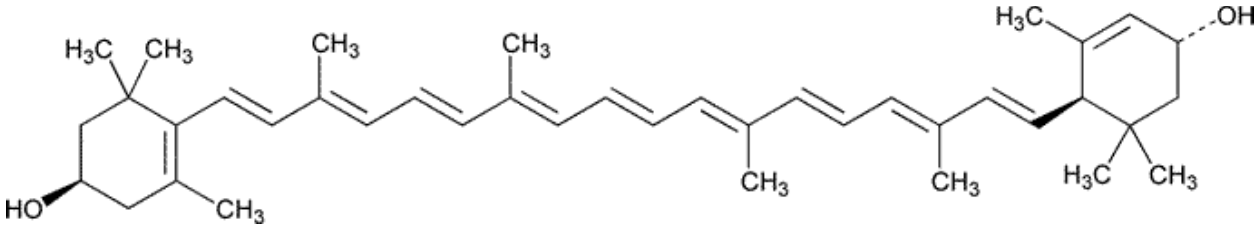
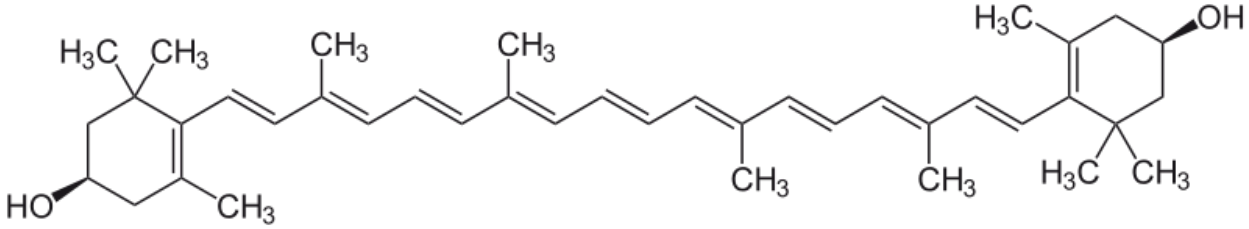


Figure 2. Chemical Structure of Zeaxanthin



II.D. Manufacturing Process of INNOBIO® Lutein Preparations

INNOBIO® Lutein preparations are naturally derived from marigold flower (*Tagetes erecta*) and consist of all trans-free lutein as the predominant constituent with a small amount of zeaxanthin. Manufacturing processes of INNOBIO® Lutein products are described as follows.

General Description of INNOBIO®'s Manufacturing Process

Current Good Manufacturing Practice (cGMP), which includes Hazard Analysis and Critical Control Point (HACCP) risk analysis as well as hygiene and transport standards, is applied throughout each manufacturing step of INNOBIO® products; continuous monitoring is used for quality control. Sampling schemes and analysis schedules are applied to raw materials, intermediate products and final products. Pesticide residues, chemical contaminants and residual solvents are measured at different stages to make sure the finished product meets the required specifications. State-of-the-art facilities and electronic monitoring systems enable precise process control at all steps. All processing aids, such as solvents and water, buffer salts and other ingredients used in the manufacturing process are food grades.

Manufacturing Process of INNOBIO® Crystalline Lutein

The manufacturing process of INNOBIO® Crystalline Lutein starts with fresh marigold flowers that are dried in a hot air dryer to prepare meal. Marigold oleoresin is obtained from the marigold meal by hexane extraction, which is unique and advanced in a few ways. First, absorbents such as activated carbon, clay and diatomite are added to the hexane extract to remove contaminants such as heavy metals and pesticide residues. Second, microfiltration is applied to eliminate impurities introduced from hexane extraction, including black specks, plant fibers, pollen, etc. Finally, marigold oleoresin is obtained by reduced pressure distillation to avoid degradation of carotenoids. The oleoresin is then subjected to saponification as described below to obtain the crystalline lutein with defined qualities. It is worth noting that only alcohol solvents (ethanol,

isopropyl alcohol, or mixture of both) but no other organic solvents are used in the saponification process to ensure the product safety.

The marigold oleoresin is mixed with the alcohol solvent and excess solid sodium hydroxide under controlled heating (50-60℃) and stirring. The resultant solution is then diluted with water to precipitate more crystalline lutein at 30-50℃. The residual sodium hydroxide is then neutralized with the acetic acid solution to make the saponification mixture slightly basic (pH 7.5-9.0). The wet cake of crystalline lutein is then obtained by filtration and purified by washing with the alcohol/water solution (V/V 40-70%) and alcohol. After mixing with a small quantity of antioxidants, the wet crystalline lutein is dried at 50-90℃ under vacuum to remove the alcohol solvent and water. At each filtration step, sodium fatty acids, alcohol soluble impurities and residual sodium hydroxide are removed from the wet cake of crystalline lutein. Finally, crystalline lutein is sieved with the 40 mesh and then packaged in a 5kg net aluminum foil bag under vacuum to get INNOBIO® Crystalline Lutein.

INNOBIO's innovative technology is environment friendly for crystalline lutein preparation. Contaminant removal increases the quality of crystalline lutein, use of only alcohol solvents guarantees the product safety, and the environment-friendly neutralization by acetic acid allows waste water to reach discharge standards.

Manufacturing Process of INNOBIO® Lutein 20% Oil Suspension

INNOBIO® Lutein 20% Oil Suspension is made by suspending the crystalline lutein in vegetable oils (e.g., such as sunflower oil, safflower oil or corn oil) to a final concentration of at least 20% of all trans-free lutein. Lutein oil suspension is milled to the D50 diameter of 80μm or less, then packaged in the 20kg net aluminum drum under nitrogen.

Manufacturing Process of INNOBIO® Lutein 5% CWS Powder, Lutein10% CWD Powder, and Lutein 20% CWD Powder

INNOBIO® Lutein 5% CWS powder is an orange red, free flowing powder microencapsulated with food grade ingredients (mixture of modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch) and contains at least 5% of all-trans free lutein. INNOBIO® Lutein 10% and 20% CWD powder are orange red, free flowing powders microencapsulated with food grade ingredients (mixture of modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch) and contain at least 10% and 20% of all-trans free lutein respectively. However, the concentrations of modified starch and gum arabic vary: 20-30% for CWS and >30% for CWD preparations. The CWS and the CWD powders have different applications but share an identical manufacturing process. The process starts from admixing modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch with water at the temperature range of 40-80℃for complete dissolution. Crystalline lutein is then added to the mixture which is stirred to form an emulsion. The emulsion of lutein is milled and then spray dried at the temperature range of 140-200℃ to form powder. After sieving and mixing, the lutein powder is packaged in a 5kg net aluminum foil bag under nitrogen.

Manufacturing Process of INNOBIO® Lutein 5% Beadlets and Lutein 10% Beadlets
INNOBIO® Lutein 5% Beadlets and 10% Beadlets are reddish brown free flowing beadlets microencapsulated with food grade ingredients (mixture of gelatin, sucrose, modified starch, gum arabic, tocopherols, ascorbyl palmitate, and corn starch) and contain at least 5% and 10% of all-trans free lutein respectively. The production starts from admixing coating materials, the emulsifier and the antioxidant with water at a temperature range of 40-80℃ for complete dissolution. Crystalline lutein is then added to the mixture and stirred to form an emulsion. The emulsion of lutein is milled and then spray dried at a temperature range of 80-180℃ to form powder, which goes through fluidized corn starch to form lutein beadlets. The beadlets are then dried at the temperature range of 10-60℃ to remove the residual water. After sieving, the lutein beadlets are packaged in a 5kg net aluminum foil bag under nitrogen.

II. E. Specifications of Lutein
Tables 2-1 - 2-7 list specifications of various lutein preparations.

Table 2-1. Specifications for INNOBIO® Crystalline Lutein

Parameter		Method
Appearance	Reddish-orange powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27
Lutein, %	70.0 Min.	USP 32/NF 27
Zeaxanthin, %	4.0 Min.	USP 32/NF 27
Moisture, %	1.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-2. Specifications for INNOBIO® Lutein 20% Oil Suspension

Parameter		Method
Appearance	Orange-red oil suspension	Visual
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27
Lutein, %	20.0 Min.	USP 32/NF 27
Zeaxanthin, %	1.0 Min.	USP 32/NF 27
Moisture, %	1.0 Max.	AOCS Td 2-64
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-3. Specifications for INNOBIO® Lutein 20% CWD Powder

Parameter		Method
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27
Lutein, %	20.0 Min.	USP 32/NF 27
Zeaxanthin, %	1.0 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467

Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-4. Specifications for INNOBIO® Lutein 10% CWD Powder

Parameter		Method
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27
Lutein, %	10.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.5 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-5. Specifications for INNOBIO® Lutein 10% Beadlets

Parameter		Method
Appearance	Orange-red, free-flowing beadlets	Visual
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27
Lutein, %	10.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.5 Min.	USP 32/NF 27
Moisture, %	7.0 Max.	USP 731
Particle Size		
-through sieve No.20	100	USP 786
-through sieve No.40	85.0 Min.	USP 786
-through sieve No.100	15.0 Max.	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-6. Specifications for Lutein 5% Beadlets

Parameter		
Appearance	Orange-red, free-flowing beadlets	Visual
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27
Lutein, %	5.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.25 Min.	USP 32/NF 27
Moisture, %	7.0 Max.	USP 731
Particle Size		
-through sieve No.20	100	USP 786
-through sieve No.40	85.0 Min.	USP 786
-through sieve No.100	15.0 Max.	USP 786
Heavy metals		

Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-7. Specifications for INNOBIO® Lutein 5% CWS Powder

Parameter		
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27
Lutein, %	5.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.25 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size	95% through 40 mesh	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Ethanol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

III. INTENDED USES AND EXPOSURE ESTIMATES

III.A. Food Sources of Lutein and Zeaxanthin

Lutein and zeaxanthin are carotenoids found in high quantities in green leafy vegetables. Other foods (Table 3) that contribute a significant level of dietary lutein include egg yolk and corn. Similarly, considerable dietary zeaxanthin is provided by egg yolks, corn, orange peppers, orange juice, oranges and honeydew melons (Sommerberg et al., 1998). The daily intake for lutein and zeaxanthin is on average lower than that of β-carotene and lycopene, but levels of approximately 0.6-2.4 mg/day are attained with a diet rich in fruits and vegetables.

Table 3. Fruit and Vegetable Sources of Lutein and Zeaxanthin

Fruit of Vegetable	Lutein + Zeaxanthin	Lutein	Zeaxanthin
Egg Yolk	89	54	35
Corn	85	60	25
Kiwi	54	54	0
Red Seedless Grapes	53	43	10
Zxucchini squash	52	47	5
Pumpkin	49	49	0
Spinach	47	47	0
Orange Pepper	45	8	37
Yellow Squash	44	44	0
Cucumber	42	38	4
Pea	41	41	0
Green Pepper	39	36	3
Red Grape	37	33	4
Butternut Squash	37	37	0
Orange Juice	35	15	20
Honeydew	35	17	18
Celery (stalks, leaves)	34	32	2
Green Grapes	32	25	7
Brussels Sprouts	29	27	2
Scallions	30	27	3
Green Beans	25	22	3
Orange	22	7	15
Broccoli	22	22	0
Apple (Red Delicious)	20	19	1
Mango	18	2	16
Green Lettuce	15	15	0
Tomato Juice	13	11	2
Peach	13	5	8
Yellow Pepper	12	12	0
Nectarine	12	6	6
Red Pepper	7	7	0

The content of the major carotenoids are given in mole %. Source: Sommerburg et al., 1998.

III.B. Intended technical effects

INNOBIO® Lutein (crystalline, powder, beadlets, or oil suspension) can be used as a nutrient as a source of lutein at concentrations consistent with cGMP.

Use of lutein preparations in the above described food categories may also incidentally contribute its own color to the product. Its intended nutritional use would thus fall outside the definition of "color additive," in accordance with 21 CFR 70.3(f), "Food ingredients ...which contribute their own natural color when mixed with other foods are not regarded as *color additives...."*

III.C. Intended use

As INNOBIO® Crystalline Lutein represents the concentrated form with ≥70% lutein and ≥4.0% zeaxanthin. Table 4 represents the quantity of lutein when used as such, or the quantity present in a standardized diluted form using food grade/GRAS ingredients.

INNOBIO® lutein preparations are intended for use as a food ingredient in the following food categories: baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg substitute 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 at use levels of 0.3 to 3 mg lutein/serving (reference amounts customarily consumed, 21CFR 101.12). A summary of use levels and food categories for lutein is presented in Table 4.
INNOBIO® Lutein will not be added to food categories that come under USDA jurisdiction.

The INNOBIO® intended uses of lutein preparation are expanded from food products listed in the GRN 000140 (FDA, 2004) by adding protein bars, low sodium tortillas, and vitamin water at a level of 2 mg lutein/Reference Amounts Customarily Consumed per eating occasion (RACC).

Table 4. Food Categories and Intended Use Levels of Lutein in the U.S.

Food Category	Proposed Food	Use Levels				
		Lutein, mg/RACC[1,2]	INNOBIO crystalline lutein (≥70%)	20% lutein, mg/RACC	10% lutein, mg/RACC	5% lutein, mg/RACC
Baked Goods and Baking Mixes	**Protein bars,** cereal bars, and energy bars	2.0	2.86	10.0	20.0	40.0
	crackers and crisp-breads	2.0	2.86	10.0	20.0	40.0
	Tortillas, low sodium	2.0	2.86	10.0	20.0	40.0
Beverages and Beverage Bases	**Vitamin water**	2.0	2.86	10.0	20.0	40.0
	Bottled water	0.5	0.72	2.5	5.0	10.0
	Carbonated beverages; Meal replacements	2.0	2.86	10.0	20.0	40.0
	Tea, ready-to-drink	0.6	0.86	3.0	6.0	12.0
Breakfast Cereals	Instant and regular hot cereals	2.0	2.86	10.0	20.0	40.0
	Ready-to-eat cereals	2.0	2.86	10.0	20.0	40.0
Chewing Gum	Chewing gum	1.0	1.43	5.0	10.0	20.0
Dairy Product Analogs	Imitation milks	2.0	2.86	10.0	20.0	40.0
	Soy milks	1.5	2.14	7.5	15.0	
Egg Substitutes	Liquid, frozen or dried egg substitutes	2.0	2.86	10.0	20.0	40.0
Fats and Oils	Margarine-like spreads, salad dressings	1.5	2.14	7.5	15.0	30.0
Frozen Dairy Desserts and Mixes	Frozen yogurt	1.0	1.43	5.0	10.0	20.0
Gravies and Sauces	Tomato based sauces	0.3	0.43	1.5	3.0	6.0
Hard Candy	Hard candy	1.0	1.43	5.0	10.0	20.0
Infant and Toddler Foods	Junior, strained and toddler-type baby foods	1.0	1.43	5.0	10.0	20.0
Milk Products	Dry milk flavored milk and milk drinks	3.0	4.29	15.0	30.0	60.0
	Fermented milk beverages	0.6	0.86	3.0	6.0	12.0
	Yogurt	3.0	4.29	15.0	30.0	60.0
	Milk based meal replacement	2.0	2.86	10.0	20.0	40.0
Processed Fruits and Fruit Juices	Energy, sport and isotonic drinks	2.0	2.86	10.0	20.0	40.0
	Fruit-flavored drinks, fruit juice, nectars	2.0	2.86	10.0	20.0	40.0
	Vegetable Juice	2.0	2.86	10.0	20.0	40.0
Soft Candy	Chewy and nougat candy; Fruit snacks	1.0	1.43	5.0	10.0	20.0
Soup and soup mixes	Canned soup	0.6	0.86	3.0	6.0	12.0

[1]Use levels are the same as those described in GRN 140 except 3 newly added items. [2]RACC Reference amounts customarily consumed per eating occasion (21 CFR § 101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC.

III.D. Exposure estimates

Estimated Daily Intakes (EDIs) before the intended use
The European Food Safety Authority (EFSA, 2010) estimated that an average intake for adults from the regular diet amounts to 0.01- 0.04 mg/kg BW/day, corresponding to 0.6-2.4 mg/person/day for a 60 kg adult.

EDIs under the intended use
Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2009-2010) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics and the Nutrition Coordinating Center, estimates of 2-day average intakes of lutein were calculated from the food code list and the survey database of diet recalls. The 2009-2010 NHANES provides the most current food consumption data available for the non-institutionalized individuals in the U.S. Table 5 shows EDIs under the intended use.

Table 5. EDIs under the intended use

Population	Sample N	EDIs				
		Unit	Mean	SE	P90	SE
All population, aged 2+y	9,042	mg	8.70	0.120	16.53	0.256
All population, aged 2+y	8,971	mg/kg bw	0.143	0.0027	0.289	0.0064
All-users, aged 2+y	8,848	mg	8.88	0.113	16.67	0.251
All-users, aged 2+y	8,780	mg/kg bw	0.146	0.0026	0.291	0.0062

P90=90th percentile; SE=standard error.

The mean intakes of lutein under the intended uses were estimated to be 8.7 mg/person/day for all population and 8.9 mg/person/day for all-users, while the 90th percentile intakes were determined as 16.5 and 16.7 mg/person/day (or 0.289 and 0.291 mg/kg BW/day), respectively. These EDIs are based on the assumption that 100% of the products listed in GRN 140 and newly added food categories (1.6 mg/person/day) are used at the maximum intended use levels. Intake from regular diets is expected to be less than 2.4 mg/day. Estimated cumulative exposure (intakes from regular diets, and expanded list of food categories) is estimated to be no more than 19.1 mg/person/day.

As reported in GRN 140, the current 90th percentile intake of zeaxanthin from specified foods were estimated to be 1.2 mg/person/day. The contribution of newly added food categories is estimated to be 0.079 mg/person/day at the 90th percentile of all-users. Even if 100% of the products listed in GRN 140 (1.2 mg/person/day) and newly added food categories are used at the maximum intended use levels, the 90th percentile intake of zeaxanthin from all GRAS-proposed use categories by users of one or more foods is 1.3 mg/person/day for all-users.

However, these estimates are highly optimistic since all foods under the intended uses will not be used at the maximum levels. For example, less than 1% of bottled water and carbonated beverages contain lutein in the market place, although EDI calculations have assumed that 100% of these products will be used at the maximum intended use levels.

These exposure estimates (EDI under the untended uses, and/or cumulative exposure) are far below the Acceptable Daily Intake (ADI) of up to 1-2 mg/kg BW/day or 60-120 mg/person/day established by The Panel on Food Additives and Nutrient Sources added to Food of the EFSA (EFSA, 2010) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), based on animal and human studies (JECFA, 2006). In addition, these exposure estimates are below the upper limit of Observed Safe Level (OSL; 20 mg/person/day) established by Shao and Hathcock (2006), based on a review of 30 human clinical studies.

IV. BASIS FOR GRAS DETERMINATION

IV.A. Current regulatory status

The FDA has received seven GRAS Notices related to food uses of lutein and lutein esters: 3 GRAS notifications on crystalline lutein to the FDA for general food applications (GRN 140; FDA, 2004; GRN 291, FDA, 2009; GRN 385, FDA, 2011), 2 notices for lutein esters for general food applications (lutein dipalmitate-GRN 110, FDA, 2003; lutein diacetate- GRN 432, FDA, 2012), and 2 notices on suspended lutein in oil for infant formula applications (GRN 221, FDA, 2007; GRN 390, FDA, 2012).

In these GRAS notices, toxicity-related studies on lutein from the literature were presented which support the safety in use of lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of lutein for the proposed food uses. The FDA did not have questions on summary of safety concluding that lutein intake up to 20 mg/person/day is safe. The safety and related information in the above mentioned GRAS Notices is hereby incorporated by reference into this independent GRAS determination. The pertinent information is available as indicated below:

GRN 140: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=140.
GRN 291: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=291.
GRN 385: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=385.
GRN 110: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=110.
GRN 432: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=432.
GRN 221: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=221.
GRN 390: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=390.

In addition, The Panel on Food Additives and Nutrient Sources added to Food of the EFSA (2010) has established an ADI of 1 mg/kg BW/day or 60 mg/day for a 60 kg adult, while JECFA established ADI for lutein from *Tagetes erecta* L. and synthetic zeaxanthin) of up to 2 mg/kg BW/day or up to 120 mg/person based on animal and human studies (JECFA, 2006).

A comparison between GRAS notices for lutein (for general food applications) and current GRAS determination is presented in Table 6.

Table 6. Summary of safety assessments of lutein reported in GRAS notices and current GRAS determination for general food applications

Parameters	GRN 140	GRN 291	GRN 385	JECFA, 2006	Current notice
	Crystalline lutein m.w. 568.88	Crystalline lutein m.w. 568.88	Crystalline lutein m.w. 568.88	Lutein	Crystalline lutein m.w. 568.88
Lutein content	76% (>74%)	>74%	>67%	Not reported	≥70.0% wt
Z content	7% (2-9%)	<8%	>13.5%; Z:MZ=1:1	Not reported	≥4.0% wt
Intended use	Expanded from GRN 110	Same as GRN 140	Same as GRN 140	Not reported	Expanded from GRN 140
EDI of lutein (mg/person/day) at 90th percentile	13.4* (or 0.28 mg/kg BW/day)	13.4* (or 0.28 mg/kg BW/day)	13.4* (or 0.28 mg/kg BW/day)	Not reported	16.7** (or 0.251 mg/kg BW/day)
Cumulative EDI of lutein (mg/person/day) at 90th percentile	Not reported	Not reported	Not reported	Not reported	19.1 (16.7 under the intended uses + 2.4 from regular diets)
EDI of Z (mg/person/day) at 90th percentile	1.2 mg (or 0.03 mg/kg BW/day)	1.2 mg (or 0.03 mg/kg BW/day)	Z-2.4 (3R,3'R-1.2; MZ, 1.2)	Not reported	1.3 (Current use, 1.2 + 0.08 from the newly added products)
ADI of lutein	Not reported	Not reported	Not reported	Up to 120 mg/day	Not reported
Safety determination	Totality of evidence	Totality of evidence	Totality of evidence	NOAEL values from animal studies and human clinical studies	Totality of evidence

Z=zeaxanthin; MZ=meso-zeaxanthin; *EDI of GRN 140 is based on the CSFII 1994-1996, 1998 database;
**EDI of the current notice is based on the NHANES 2009-2010 database.

IV.B. Review of safety data

As noted above, the FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. For each of these notices, after FDA's review, the agency issued letters indicating that it had no questions concerning the safety of lutein and its esters. As the lutein in this GRAS determination is similar in specifications compared to the other luteins in the FDA GRAS Notices, it is recognized that the information and data in the other GRAS Notices is pertinent to the safety of the lutein in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRNs 110, 140, 221, 291, 385, 390, and 432. Additionally, this notice discusses additional studies that have been published since the FDA's last reviews of 2011-2012. The subject of present GRAS assessment is lutein.

IV.B.1. Metabolism of lutein

Oral ingestions of lutein and zeaxanthin are likely to follow the same digestion and intestinal absorption pathways as dietary fat (Borel et al., 2007). Upon absorption, both lutein and zeaxanthin are incorporated into chylomicrons, and are approximately evenly distributed between high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in circulation (Goulinet and Chapman, 1997). This is in contrast with hydrocarbon carotenoids (lycopene, α- and β-carotene) which are transported in LDL (45% and 76%, respectively). Lutein appears to be distributed to tissue following the interaction of lipoprotein particles with receptors, and degradation of lipoproteins by extra hepatic enzymes such as lipoprotein lipase. Uptake and transport of lutein and zeaxanthin (xanthophyll carotenoids) from the bloodstream into the human macula are mediated by specific xanthophyll-binding proteins and human retinal lutein-binding protein may facilitate lutein's localization to human macula (Bhosale et al., 2009). Leo et al. (1995) suggested that most of lutein is likely to undergo biliary excretion into the feces in humans, that biliary concentrations reflect plasma levels in both normal and pathologic states, and that decreased biliary excretion of carotenoids does not increase plasma concentrations.

IV.B.2. Mutagenicity and Genotoxicity studies

In the previous GRAS Notices to the FDA, the safety of lutein has been established in mutagenicity and genotoxicity studies. Since the last review of 2012, no new mutagenicity studies have been published. Thus, this GRAS Notice summarizes the studies already reviewed in previous GRAS notices. As shown in Table 7, lutein was not mutagenic or clastogenic (Kruger et al., 2002; Kuttan and Sabu, 2004; Ravikrishan et al., 2011; Wang et al., 2006). Also, an *in vivo* rat study showed that lutein acted as an inhibitor during promotion, but not initiation of hepatocarcinogenesis (Moreno et al., 2007).

A recent mutagenicity test by Ravikkrishan et al. (2011) employed *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) with and without metabolic activation (S9). Lutein/zeaxanthin concentrate (67% lutein +13.5% 1:1 mixture of zeaxanthin and meso-zeaxanthin) was dissolved in dimethyl sulfoxide (DMSO) and was tested in two independent experiments at concentrations of 31, 63, 125, 250 and 500 ug/plate. No substantial increases in the revertant colony count in any of the five strains were reported at any of the test concentrations in the presence or absence of metabolic activation (S9 mix). Positive controls resulted in significant increases in the revertant count. The spontaneous reversion rates in the negative and positive control were within the range of historical data. In the second experiment, no biologically relevant increase in the revertant counts was observed in any of the five tester

strains pre-incubated with the test item. The results of these investigations suggest that under the experimental conditions, lutein/zeaxanthin concentrate did not induce gene mutation.

However, an *in vitro* comet assay showed conflicting results. Santocono et al. (2006) determined whether these compounds were capable of minimizing DNA damage and influencing the kinetics of DNA repair. DNA damage was assessed using the Comet assay to detect primary DNA damage in individual cells. The addition of zeaxanthin (after 60 min incubation) and lutein (after 180 min incubation) to rat epithelial cells after 2 min of UVA exposition showed a genotoxic effect. The addition of carotenoids to neuroblastoma cells after 30 min UVA exposition positively influenced the kinetics of DNA repair in the first 15min of incubation. At longer exposition times, while the behaviour measured was not constant, a genotoxic effect was not observed. Another Comet assay of serum lymphocytes from humans did not show any DNA damage in response to lutein intakes at a dose of 15 mg/day for 12 weeks (Collins et al., 1998). In this study, comet assay (single cell alkaline gel electrophoresis) was employed to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes from 40 volunteers supplemented with alpha/beta-carotene, lutein, lycopene or placebo. There was a significant negative correlation between basal concentrations of lutein and oxidized pyrimidines. Overall, there is no evidence that lutein is mutagenic, genotoxic, or clastogenic.

Table 7. Summary of mutagenicity and genotoxicity studies

Test	Study condition	Results	Reference
Ames test; *in vitro* mutagenic potential	Lutein at conc. of 334, 668 and 1335 ug/plate; (*Salmonella typhimurium* strains TA97, TA98, TA100 and TA102) in the presence and absence of S9 mix.	Dose-related anti-mutagenic effect	Wang et al. 2006
Ames test; *in vitro* mutagenic potential	Crystalline lutein (75% purity) at conc. of 31-500 ug/plate; (*Salmonella typhimurium* strains TA98 andTA100) in the presence and absence of S9 mix.	Not mutagenic	Ravikrishan et al., 2011
Ames test; *in vitro* mutagenic potential	*Salmonella typhimurium* strains (TA98, TA100 and TA1535) in the presence and absence of S9 mix.	Dose-related anti-mutagenic effect	Kuttan and Sabu, 2004; cited in GRN 385
Ames test; *in vitro* mutagenic potential	Crystalline lutein 10% beadlet at conc. of 100-5,000 ug/plate; (*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA 1535) in the presence and absence of S9 mix.	Not mutagenic	Kruger et al., 2002
In vitro clastogenic potential	Standard chromosome aberration test using Chinese hamster ovary (CHO) cells; lutein (66.8, 133.5 and 267.0 mg/L	Not clastogenic	Wang et al., 2006
In vitro comet assay assessing DNA damage	UVA (2 min)-induced DNA damage in rat tracheal epithelial cells and in human neuroblastoma cells The presence of carotenoids reduced DNA damage when rat epithelial cells were exposed to UVA radiation for 2min.	Lutein (after 180 min incubation) showed a genotoxic effect. At longer exposure times, a genotoxic effect was not observed.	Santocono et al., 2006
Comet assay of lymphocytes from humans	Consume 15 mg/day lutein for 12 weeks and then comet assay of blood lymphocytes to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes	Lutein has anti-genotoxic effects (decreased oxidized pyrimidines)	Collins et al., 1998

IV.B.3. Animal Toxicity Studies

Previous seven GRAS notifications on lutein and its esters (GRN 140, 221, 385, 291, 390 110, 432) extensively reviewed animal toxicity data for lutein and its esters. This GRAS notice

included an additional study (Nidhi and Baskaran, 2013) since the submission of GRN 385 and 432. As shown in Tables 8-9, the safety of lutein has been proven in various animal models.

IV.B.3.1. Acute and subacute toxicity studies

Harikumar et al. (2008) reported that the oral LD_{50} of lutein (85% purity in sunflower oil) isolated from marigold was >4,000 mg/kg BW in female Wistar rats. The oral LD_{50} for a purified plant extract containing 70 to 85% lutein was determined to be >2,000 mg/kg BW in male and female Wistar rats (JECFA, 2006; Ravikkrishan et al., 2011). In a preliminary acute toxicity study of Nidhi and Baskaran (2013), LD_{50} of lutein from *Tagetes erecta* in lutein-deficient male mice was determined to be higher than 10,000 mg/kg BW, the highest dose tested.

In a 4 week subacute toxicity study, the administration of lutein via the diet or by gavage was not associated with toxicologically-significant adverse effects at doses up to 639 mg/kg BW/day for lutein in rats, the highest level tested (Kruger et al., 2002). The study of Harikuma et al. (2008) reported the no-observed-adverse-effect level (NOAEL) for lutein as 400 mg/kg BW/day, the highest dose tested in this rat study. In a recent subacute study of Nidhi and Baskaran (2013), male mice were gavaged with 0, 100, 1,000 mg/kg BW/day for a period of 4 weeks. Plasma lutein levels increased dose dependently ($P < 0.01$) after acute and subacute feeding of lutein in lutein-deficient mice. Compared to the control (peanut oil without lutein) group, no treatment-related toxicologically significant effects of lutein were prominent in clinical observation, ophthalmic examinations, body and organ weights as well as hematological, histopathological, and other clinical chemistry parameters. The authors concluded that the NOAEL for lutein in lutein-deficient mice was determined as 1,000 mg/kg BW/day, the highest dose tested.

Thus, it is summarized that LD_{50} of lutein is higher than 10,000 mg/kg BW in lutein-deficient male mice (Nidhi and Baskaran, 2013) and the NOAEL for lutein exceeds 1,000 mg/kg BW/day in male mice (Nidhi and Baskaran, 2013), the highest dose tested. In rats, the NOAEL for lutein exceeds 639 mg/kg BW/day, the highest dose tested (Kruger et al., 2002).

IV.B.3.2. Subchronic toxicity studies

In 13 week subchronic toxicity studies, the administration of lutein via the diet or by gavage was not associated with toxicologically-significant adverse effects at doses up to 400 mg/kg BW/day in rats, the highest level tested (Harikuma et al., 2008; Kruger et al., 2002; Ravikkrishan et al., 2011). The 13 week rat study of Kruger et al. (2002) reported the NOAEL of 260 mg/kg BW/day, the highest dose tested. In 90 day subchronic rat studies of Harikuma et al., (2008) and Ravikkrishan et al. (2011), the NOAEL was determined to be 400 mg/kg BW/day, the highest level tested. Thus, it is summarized that the NOAEL for lutein exceeds 400 mg/kg BW/day, the highest level tested in rats (Harikuma et al., 2008; Ravikkrishan et al., 2011).

In addition, consumption of lutein at a dose of 9.3 mg/kg BW/day for 1 year did not result in adverse effects in monkeys (Khachik et al., 2006).

IV.B.3.3. 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 BW/day on gestation days 6 to 20. No maternal toxicity or embryotoxicity/teratogenicity reported (Edwards et al., 2002; cited in GRN 390).

Table 8. Summary of animal toxicity studies of lutein

	Daily dose, mg/kg BW	Duration	NOAEL, mg/kg BW/day	Reference
13 Rhesus monkeys	Lutein 9.34 +Z 0.66 mg/kg BW; Z, 10 mg/kg BW; Lutein+Z, 0.5 mg/kg BW each; or control	12 mo	NOAEL- lutein, 9.34 mg/kg BW; Z, 10 mg/kg BW, the highest levels tested	Khachik et al., 2006
70 young adult female Wistar rats	Lutein* (85% purity in sunflower oil); 1,000, 2,000, or 4,000 mg/kg BW	Single dose; observed 2 wk	No mortality at 4,000 mg/kg BW	Harikuma et al., 2008
6 young (9-10 wk old) female Wistar rats	Lutein* (crystalline; >67% purity+13.5% Z); 2,000 mg/kg BW	Single dose; observed 2 wk	No mortality at 2,000 mg/kg BW	Ravikkrishan et al., 2011
50 lutein-deficient mice	0.57 (physiological dose), 100, 1,000, and 10,000 mg/kg BW	Single dose; observed 2 wk	$LD_{50,}$ >10,000 mg/kg BW, the highest dose tested	Nidhi and Baskaran, 2013
84 male and female Wistar rats	2.6, 7.7, 26.0, 77.3, 260, or 773.2 mg crystalline lutein+Z /kg BW (>77% purity; >75% lutein +>2% Z); corresponding to 2.1, 6.4, 21.3, 63.9, 213 or 638.7 mg/kg BW for pure lutein+Z	4 wk	NOAEL- 773 mg/kg BW for crystalline lutein+Z with >77% purity (>75% lutein+>2% Z); or 638.7 mg/kg BW for pure lutein+Z	Kruger et al., 2002
30 male mice	100, or 1,000 mg/kg BW	4 wk	NOAEL- 1,000 mg/kg BW/day	Nidhi and Baskaran, 2013
50 young (~4 wk) male and female Wistar rats	2.6, 26, or 260 mg/kg BW for crystalline lutein+Z (>77% purity; >75% lutein +>2% Z); corresponding to 2.1, 22.4, or 208 pure lutein+Z	13 wk	NOAEL- 260 mg/kg BW for crystalline lutein+Z with >77% purity (>75% lutein+>2% Z); or 208 mg/kg BW for pure lutein+Z	Kruger et al., 2002
80 young (6-9 wk old) male and female Wistar rats	4, 40, and 400 mg/kg BW crystalline lutein (>67% lutein +13.5% Z with both Z isomers)	90 d	NOAEL- 400 mg/kg BW/day	Ravikkrishan et al., 2011
70 young adult male and female Wistar rats	Lutein (85% purity in sunflower oil); 4, 40, and 400 mg/kg BW	4 wk	NOAEL- 400 mg/kg BW/day	Harikuma et al., 2008
70 young adult male and female Wistar rats	Lutein (85% purity in sunflower oil); 4, 40, and 400 mg/kg BW	13 wk	NOAEL- 400 mg/kg BW/day	Harikuma et al., 2008

Z=zeaxanthin

IV.B.4. Human clinical studies

This notice incorporates by reference the safety and metabolism studies discussed in GRNs 385 and 432. This notice discusses additional studies that have been published since the submission of GRN 385 and 432. The notified substance in GRN 432 was lutein ester, but it included the safety studies of lutein as the basis of discussing the safety of lutein. As shown in Table 9, all of the 24 studies (26 articles) published since 2011 reported no adverse effects of lutein and no studies reported results inconsistent with the FDA's reviews of 2011-2012.

In these recent studies, doses tested for lutein were up to 21 mg/day and the duration of the studies were up to 12 months (AREDS Research group, 2013, 2014; Berrow et al., 2013; Connolly et al., 2011; Evans et al., 2013; Garcia-Layana et al., 2013; Graydon et al., 2012; Hammond et al, 2012; Hu et al., 2011; Huang et al., 2013; Ma et al., 2012a, 2012b; Murray et al., 2013; Nolan et al., 2011, 2012; Richer et al., 2011; Sasamoto et al., 2011; Tanito et al., 2012; Thomson et al., 2014; Tian et al., 2013; Wang et al., 2013; Weigert et al., 2011; Xu et al., 2013; Yao et al., 2013; Zeimer et al., 2012; Zou et al., 2014). No adverse effects were observed in any of the clinical trials. Repeated daily doses of 20-21 mg lutein were not associated with adverse effects (Huang et al., 2012; Ma et al., 2012a, 2012b; Nolan et al., 2012; Thompson et al., 2014; Wang et al., 2013; Weigert et al., 2011; Xu et al., 2013; Yao et al., 2013; Zou et al., 2014). In particular, longer term administration of 20 mg lutein/day for 1 year resulted in no adverse effects (Yao et al., 2013; Zhou et al., 2014).

In addition, meta analysis of 6 prospective cohort studies involving 4,416 cases and 41,999 participants revealed that dietary lutein and zeaxanthin intake had a significant inverse association with nuclear cataract (Relative risk=0.75; 95 % CI: 0.65, 0.85), but not with cortical cataract and posterior subcapsular cataract (Ma et al., 2014). Dose-response analysis showed that every 300 μg/day increment in dietary lutein and zeaxanthin intake was associated with a 3 %, 1 %, or 3 % reduction in the risk of nuclear cataract, cortical cataract, or posterior subcapsular cataract, respectively. No adverse effects of lutein and zeaxanthin were found. The absence of any adverse effects in these clinical trials provides support for the safety of lutein. The AREDS 2 Research Group (2013, 2014) also reported that intake of 10 mg lutein plus 2 mg zeaxanthin for an average of 4.8 years did not result in any adverse effects.

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
14 subjects with early AMD (8 participants aged 56-81 years in the treated group and 6 participants aged 61-83 years in the non-treated group)	Supplement (12 mg L+0.6 mg Z+ 150 mg Vit C +15 mg Vit E+ 1080 mg omega 3 FA); or no supplement (control group)	40 weeks. At 60 weeks, the treated group attended a fourth visit following 20 weeks of supplement withdrawal	Retinal and visual function in people with early stages of AMD	No differences between the treated and control groups during supplementation. Multifocal electroretinography (mfERG) ring 3 N2 latency (P=0.041) and ring 4 P1 latency (P=0.016) increased, and a trend for reduction of mfERG amplitudes was observed in rings 1, 3 and 4 on supplement withdrawal, suggesting a potentially beneficial effect of lutein based supplementation in AMD-affected eyes.	Berrow et al., 2013
144 subjects with subclinical atherosclerosis aged 45-68 years	20 mg L (n=48); 20 mg L+20 mg lycopene (n=48); or placebo	12 months, P	Carotid artery intima-media thickness (CAIMT)	Serum lutein concentrations increased significantly from 0.34 to 1.96 µmol/l in the lutein group (P< 0.001) and from 0.35 to 1.66 µmol/l in the combination group (P< 0·001). The mean values of CAIMT decreased significantly by 0.035 mm (P= 0.042) and 0.073 mm (P< 0.001) in the lutein and combination groups, respectively.	Zou et al., 2014
39 older adults (BMI, 25.3; mean age, 68.8 year) not meeting Australian physical activity guidelines	21 mg L; or placebo	4 weeks, P	Physical activity	Plasma lutein were inversely associated with absolute (r = -0.36, p = 0.03) and percentage changes (r = -0.39, p = 0.02) in sedentary time and positively associated with the percentage change in average daily activity counts (r = 0.36, p = 0.03).	Thomson et al., 2014

AUC=Area Under the Curve; AREDS2=Age-Related Eye Disease Study 2; BMI=body mass index; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; L=lutein; mfERG = multifocal electroretinography; MP =Macular pigment; MZ=meso-zeaxanthin; P=parallel design; VA=visual acuity; X=cross over design; Z=zeaxanthin.

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
126 patients with AMD (AREDS [Age-related Eye Disease Study] stages 2, 3, and 4)	1 to 3-20 mg L in 1-3 months and 10 mg L in 4 - 6 months; or placebo	6 months, P	MPOD and visual acuity (VA)	Lutein significantly increased MPOD by 27.9% ± 2.9% ($p < 0.001$ vs. placebo). No significant effect of lutein supplementation on mean differential light threshold (MDLT) or VA was seen, although a tendency toward an increase was seen for both parameters (MDLT, $p = 0.096$ vs. placebo; VA, $p = 0.070$ vs. placebo). However, a significant correlation was found between the increase in MPOD and the increase in MDLT after 6 months ($r = 0.25$, $p = 0.027$), as well as between the increase in MPOD after 6 months and the increase in VA after 6 months ($r = 0.27$, $p = 0.013$).	Weigert et al., 2011
65 early athero-sclerosis patients	20 mg L (n= 34); or placebo (n= 31)	3 months, P	Serum cytokines, apoE and lipoprotein profiles	Lutein supplements significantly decreased in serum interleukin-6 and monocyte chemoattractant protein-1 (MCP-1) at 3 month ($p<0.05$). Intragroup comparison revealed a significant difference in changes of serum MCP-1 between the 2 groups ($p= 0.021$). The serum LDL and triglycerides (TG) significantly decreased in lutein group ($p<0.05$).	Xu et al., 2013
48 healthy volunteers	Single dose of 20 mg of lutein as either a starch-matrix (lutein 5 %); or as a cross-linked alginate-matrix beadlet (lutein 20 %) formulation.	Up to 672 h, X	Plasma kinetics of lutein and Z provided as two different beadlet formulations	By 14 h post-dose, total plasma lutein increased by 7 % with alginate matrix beadlets and by 126% with starch-matrix. Total lutein AUC (0-72h) and AUC (0-672h) were 1.8-fold and 1.3-fold higher, respectively, for starch-matrix compared to alginate-matrix beadlet. Both formulations were well tolerated by subjects in this study.	Evans et al., 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Daily dose of L and/or Z	Duration	Measured Outcomes	Results	Reference
117 healthy nonsmokers	10 L; 20 mg L; or placebo	12 weeks, P	Levels of plasma carotenoid, total antioxidant capacity (TAOC), lipoprotein profile, and antioxidant enzymes activities	Plasma lutein and TAOC significantly increased in both active treatment groups. A significant reduction was found in malondialdehyde in the 20 mg lutein group.	Wang et al., 2013
108 subjects with early AMD	10 mg L; 20 mg L; 10 mg L+10 mg Z; or placebo	48 weeks, P	Retinal function using mfERG or MPOD	The negative trough and first positive peak (N1P1) response densities significantly increased in ring 1 for the 20 mg lutein group and for the lutein and Z group, and in ring 2 for the 20 mg lutein group, indicating improvement in renal function. The increases in MPOD related positively to the increases in N1P1 response density in ring 1 and ring 2 for nearly all active treatment groups. N1P1 response densities in ring 3 to ring 6 or P1 peak latencies in all rings did not change significantly in any group.	Ma et al., 2012a, 2012b
108 patients with early AMD (aged >50 years)	10 mg L; 20 mg L; 10 mg L+10 mg Z; or placebo	48 weeks, P	Serum and macular (in vivo measured MPOD) responses	Serum lutein levels in the all lutein and L+Z groups increased 4.24-, 4.66-, and 6.23-fold during the trial, respectively (all $P < 0.001$). MPOD and serum lutein levels increased linearly with the dosage and their increasing rates were statistically correlated (all $P < 0.05$). No notable changes were detected in the placebo group for MPOD and serum concentrations.	Huang et al, 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
120 healthy drivers with long-term light exposure (drivers).	20 mg L; or placebo	1year, P	VA, MPOD, and visual performance	Significant increase in serum lutein and central MPOD in the treatment group; no change was observed in the placebo group. Significant improvements in the score of the National Eye Institute 25-Item Visual Functioning Questionnaire driving subscale in the a treatment group.	Yao et al., 2013
31 healthy subjects with such atypical MP spatial profiles	20 mg L + 2 mg Z; 10 mg MZ+ 10 mg L+ 2 mg Z; 17 mg MZ+ 3 mg L+ 2 mg Z; or placebo	8 weeks, P	MP in subjects where the profile does not exhibit the typical central peak (i.e. peaked MP at foveal epicentre).	No significant increase in MP at any eccentricity in the 20 mg L + 2 mg Z group. There was a trend towards an increase in MP at all eccentricities in, with a significant increase found at 0.25° and 0.50° 10 mg in the MZ+ 10 mg L+ 2 mg Z group. A significant increase in MP at 0.25° in the 17 mg MZ+ 3 mg L+ 2 mg Z group 3, but at no other eccentricity. The typical central peak of MP can be realised in subjects with atypical spatial profiles, following supplementation with all three macular carotenoids, but not with a supplement lacking MZ.	Nolan et al., 2012
322 healthy female twin volunteers, aged 16–50 yr	18 mg L + 2.4 mg Z; or placebo	6 months, P	Heritability of MP augmentation in response to supplemental L and Z	Serum L and Z levels were raised significantly from baseline following 3 months' supplementation (mean increase 223% and 633%, respectively), with no MPOD increase. After 6 months' supplementation, a small increase in MPOD was seen (mean increase 0.025, P = 0.02). Genetic factors explained 27% (95% confidence interval [CI] 7–45) of the variation in MPOD response. Distribution profiles of MP did not change in response to supplementation.	Hammond et al, 2012

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
44 patients with AMD	12 mg L+ 280 mg DHA daily; or placebo	1 year, P	MPOD	At baseline, the MPOD in placebo was 0.286 meanwhile it was 0.291 in the supplemented group. One year later, the mean MPOD was increased by 0.059 in the placebo group and by 0.162 in patients receiving lutein + DHA ($p < 0.05$).	Garcia-Layana et al., 2013
97 subjects	12 mg L + 1 mg Z; no control group	6-mo intervention, 3 mo FU	MPOD, a ring structure	L and Z intake led to a general shift toward higher MP values in eyes without ring structure. Ring structures were neither attenuated nor generated de novo following supplementation.	Zeimer et al., 2012
4203 elderly at risk for developing late AMD 50 -85 y	10 mg L +2 mg Z; 1.0 g omega-3 FA; the combination; or placebo	median of 4.8 years, P	development of late AMD	In exploratory analysis of lutein/zeaxanthin vs no lutein/zeaxanthin, the hazard ratio of the development of late AMD was 0.90 (95% CI, 0.82-0.99; P = 0.04). No differences in adverse events or serious adverse event among the groups.	AREDS 2 Research Group, 2013, 2014
72 patients with early AMD (mean age 70.5 ± 8.7)	10 mg L ester; or a placebo	12 months; P	MPOD and VA	At the end of the trial, an overall increase in the mean MPOD level was found for the L group from 0.38 ± 0.19 to 0.53 ± 0.22 optical density units. No change in MPOD was found for the control group. In the L subgroup (n = 19),there was a mean improvement in VA from 0.23 ± 0.12 at baseline to 0.16 ± 0.10 at visit 4 ($P < 0.05$). In the placebo subgroup (n = 14), there was a small deterioration from 0.18 ± 0.13 to 0.19 ± 0.12 (P = 0.70).	Murray et al., 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
44 healthy subjects	10.6 mg MZ; 5.9 mg L+ 1.2 mg Z; or placebo	6 months, P	The spatial profile of MPOD, and serum conc. of L and Z	The test group had significantly increased serum concentrations of L and Z central MPOD (0.25°: P = 0.001; 0.5°: P = 0.001), with no significant change in the control group. Clinical pathology analysis confirmed that all variables remained within the normal reference range.	Connolly et al., 2011
22 healthy volunteers without high myopia	10 mg L; or 10 mg Z	3 months, P	MPOD	MPOD resonance Raman spectrophotometry (RRS) and MPOD autofluorescence imaging (AFI) levels increased >20 % from baseline at 2 and 3 months after lutein supplementation. In the Z group, MPOD (RRS) levels remained unchanged in those with and without high myopia. The authors concluded that lutein supplementation increased MPOD levels within the fovea more effectively than did zeaxanthin.	Tanito et al., 2012
72 subjects with signs of early AMD	10 mg L; or placebo	12 months, P	Complement factor D (CFD) and its products C5a and C3d	Lutein supplementation markedly decreases circulating levels of the complement factors CFD, C5a and C3d levels, suggesting that lutein supplementation may be a powerful tool in the prevention and treatment of AMD.	Tian et al., 2013
43 eyes of 43 subjects, with central serous chorioretinopathy (CSC), and 33 fellow eyes with AMD	6 mg L (no control group)	1 year	1-year changes in MPOD and visual function	Daily supplementation with 6 mg of lutein did not affect the MPOD level for 1 year, but supplementation seems to improve visual functions such as contrast sensitivity and retinal sensitivity.	Sasamoto et al., 2011

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Daily dose	Duration	Measured Outcomes	Results	Reference
121 normal subjects (18-41 years)	12 mg L+ 1mg Z	1year, P	Visual performance	Supplementation of L+Z improved MPOD.	Nolan et al., 2011
60 subjects with mild-to moderate AMD (57 men, 3 women; mean 74.9 years)	8 mg Z; 8 mg Z +9 mg L; or 9 mg L- Faux placebo- control group	1year, P	MPOD	There were no intergroup differences in 3 major AMD risk factors (age, smoking, and body mass index as well as disease duration and Visual Function Questionnaire 25 composite score differences) and MPOD.	Richer et al., 2011
Study 1) 52 healthy subjects Study 2) 75 healthy subjects	Either carrot juice (a carotene-rich food) or spinach powder (L+Z-rich food); supplements containing L + Z, β-carotene; or placebo	Two 8-week studies, P	MP level, serological markers of endothelial activation, and inflammation and oxidation	No effects on MP level or markers of endothelial activation, inflammation or oxidation were observed. However, the change in serum lutein and Z was associated or tended to be associated with the change in MP levels in those receiving lutein rich foods (L: r=0.40, P = 0.05; Z: r=0.30, P = 0.14) or the lutein and Z supplement (L; r=0.43, P = 0.03; Z: r=0.22, P = 0.28).	Graydon et al., 2012
90 nonproliferative diabetic retinopathy (NDR) patients divided evenly into 3 groups	6mg L+ 0.5 mg Z; No L/Z supplementation (NDR control group and control group).	3 months, P	Serum L/Z concentrations visual function between patients with NDR and normal subjects	Serum L/Z concentrations of NDR group increased significantly by 2 times (P=0.000) and VA improved significantly after supplementation. Average contrast sensitivity (CS) values after 3 months increased significantly and the foveal thickness decreased (P =0.05).	Hu et al., 2011

AUC=Area Under the Curve; AREDS2=Age-Related Eye Disease Study 2; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; L=lutein; MPOD= macular pigment optical density; MZ=meso-zeaxanthin; MP =Macular pigment; VA=visual acuity; Z=zeaxanthin

V. SAFETY DETERMINATION

Numerous human and animal studies have reported health benefits of lutein with no major adverse effects. INNOBIO® uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of lutein and its ester. This GRAS determination is based on the data and information generally available and consented opinion about the safety of lutein. The literature indicates that lutein offers consumers health benefits without serious adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of lutein as well as appropriate corroborative data.

1. INNOBIO's lutein will be used as a food ingredient (nutrient) at concentrations of up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12) in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute 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; soups and soup mixes. Intended use excludes food categories such as egg products and soup and soup mixes that come under USDA. Compositional analysis of INNOBIO lutein demonstrated that product primarily contains ≥70% lutein with small amount of ≥4% zeaxanthin, carbohydrate, and protein. The intended use of lutein in the specified food categories will result in cumulative 90th percentile EDIs (intakes from regular diets, current exposure, and newly added food categories) of 19.1 mg lutein/person/day and 1.3 mg zeaxanthin/person/day, respectively.

2. These exposure estimates (EDI under the untended uses, and/or cumulative exposure) are far below the ADI of up to 1 mg/kg BW/day or 60 mg/person/day established by The Panel on Food Additives and Nutrient Sources added to Food of EFSA as well as ADI of up to 2 mg/kg BW/day or 120 mg/person/day determined by JECFA, based on animal and human studies. In addition, these exposure estimates are below the upper limit of Observed Safe Level (OSL; 20 mg/person/day) established by Shao and Hathcock (2006), based on a review of 30 human clinical studies.

3. In response to several GRAS notifications on lutein (crystalline lutein - GRN 140, 291, 385; suspended lutein - GRN 221, 390; lutein ester - GRN 110 and 432), the FDA did not question the safety of lutein and lutein esters for the specified food uses. The FDA responses to GRAS notifications on lutein indicate that the FDA is satisfied with the safety-in-use of the lutein, as long as consumption is below 20 mg/person/day.

4. In the previous GRAS notices to the FDA, the safety of lutein has been established in toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. Additionally, the safety is corroborated by additional studies conducted with various sources of lutein, lutein-rich foods, and lutein supplements. Furthermore, historical consumption of eggs and fruits and vegetables where these carotenoids predominate (e.g., green leafy vegetables, such as spinach and kale) support the safety of

lutein and zeaxanthin. Additional studies published subsequent to the FDA GRAS notices continue to support safety of lutein as a food ingredient.

VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF LUTEIN (CRYSTALLINE, SUSPENDED OIL, POWDER, AND BEADLETS)

The intended uses of Lutein (crystalline, suspended oil, powder, and beadlets) have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

On behalf of INNOBIO® Limited (INNOBIO®), we, the undersigned expert panel members, Susan Cho, Ph.D., Robert Martin, Ph.D., Madhusudan Soni, Ph.D., have independently evaluated the materials summarized in the lutein Generally Recognized as Safe (GRAS) report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that lutein, produced consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of lutein are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

_____ _____

Susan Cho, Ph.D. Date
NutraSource, Inc.

_____ _____

Robert Martin, Ph.D. Date

_____ _____

Madhusudan Soni, Ph.D. Date
Soni and Associates

VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF LUTEIN (CRYSTALLINE, SUSPENDED OIL, POWDER, AND BEADLETS)

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(b) (6)

Susan Cho, Ph.D.
NutraSource, Inc.

9/4/2014
Date

(b) (6)

Robert Martin, Ph.D.

Sept. 3, 2014
Date

(b) (6)

Madhusudan Soni, Ph.D.
Soni and Associates, Inc.

August 28, 2014
Date

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APPENDIX A. CERTIFICATE OF ANALYSIS

INNOBIO® Crystalline Lutein
Lot No.:30201-B318F1 Date of Manufacture: 06/18/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	77.1
Lutein (%)	70.0 Min.	USP 32/NF 27	70.9
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.5
Moisture (%)	1.0 Max.	USP 731	0.04
Particle Size	95% through 40 mesh	USP 786	96.5
Lead (ppm)	1.0 Max.	EN ISO 17294	0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	43.1
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:_____ (b) (6) Date: 07/01/2013

INNOBIO® Crystalline Lutein

Lot No.:30201-B324F1 Date of Manufacture: 06/24/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	77.9
Lutein (%)	70.0 Min.	USP 32/NF 27	71.7
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.6
Moisture (%)	1.0 Max.	USP 731	0.1
Particle Size	95% through 40 mesh	USP 786	95.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.01
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.05
Mercury (ppm)	0.1 Max.	BS EN 13806	<10
Hexane (ppm)	10.0 Max.	USP 467	3.9
Ethanol (ppm)	50.0 Max.	USP 467	33.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:____ (b) (6) Date: 07/03/2013

INNOBIO® Crystalline Lutein

Lot No.:30201-B325F1 Date of Manufacture: 06/25/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	78.2
Lutein (%)	70.0 Min.	USP 32/NF 27	71.9
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.7
Moisture (%)	1.0 Max.	USP 731	0.1
Particle Size	95% through 40 mesh	USP 786	95.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.01
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.05
Mercury (ppm)	0.1 Max.	BS EN 13806	<10
Hexane (ppm)	10.0 Max.	USP 467	5.7
Ethanol (ppm)	50.0 Max.	USP 467	36.3
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:_____ (b) (6) Date: 07/03/2013

INNOBIO® Lutein 20% Oil Suspension
Lot No.: 30215-D313F1 Date of Manufacture: 06/13/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.6
Lutein (%)	20.0 Min.	USP 32/NF 27	20.4
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.4
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	10.8
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

Approval: (b) (6) Date: 06/24/2013

INNOBIO® Lutein 20% Oil Suspension

Lot No.: 30215-D315F1 Date of Manufacture: 06/15/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.9
Lutein (%)	20.0 Min.	USP 32/NF 27	20.6
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.5
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.3
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.8
Ethanol (ppm)	50.0 Max.	USP 467	17.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	50
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

(b) (6)

Approval: Date: 06/24/2013

INNOBIO® Lutein 20% Oil Suspension
Lot No.: 30215-D317F1 Date of Manufacture: 06/17/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.1
Lutein (%)	20.0 Min.	USP 32/NF 27	20.3
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.1
Lead (ppm)	1.0 Max.	EN ISO 17294	0.2
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	27.3
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

Approval: (b) (6) Date: 06/26/2013

INNOBIO® Lutein 20% CWD Powder

Code: 30202-20
Lot No.: 30202-318D1 Date of Manufacture: 04/18/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	23.9
Lutein (%)	20.0 Min.	USP 32/NF 27	22.0
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	5.0 Max.	USP 731	2.1
Particle Size	95% through 40 mesh	USP 786	98.5
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.3
Ethanol (ppm)	50.0 Max.	USP 467	10.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____(b) (6) Date: 04/25/2013

INNOBIO® Lutein 20% CWD Powder

Code: 30202-20
Lot No.: 30202-328A1 Date of Manufacture: 01/28/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	24.2
Lutein (%)	20.0 Min.	USP 32/NF 27	22.3
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	5.0 Max.	USP 731	2.2
Particle Size	95% through 40 mesh	USP 786	98.2
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.9
Ethanol (ppm)	50.0 Max.	USP 467	20.4
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	50
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____(b) (6)_____ Date: 02/25/2013

INNOBIO® Lutein 20% CWD Powder

Lot No.: 30202-D307K1 Date of Manufacture: 11/07/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	24.6
Lutein (%)	20.0 Min.	USP 32/NF 27	22.5
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.5
Moisture (%)	5.0 Max.	USP 731	2.5
Particle Size	95% through 40 mesh	USP 786	97.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.8
Ethanol (ppm)	50.0 Max.	USP 467	12.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	60
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 11/18/2013

INNOBIO® Lutein 10% CWD Powder

Lot No.: 13050901 Date of Manufacture: 05/09/2013

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.9
Lutein (%)	10.0 Min.	USP 32/NF 27	10.9
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.9
Particle Size	95% through 40 mesh	USP 786	96.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.4
Ethanol (ppm)	50.0 Max.	USP 467	11.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 05/19/2013

INNOBIO® Lutein 10% CWD Powder

Lot No.: 30202-B108C1 Date of Manufacture: 03/08/2011

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.4
Lutein (%)	10.0 Min.	USP 32/NF 27	10.5
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.3
Particle Size	95% through 40 mesh	USP 786	96.2
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.9
Ethanol (ppm)	50.0 Max.	USP 467	10.9
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 03/15/2011

INNOBIO® Lutein 10% CWD Powder

Lot No.: 30202-326D1 Date of Manufacture: 04/26/2013

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.5
Lutein (%)	10.0 Min.	USP 32/NF 27	10.6
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.1
Particle Size	95% through 40 mesh	USP 786	97.1
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.7
Ethanol (ppm)	50.0 Max.	USP 467	8.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 05/03/2013

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-215E1 Date of Manufacture:05/15/2012

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.3
Lutein (%)	10.0 Min.	USP 32/NF 27	10.4
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.68
Moisture (%)	7.0 Max.	USP 731	0.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.9
-through sieve No.100	15.0 Max.	USP 786	18.6
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.6
Ethanol (ppm)	50.0 Max.	USP 467	19.5
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 06/20/2012

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-303D1 Date of Manufacture: 04/03/2013

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conform
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.5
Lutein (%)	10.0 Min.	USP 32/NF 27	10.6
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.69
Moisture (%)	7.0 Max.	USP 731	3.1
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	99.5
-through sieve No.100	15.0 Max.	USP 786	17.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.0
Ethanol (ppm)	50.0 Max.	USP 467	12.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 04/08/2013

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-327C1 Date of Manufacture: 03/27/2013

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conform
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.8
Lutein (%)	10.0 Min.	USP 32/NF 27	10.9
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	7.0 Max.	USP 731	3.2
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.5
-through sieve No.100	15.0 Max.	USP 786	18.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	4.2
Ethanol (ppm)	50.0 Max.	USP 467	9.8
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 04/03/2013

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-215G1 Date of Manufacture: 07/15/2012

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.6
Lutein (%)	5.0 Min.	USP 32/NF 27	5.1
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.33
Moisture (%)	5.0 Max.	USP 731	3.9
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.4
-through sieve No.100	15.0 Max.	USP 786	19.7
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.6
Ethanol (ppm)	50.0 Max.	USP 467	10.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___ (b) (6) Date: 07/25/2012

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-311C1 Date of Manufacture: 03/11/2013

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.7
Lutein (%)	5.0 Min.	USP 32/NF 27	5.2
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.34
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.2
-through sieve No.100	15.0 Max.	USP 786	19.3
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	2.4
Ethanol (ppm)	50.0 Max.	USP 467	8.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___(b) (6) Date: 03/27/2013

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-311C1 Date of Manufacture: 03/11/2013

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.7
Lutein (%)	5.0 Min.	USP 32/NF 27	5.2
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.33
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.5
-through sieve No.100	15.0 Max.	USP 786	18.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.9
Ethanol (ppm)	50.0 Max.	USP 467	9.0
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___ (b) (6) Date: 03/27/2013

INNOBIO® Lutein 5% CWS Powder

Lot No.: 30202-224B1 Date of Manufacture: 02/24/2012

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	6.2
Lutein (%)	5.0 Min.	USP 32/NF 27	5.7
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.37
Moisture (%)	5.0 Max.	USP 731	1.1
Particle Size	95% through 40 mesh	USP 786	99.4
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.5
Ethanol (ppm)	50.0 Max.	USP 467	6.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	80
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___ (b) (6) Date: 03/09/2012

INNOBIO® Lutein 5% CWS Powder

Code: 30202-05
Lot No.: 30202-D311F1 Date of Manufacture: 06/11/2013

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.9
Lutein (%)	5.0 Min.	USP 32/NF 27	5.4
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.35
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size	95% through 40 mesh	USP 786	99.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.7
Ethanol (ppm)	50.0 Max.	USP 467	9.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 06/21/2013

INNOBIO® Lutein 5% CWS Powder

Code: 30202-05
Lot No.: 30202-213F1 Date of Manufacture: 06/13/2013

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.8
Lutein (%)	5.0 Min.	USP 32/NF 27	5.4
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.34
Moisture (%)	5.0 Max.	USP 731	2.0
Particle Size	95% through 40 mesh	USP 786	98.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.2
Ethanol (ppm)	50.0 Max.	USP 467	3.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 06/27/2013

Pages 000071-000452 have been removed in accordance with copyright laws. Please see the bibliography list of the references that have been removed from this request on pages 000044-000049.

SUBMISSION END

GRAS EXEMPTION CLAIM for INNOBIO® LUTEIN

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336;
Susanscho1@yahoo.com

GRAS EXEMPTION CLAIM

A. Lutein: Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 5 170.36(1)

INNOBIO® Limited (INNOBIO®; the notifier) has determined that its lutein (crystalline, oil suspension, powder, and beadlet forms) are 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 described in the following sections. Since these procedures specify the conditions of its intended use in food, the use of lutein is exempt from the requirement of premarket approval.

Signed

Susan Cho Date:
Agent for
INNOBIO® Limited (INNOBIO®)

B. Notifier's Name and Address

Notifier's name: INNOBIO® Limited (INNOBIO®)
Phone number: 86-411-87406671
Fax number: 86-411-87406801
Physical address: No.49, DDA, Dalian, Liaoning, 116600, China
E mail address: sunx@INNOBIO.cn

C. Name of GRAS Substance

The common name of the notified substance is Lutein. The trade names are:
INNOBIO® Crystalline Lutein,
INNOBIO® Lutein 20% Oil Suspension,
INNOBIO® Lutein 20% Cold Water Dispersible (CWD) Powder,
INNOBIO® Lutein 10% CWD Powder,
INNOBIO® Lutein 10% Beadlets,
INNOBIO® Lutein 5% Beadlets, and
INNOBIO® Lutein 5% Cold Water Soluble (CWS) Powder

D. Product Description

Lutein and zeaxanthin belong to the class of plant compounds called carotenoids, and are present in a wide variety of fruits and vegetables. Interestingly, while other tissues contain a mixture of other carotenoids, the macular pigment in the human retina contains only lutein and zeaxanthin, often referred as xanthophylls or macular pigment. Scientific evidence shows that supplementing diets with lutein and zeaxanthin daily keeps eyes healthy, improves visual performance, and helps reduce the risk of certain eye conditions. The human body does not produce the dietary zeaxanthin and lutein it needs daily, so it is essential to get these nutrients through diet and/or supplementation. Unlike β-carotene, these two carotenoids do not have vitamin A activity. While there is no RDA for lutein and zeaxanthin, intakes of approximately 6-10 mg/day have been associated with a decreased risk of age related macular degeneration (AMD).

The current intakes of lutein and zeaxanthin among adults 50 years of age falls well below this level, with an intake range of lutein at 0.6-2.4 mg/day. Only men in the 99th percentile of lutein/zeaxanthin intake and women in the 95th percentile meet the dietary intakes that have been associated with decreased risk of AMD. Most Americans consume only 10 percent of the zeaxanthin and lutein needed each day to protect their eyes and keep them healthy.

E. Applicable Conditions for Use of the Notified Substance

E.1. Current Regulatory Status and Background Dietary Intake of Lutein

The FDA has received seven GRAS Notices related to food uses of lutein and lutein esters: 3 separate GRAS notifications on crystalline lutein to the FDA for general food applications (GRN 140; FDA, 2004; GRN 291, FDA, 2009; GRN 385, FDA, 2011), 2 notices for lutein esters for general food applications (lutein dipalmitate-GRN 110, FDA, 2003; lutein diacetate- GRN 432, FDA, 2012) and 2 notices on suspended lutein in oil for infant formula applications (GRN 221, FDA, 2007; GRN 390, FDA, 2012). In these GRAS notices, toxicity-related studies on lutein from the literature were presented which support the safety in use of lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of lutein for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that lutein intake up to 20-22 mg/person/day is safe. In addition, The Panel on Food Additives and Nutrient Sources added to Food of the European Food Safety Authority (EFSA, 2010) has established an ADI of 1 mg/kg body weight (BW)/day or 60 mg/person/day for a 60 kg adult, while Joint FAO/WHO Expert Committee on Food Additives (JECFA) established ADI for lutein from *Tagetes erecta* L. and synthetic zeaxanthin) of up to 2 mg/kg BW/day or up to 120 mg/person/day based on animal and human studies (JECFA, 2006).

Intake from regular diets is expected to be less than 2.4 mg/day.

E.2. Intended Use of Lutein and Levels of Use in Foods

As INNOBIO® crystalline lutein represents the concentrated form with ≥70% lutein and ≥4.0% zeaxanthin, Table 1 represents the quantity of lutein when used as such, or the quantity present in a standardized diluted form using food grade/GRAS ingredients.

The intended use of INNOBIO® Lutein (crystalline, suspended oil, powder, and beadlets) is expanded from food products listed in the GRN 000140 by adding protein bars, low sodium tortillas, and vitamin water. INNOBIO® Lutein is intended for use as a food ingredient (a nutrient) in the following food categories: baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute 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 at use levels of 0.3 to 3 mg lutein/serving (reference amounts customarily consumed, 21CFR 101.12). INNOBIO® Lutein will not be added to food categories that come under USDA jurisdiction.

E.3. Estimated Dietary Intakes (EDIs) of Lutein Based upon Intended Food Uses

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2009-2010), estimates of 2-day average intakes of lutein were calculated from the food code list and the survey database of diet recalls. The mean intakes of lutein under the intended use were estimated to be 8.7 mg/person/day for all population and 8.9 mg/person/day for all-users, while the 90th percentile intakes were determined as 16.5 and 16.7 mg/person/day (or 0.289 and 0.291 mg/kg BW/day), respectively. These EDIs are based on the assumption that 100% of the products listed in GRN 140 and newly added food categories (1.6 mg/person/day) will be used at the maximum intended use levels. Intake from regular diets is expected to be less than 2.4 mg/day. Estimated cumulative exposure (intakes from regular diets, and expanded list of food categories) is estimated to be no more than 19.1 mg/person/day.

As reported in GRN 140, the current 90th percentile intake of zeaxanthin from specified foods was estimated to be 1.2 mg/person/day. The contribution of newly added food categories is estimated to be 0.079 mg/person/day at the 90the percentile of all-users. Even if 100% of the products listed in GRN 140 (1.2 mg/person/day) and newly added food categories are used at the maximum intended use levels, the 90th percentile intake of zeaxanthin from all GRAS-proposed use categories by users of one or more foods are 1.3 mg/person/day for all-users.

Table 1. Food Categories and Intended Use Levels of Lutein in the United States.

Food Category	Proposed Food	Use Levels				
		Lutein, mg/RACC[1,2]	INNOBIO® Crystalline Lutein (≥70%)	20% lutein, mg/RACC	10% lutein, mg/RACC	5% lutein, mg/RACC
Baked Goods and Baking Mixes	**Protein bars,** cereal bars, and energy bars	2.0	2.86	10.0	20.0	40.0
	Crackers and crisp-breads	2.0	2.86	10.0	20.0	40.0
	Tortillas, low sodium	2.0	2.86	10.0	20.0	40.0
Beverages and Beverage Bases	**Vitamin water**	2.0	2.86	10.0	20.0	40.0
	Bottled water	0.5	0.72	2.5	5.0	10.0
	Carbonated beverages; Meal replacements	2.0	2.86	10.0	20.0	40.0
	Tea, ready-to-drink	0.6	0.86	3.0	6.0	12.0
Breakfast Cereals	Instant and regular hot cereals	2.0	2.86	10.0	20.0	40.0
	Ready-to-eat cereals	2.0	2.86	10.0	20.0	40.0
Chewing Gum	Chewing gum	1.0	1.43	5.0	10.0	20.0
Dairy Product Analogs	Imitation milks	2.0	2.86	10.0	20.0	40.0
	Soy milks	1.5	2.14	7.5	15.0	
Egg Products	Liquid, frozen or dried egg substitutes	2.0	2.86	10.0	20.0	40.0
Fats and Oils	Margarine-like spreads, salad dressings	1.5	2.14	7.5	15.0	30.0
Frozen Dairy Desserts and Mixes	Frozen yogurt	1.0	1.43	5.0	10.0	20.0
Gravies and Sauces	Tomato based sauces	0.3	0.43	1.5	3.0	6.0
Hard Candy	Hard candy	1.0	1.43	5.0	10.0	20.0
Infant and Toddler Foods	Junior, strained and toddler-type baby foods	1.0	1.43	5.0	10.0	20.0
Milk Products	Dry milk flavored milk and milk drinks	3.0	4.29	15.0	30.0	60.0
	Fermented milk beverages	0.6	0.86	3.0	6.0	12.0
	Yogurt	3.0	4.29	15.0	30.0	60.0
	Milk based meal replacement	2.0	2.86	10.0	20.0	40.0
Processed Fruits and Fruit Juices	Energy, sport and isotonic drinks	2.0	2.86	10.0	20.0	40.0
	Fruit-flavored drinks, fruit juice, nectars	2.0	2.86	10.0	20.0	40.0
	Vegetable Juice	2.0	2.86	10.0	20.0	40.0
Soft Candy	Chewy and nougat candy; Fruit snacks	1.0	1.43	5.0	10.0	20.0
Soup and soup mixes	Canned soup	0.6	0.86	3.0	6.0	12.0

[1]Use levels are the same as those described in GRN 140 except 3 newly added items. [2]RACC Reference amounts customarily consumed per eating occasion (21 CFR § 101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC.

E.4. Basis for the GRAS Determination
As noted above, the FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. For each of these notices, after the FDA's review, the agency issued letters indicating that it had no questions concerning the safety of lutein and its esters. As the lutein in this GRAS determination is similar in specifications compared to the other luteins in the FDA GRAS Notices, it is recognized that the information and data in the other GRAS Notices is pertinent to the safety of the lutein in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 110, 140, 221, 291, 385, 390, and 432. Additionally, this notice discusses additional studies that have been published since the FDA's last reviews of 2011-2012. The subject of present GRAS assessment is lutein.

Pursuant to 21 CFR 9170.35, INNOBIO® Lutein (crystalline, suspended oil, powder, and beadlets) have been determined to be GRAS on the basis of scientific procedures. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of Lutein as a component of food. Expert Panel members, Susan S. Cho, Ph.D., Robert L. Martin, Ph.D., Madhusudan Soni, Ph.D., have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that lutein, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the specifications described herein, is safe under its intended conditions of use. The Expert Panel further unanimously concludes that these uses of lutein are GRAS based on scientific procedures, and that other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

F. Availability of Information
The detailed data and information that serve as a basis for this GRAS determination will be provided to the U. S. FDA upon request, or are available for the FDA's review and copying during reasonable business hours at the offices of NutraSource:
6309 Morning Dew Ct., Clarksville, MD 21029, USA.

G. Basis of GRAS determination: Through scientific procedures.

H. Review and copying statement: The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of notifier, or will be sent to FDA upon request.

EXPERT PANEL STATEMENT

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF INNOBIO® LUTEIN AS A FOOD INGREDIENT

Prepared for INNOBIO®

Prepared by: NutraSource, Inc.
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1. INTRODUCTION

The undersigned, an independent panel of recognized experts (herein after referred to as the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by NutraSource, Inc., at the request of INNOBIO® Limited (INNOBIO®), to determine the Generally Recognized As Safe (GRAS) status of its lutein as a food ingredient (a nutrient) [21CFR 170.3(o)(20)] in selected food products (baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute products; fats and oils; frozen dairy desserts and mixes; gravies and sauces; hard candy; infant and toddler foods; milk products; processed fruits and fruit juices; soft candy; soups and soup mixes) at use levels of 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on lutein was conducted and made available to the Expert Panel members. The Expert Panel members independently and critically evaluated materials submitted by INNOBIO® and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel unanimously agreed to the decision described herein.

II. INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Common or Trade Name: Lutein produced by INNOBIO®
The trade names are:
INNOBIO® Crystalline Lutein,
INNOBIO® Lutein 20% Oil Suspension,
INNOBIO® Lutein 20% Cold Water Dispersible (CWD) Powder,
INNOBIO® Lutein 10% CWD Powder,
INNOBIO® Lutein 10% Beadlets,
INNOBIO® Lutein 5% Beadlets, and
INNOBIO® Lutein 5% Cold Water Soluble (CWS) Powder

II.B. Background

Lutein and zeaxanthin belong to the class of plant compounds called carotenoids, and are present in a wide variety of fruits and vegetables (Sommerberg et al., 1998). Interestingly, while other tissues contain a mixture of other carotenoids, the macular pigment in the human retina contains only lutein and zeaxanthin, often referred as xanthophylls or macular pigment (MP; Sommerberg et al., 1998). The lutein and zeaxanthin concentration in the eye can be 500-fold higher than in other tissues (Rasmussen and Johnson, 2013).

Lutein is an important nutrient with a recognized role in providing protection of the macula from photo-oxidative stress and evidence indicates that lutein protects the retina from the onset of age related macular degeneration (AMD; Bone and Landrum, 2010; Gao et al., 2011; Landrum and Bone, 2001; Mares et al., 2011). There is accumulating evidence to suggest that lutein and zeaxanthin intakes are positively related to macular pigment optical density (MPOD)—an increased concentration of retinal carotenoids in the MP. In fact, elevated MPOD has been directly associated with foveal function in patients with maculopathy, to preserved visual sensitivity in healthy elderly individuals and to a decreased risk of AMD (Mares-Perlman et al., 2002). AMD is the leading cause of irreversible vision loss in the elderly population.

The human body does not produce the dietary zeaxanthin and lutein it needs daily, so it is essential to get these nutrients through diet and/or supplementation. Unlike β-carotene, these two carotenoids do not have vitamin A activity (IOM, 2001). Of the 20–30 carotenoids found in human blood and tissues, only lutein and zeaxanthin are found in the lens and retina (Yeum et al., 1999). Lutein and zeaxanthin are concentrated in the macula or central region of the retina, and are referred to as macular pigment. In addition to their role as antioxidants, lutein and zeaxanthin are believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching reactive oxygen species (Krinksy, 2002). While there is no RDA for lutein and zeaxanthin, intakes of approximately 6-10 mg/day have been associated with a decreased risk of AMD (Seddon et al., 1994).

The current intakes of lutein and zeaxanthin among adults 50 years of age falls well below this level, with an intake range of lutein at 0.6-2.4 mg/day. Only men in the 99th percentile of lutein/zeaxanthin intake and women in the 95th percentile meet the dietary intakes that have been associated with decreased risk of AMD (Rasmussen and Johnson, 2013). Most Americans consume only 10 percent of the zeaxanthin and lutein needed each day to protect their eyes and keep them healthy.

Zeaxanthin, like lutein, acts as a protective antioxidant in the eye and is proven to promote eye health (Rasmussen and Johnson, 2013). Scientific evidence shows that supplementing the diets with lutein and zeaxanthin daily keeps eyes healthy, improves visual performance, and helps reduce the risk of certain eye conditions.

II.C. Chemistry, Physico-chemical Properties, and Structure

Table 1. Chemical and Physical Characteristics of Lutein and Zeaxanthin (free form)

Property/Parameter	Lutein	Zeaxanthin
CAS Registry No.	127-40-2	144-68-3
Chemical Names	Xanthophyll; beta, e-carotene-3,3'-diol (3*R*,3′*R*,6′*R*); *all-trans*-(+)-xanthophyll; alltrans-lutein	Beta 3, 6-carotene-3,3'-diol; all-trans-I3-carotene-3,3'-diol; (3R,3' R)-dihydroxy-beta-carotene;
Empirical formula	$C_{40}H_{56}0_2$	$C_{40}H_{56}0_2$
Molecular weight	568.88	568.88
Physical state	Crystalline	Crystalline
Melting Point	177-178°C	207-215.5°C
Density	0.35-0.40 g/mL	0.38-0.41 g/mL
Solubility in water at 5°C	Insoluble	Insoluble

*Based on information from INNOBIO® and other publicly available sources

Figure 1. Chemical Structure of Lutein

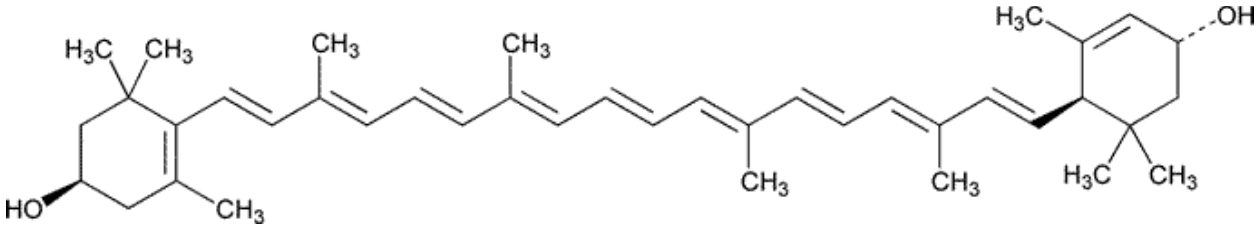
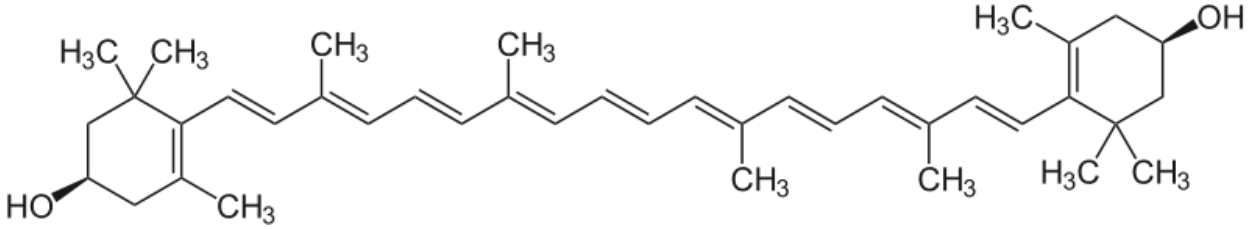


Figure 2. Chemical Structure of Zeaxanthin



II.D. Manufacturing Process of INNOBIO® Lutein Preparations

INNOBIO® Lutein preparations are naturally derived from marigold flower (*Tagetes erecta*) and consist of all trans-free lutein as the predominant constituent with a small amount of zeaxanthin. Manufacturing processes of INNOBIO® Lutein products are described as follows.

General Description of INNOBIO®'s Manufacturing Process

Current Good Manufacturing Practice (cGMP), which includes Hazard Analysis and Critical Control Point (HACCP) risk analysis as well as hygiene and transport standards, is applied throughout each manufacturing step of INNOBIO® products; continuous monitoring is used for quality control. Sampling schemes and analysis schedules are applied to raw materials, intermediate products and final products. Pesticide residues, chemical contaminants and residual solvents are measured at different stages to make sure the finished product meets the required specifications. State-of-the-art facilities and electronic monitoring systems enable precise process control at all steps. All processing aids, such as solvents and water, buffer salts and other ingredients used in the manufacturing process are food grades.

Manufacturing Process of INNOBIO® Crystalline Lutein

The manufacturing process of INNOBIO® Crystalline Lutein starts with fresh marigold flowers that are dried in a hot air dryer to prepare meal. Marigold oleoresin is obtained from the marigold meal by hexane extraction, which is unique and advanced in a few ways. First, absorbents such as activated carbon, clay and diatomite are added to the hexane extract to remove contaminants such as heavy metals and pesticide residues. Second, microfiltration is applied to eliminate impurities introduced from hexane extraction, including black specks, plant fibers, pollen, etc. Finally, marigold oleoresin is obtained by reduced pressure distillation to avoid degradation of carotenoids. The oleoresin is then subjected to saponification as described below to obtain the crystalline lutein with defined qualities. It is worth noting that only alcohol solvents (ethanol,

isopropyl alcohol, or mixture of both) but no other organic solvents are used in the saponification process to ensure the product safety.

The marigold oleoresin is mixed with the alcohol solvent and excess solid sodium hydroxide under controlled heating (50-60℃) and stirring. The resultant solution is then diluted with water to precipitate more crystalline lutein at 30-50℃. The residual sodium hydroxide is then neutralized with the acetic acid solution to make the saponification mixture slightly basic (pH 7.5-9.0). The wet cake of crystalline lutein is then obtained by filtration and purified by washing with the alcohol/water solution (V/V 40-70%) and alcohol. After mixing with a small quantity of antioxidants, the wet crystalline lutein is dried at 50-90℃ under vacuum to remove the alcohol solvent and water. At each filtration step, sodium fatty acids, alcohol soluble impurities and residual sodium hydroxide are removed from the wet cake of crystalline lutein. Finally, crystalline lutein is sieved with the 40 mesh and then packaged in a 5kg net aluminum foil bag under vacuum to get INNOBIO® Crystalline Lutein.

INNOBIO's innovative technology is environment friendly for crystalline lutein preparation. Contaminant removal increases the quality of crystalline lutein, use of only alcohol solvents guarantees the product safety, and the environment-friendly neutralization by acetic acid allows waste water to reach discharge standards.

Manufacturing Process of INNOBIO® Lutein 20% Oil Suspension

INNOBIO® Lutein 20% Oil Suspension is made by suspending the crystalline lutein in vegetable oils (e.g., such as sunflower oil, safflower oil or corn oil) to a final concentration of at least 20% of all trans-free lutein. Lutein oil suspension is milled to the D50 diameter of 80μm or less, then packaged in the 20kg net aluminum drum under nitrogen.

Manufacturing Process of INNOBIO® Lutein 5% CWS Powder, Lutein10% CWD Powder, and Lutein 20% CWD Powder

INNOBIO® Lutein 5% CWS powder is an orange red, free flowing powder microencapsulated with food grade ingredients (mixture of modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch) and contains at least 5% of all-trans free lutein. INNOBIO® Lutein 10% and 20% CWD powder are orange red, free flowing powders microencapsulated with food grade ingredients (mixture of modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch) and contain at least 10% and 20% of all-trans free lutein respectively. However, the concentrations of modified starch and gum arabic vary: 20-30% for CWS and >30% for CWD preparations. The CWS and the CWD powders have different applications but share an identical manufacturing process. The process starts from admixing modified starch, gum arabic, solid corn syrup, tocopherols, ascorbyl palmitate, sodium ascorbate, and corn starch with water at the temperature range of 40-80℃for complete dissolution. Crystalline lutein is then added to the mixture which is stirred to form an emulsion. The emulsion of lutein is milled and then spray dried at the temperature range of 140-200℃ to form powder. After sieving and mixing, the lutein powder is packaged in a 5kg net aluminum foil bag under nitrogen.

Manufacturing Process of INNOBIO® Lutein 5% Beadlets and Lutein 10% Beadlets
INNOBIO® Lutein 5% Beadlets and 10% Beadlets are reddish brown free flowing beadlets microencapsulated with food grade ingredients (mixture of gelatin, sucrose, modified starch, gum arabic, tocopherols, ascorbyl palmitate, and corn starch) and contain at least 5% and 10% of all-trans free lutein respectively. The production starts from admixing coating materials, the emulsifier and the antioxidant with water at a temperature range of 40-80℃ for complete dissolution. Crystalline lutein is then added to the mixture and stirred to form an emulsion. The emulsion of lutein is milled and then spray dried at a temperature range of 80-180℃ to form powder, which goes through fluidized corn starch to form lutein beadlets. The beadlets are then dried at the temperature range of 10-60℃ to remove the residual water. After sieving, the lutein beadlets are packaged in a 5kg net aluminum foil bag under nitrogen.

II. E. Specifications of Lutein
Tables 2-1 - 2-7 list specifications of various lutein preparations.

Table 2-1. Specifications for INNOBIO® Crystalline Lutein

Parameter		Method
Appearance	Reddish-orange powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27
Lutein, %	70.0 Min.	USP 32/NF 27
Zeaxanthin, %	4.0 Min.	USP 32/NF 27
Moisture, %	1.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-2. Specifications for INNOBIO® Lutein 20% Oil Suspension

Parameter		Method
Appearance	Orange-red oil suspension	Visual
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27
Lutein, %	20.0 Min.	USP 32/NF 27
Zeaxanthin, %	1.0 Min.	USP 32/NF 27
Moisture, %	1.0 Max.	AOCS Td 2-64
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-3. Specifications for INNOBIO® Lutein 20% CWD Powder

Parameter		Method
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27
Lutein, %	20.0 Min.	USP 32/NF 27
Zeaxanthin, %	1.0 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467

Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-4. Specifications for INNOBIO® Lutein 10% CWD Powder

Parameter		Method
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27
Lutein, %	10.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.5 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size, through 40 mesh	95%	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-5. Specifications for INNOBIO® Lutein 10% Beadlets

Parameter		Method
Appearance	Orange-red, free-flowing beadlets	Visual
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27
Lutein, %	10.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.5 Min.	USP 32/NF 27
Moisture, %	7.0 Max.	USP 731
Particle Size		
-through sieve No.20	100	USP 786
-through sieve No.40	85.0 Min.	USP 786
-through sieve No.100	15.0 Max.	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-6. Specifications for Lutein 5% Beadlets

Parameter		
Appearance	Orange-red, free-flowing beadlets	Visual
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27
Lutein, %	5.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.25 Min.	USP 32/NF 27
Moisture, %	7.0 Max.	USP 731
Particle Size		
-through sieve No.20	100	USP 786
-through sieve No.40	85.0 Min.	USP 786
-through sieve No.100	15.0 Max.	USP 786
Heavy metals		

Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

Table 2-7. Specifications for INNOBIO® Lutein 5% CWS Powder

Parameter		
Appearance	Orange-red, free-flowing powder	Visual
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27
Lutein, %	5.0 Min.	USP 32/NF 27
Zeaxanthin, %	0.25 Min.	USP 32/NF 27
Moisture, %	5.0 Max.	USP 731
Particle Size	95% through 40 mesh	USP 786
Heavy metals		
Lead, ppm	1.0 Max.	EN ISO 17294
Arsenic, ppm	0.2 Max.	EN ISO 17294/ AOAC 2013.06
Cadmium, ppm	1.0 Max.	EN ISO 17294
Mercury, ppm	0.1 Max.	BS EN 13806
Residual solvents		
Hexane, ppm	10.0 Max.	USP 467
Residual alcohol, ppm	50.0 Max.	USP 467
Microbiological		
Total plate count, cfu/g	1,000 Max.	ISO 4833
Yeast and molds, cfu/g	100 Max.	ISO 7954
Coliform , cfu/g	10 Max.	ISO 4832
E. coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Staphyloccous aureus	Negative/g	ISO 6888

III. INTENDED USES AND EXPOSURE ESTIMATES

III.A. Food Sources of Lutein and Zeaxanthin

Lutein and zeaxanthin are carotenoids found in high quantities in green leafy vegetables. Other foods (Table 3) that contribute a significant level of dietary lutein include egg yolk and corn. Similarly, considerable dietary zeaxanthin is provided by egg yolks, corn, orange peppers, orange juice, oranges and honeydew melons (Sommerberg et al., 1998). The daily intake for lutein and zeaxanthin is on average lower than that of β-carotene and lycopene, but levels of approximately 0.6-2.4 mg/day are attained with a diet rich in fruits and vegetables.

Table 3. Fruit and Vegetable Sources of Lutein and Zeaxanthin

Fruit of Vegetable	Lutein + Zeaxanthin	Lutein	Zeaxanthin
Egg Yolk	89	54	35
Corn	85	60	25
Kiwi	54	54	0
Red Seedless Grapes	53	43	10
Zxucchini squash	52	47	5
Pumpkin	49	49	0
Spinach	47	47	0
Orange Pepper	45	8	37
Yellow Squash	44	44	0
Cucumber	42	38	4
Pea	41	41	0
Green Pepper	39	36	3
Red Grape	37	33	4
Butternut Squash	37	37	0
Orange Juice	35	15	20
Honeydew	35	17	18
Celery (stalks, leaves)	34	32	2
Green Grapes	32	25	7
Brussels Sprouts	29	27	2
Scallions	30	27	3
Green Beans	25	22	3
Orange	22	7	15
Broccoli	22	22	0
Apple (Red Delicious)	20	19	1
Mango	18	2	16
Green Lettuce	15	15	0
Tomato Juice	13	11	2
Peach	13	5	8
Yellow Pepper	12	12	0
Nectarine	12	6	6
Red Pepper	7	7	0

The content of the major carotenoids are given in mole %. Source: Sommerburg et al., 1998.

III.B. Intended technical effects

INNOBIO® Lutein (crystalline, powder, beadlets, or oil suspension) can be used as a nutrient as a source of lutein at concentrations consistent with cGMP.

Use of lutein preparations in the above described food categories may also incidentally contribute its own color to the product. Its intended nutritional use would thus fall outside the definition of "color additive," in accordance with 21 CFR 70.3(f), "Food ingredients ...which contribute their own natural color when mixed with other foods are not regarded as *color additives...."*

III.C. Intended use

As INNOBIO® Crystalline Lutein represents the concentrated form with ≥70% lutein and ≥4.0% zeaxanthin. Table 4 represents the quantity of lutein when used as such, or the quantity present in a standardized diluted form using food grade/GRAS ingredients.

INNOBIO® lutein preparations are intended for use as a food ingredient in the following food categories: baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg substitute 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 at use levels of 0.3 to 3 mg lutein/serving (reference amounts customarily consumed, 21CFR 101.12). A summary of use levels and food categories for lutein is presented in Table 4. INNOBIO® Lutein will not be added to food categories that come under USDA jurisdiction.

The INNOBIO® intended uses of lutein preparation are expanded from food products listed in the GRN 000140 (FDA, 2004) by adding protein bars, low sodium tortillas, and vitamin water at a level of 2 mg lutein/Reference Amounts Customarily Consumed per eating occasion (RACC).

Table 4. Food Categories and Intended Use Levels of Lutein in the U.S.

Food Category	Proposed Food	Use Levels				
		Lutein, mg/RACC[1,2]	INNOBIO crystalline lutein (≥70%)	20% lutein, mg/RACC	10% lutein, mg/RACC	5% lutein, mg/RACC
Baked Goods and Baking Mixes	**Protein bars,** cereal bars, and energy bars	2.0	2.86	10.0	20.0	40.0
	crackers and crisp-breads	2.0	2.86	10.0	20.0	40.0
	Tortillas, low sodium	2.0	2.86	10.0	20.0	40.0
Beverages and Beverage Bases	**Vitamin water**	2.0	2.86	10.0	20.0	40.0
	Bottled water	0.5	0.72	2.5	5.0	10.0
	Carbonated beverages; Meal replacements	2.0	2.86	10.0	20.0	40.0
	Tea, ready-to-drink	0.6	0.86	3.0	6.0	12.0
Breakfast Cereals	Instant and regular hot cereals	2.0	2.86	10.0	20.0	40.0
	Ready-to-eat cereals	2.0	2.86	10.0	20.0	40.0
Chewing Gum	Chewing gum	1.0	1.43	5.0	10.0	20.0
Dairy Product Analogs	Imitation milks	2.0	2.86	10.0	20.0	40.0
	Soy milks	1.5	2.14	7.5	15.0	
Egg Substitutes	Liquid, frozen or dried egg substitutes	2.0	2.86	10.0	20.0	40.0
Fats and Oils	Margarine-like spreads, salad dressings	1.5	2.14	7.5	15.0	30.0
Frozen Dairy Desserts and Mixes	Frozen yogurt	1.0	1.43	5.0	10.0	20.0
Gravies and Sauces	Tomato based sauces	0.3	0.43	1.5	3.0	6.0
Hard Candy	Hard candy	1.0	1.43	5.0	10.0	20.0
Infant and Toddler Foods	Junior, strained and toddler-type baby foods	1.0	1.43	5.0	10.0	20.0
Milk Products	Dry milk flavored milk and milk drinks	3.0	4.29	15.0	30.0	60.0
	Fermented milk beverages	0.6	0.86	3.0	6.0	12.0
	Yogurt	3.0	4.29	15.0	30.0	60.0
	Milk based meal replacement	2.0	2.86	10.0	20.0	40.0
Processed Fruits and Fruit Juices	Energy, sport and isotonic drinks	2.0	2.86	10.0	20.0	40.0
	Fruit-flavored drinks, fruit juice, nectars	2.0	2.86	10.0	20.0	40.0
	Vegetable Juice	2.0	2.86	10.0	20.0	40.0
Soft Candy	Chewy and nougat candy; Fruit snacks	1.0	1.43	5.0	10.0	20.0
Soup and soup mixes	Canned soup	0.6	0.86	3.0	6.0	12.0

[1]Use levels are the same as those described in GRN 140 except 3 newly added items. [2]RACC Reference amounts customarily consumed per eating occasion (21 CFR § 101.12). When a range of use-levels (%) is reported for a proposed food use, particular foods within that food-use may differ with respect to their RACC.

III.D. Exposure estimates

Estimated Daily Intakes (EDIs) before the intended use

The European Food Safety Authority (EFSA, 2010) estimated that an average intake for adults from the regular diet amounts to 0.01- 0.04 mg/kg BW/day, corresponding to 0.6-2.4 mg/person/day for a 60 kg adult.

EDIs under the intended use

Based on food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2009-2010) compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics and the Nutrition Coordinating Center, estimates of 2-day average intakes of lutein were calculated from the food code list and the survey database of diet recalls. The 2009-2010 NHANES provides the most current food consumption data available for the non-institutionalized individuals in the U.S. Table 5 shows EDIs under the intended use.

Table 5. EDIs under the intended use

Population	Sample N	EDIs				
		Unit	Mean	SE	P90	SE
All population, aged 2+y	9,042	mg	8.70	0.120	16.53	0.256
All population, aged 2+y	8,971	mg/kg bw	0.143	0.0027	0.289	0.0064
All-users, aged 2+y	8,848	mg	8.88	0.113	16.67	0.251
All-users, aged 2+y	8,780	mg/kg bw	0.146	0.0026	0.291	0.0062

P90=90th percentile; SE=standard error.

The mean intakes of lutein under the intended uses were estimated to be 8.7 mg/person/day for all population and 8.9 mg/person/day for all-users, while the 90th percentile intakes were determined as 16.5 and 16.7 mg/person/day (or 0.289 and 0.291 mg/kg BW/day), respectively. These EDIs are based on the assumption that 100% of the products listed in GRN 140 and newly added food categories (1.6 mg/person/day) are used at the maximum intended use levels. Intake from regular diets is expected to be less than 2.4 mg/day. Estimated cumulative exposure (intakes from regular diets, and expanded list of food categories) is estimated to be no more than 19.1 mg/person/day.

As reported in GRN 140, the current 90th percentile intake of zeaxanthin from specified foods were estimated to be 1.2 mg/person/day. The contribution of newly added food categories is estimated to be 0.079 mg/person/day at the 90th percentile of all-users. Even if 100% of the products listed in GRN 140 (1.2 mg/person/day) and newly added food categories are used at the maximum intended use levels, the 90th percentile intake of zeaxanthin from all GRAS-proposed use categories by users of one or more foods is 1.3 mg/person/day for all-users.

However, these estimates are highly optimistic since all foods under the intended uses will not be used at the maximum levels. For example, less than 1% of bottled water and carbonated beverages contain lutein in the market place, although EDI calculations have assumed that 100% of these products will be used at the maximum intended use levels.

These exposure estimates (EDI under the untended uses, and/or cumulative exposure) are far below the Acceptable Daily Intake (ADI) of up to 1-2 mg/kg BW/day or 60-120 mg/person/day established by The Panel on Food Additives and Nutrient Sources added to Food of the EFSA (EFSA, 2010) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), based on animal and human studies (JECFA, 2006). In addition, these exposure estimates are below the upper limit of Observed Safe Level (OSL; 20 mg/person/day) established by Shao and Hathcock (2006), based on a review of 30 human clinical studies.

IV. BASIS FOR GRAS DETERMINATION

IV.A. Current regulatory status

The FDA has received seven GRAS Notices related to food uses of lutein and lutein esters: 3 GRAS notifications on crystalline lutein to the FDA for general food applications (GRN 140; FDA, 2004; GRN 291, FDA, 2009; GRN 385, FDA, 2011), 2 notices for lutein esters for general food applications (lutein dipalmitate-GRN 110, FDA, 2003; lutein diacetate- GRN 432, FDA, 2012), and 2 notices on suspended lutein in oil for infant formula applications (GRN 221, FDA, 2007; GRN 390, FDA, 2012).

In these GRAS notices, toxicity-related studies on lutein from the literature were presented which support the safety in use of lutein. The FDA did not question the acceptability and suitability of these studies to establish the safety of lutein for the proposed food uses. The FDA did not have questions on summary of safety concluding that lutein intake up to 20 mg/person/day is safe. The safety and related information in the above mentioned GRAS Notices is hereby incorporated by reference into this independent GRAS determination. The pertinent information is available as indicated below:

GRN 140: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=140.
GRN 291: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=291.
GRN 385: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=385.
GRN 110: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=110.
GRN 432: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=432.
GRN 221: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=221.
GRN 390: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=390.

In addition, The Panel on Food Additives and Nutrient Sources added to Food of the EFSA (2010) has established an ADI of 1 mg/kg BW/day or 60 mg/day for a 60 kg adult, while JECFA established ADI for lutein from *Tagetes erecta* L. and synthetic zeaxanthin) of up to 2 mg/kg BW/day or up to 120 mg/person based on animal and human studies (JECFA, 2006).

A comparison between GRAS notices for lutein (for general food applications) and current GRAS determination is presented in Table 6.

Table 6. Summary of safety assessments of lutein reported in GRAS notices and current GRAS determination for general food applications

Parameters	GRN 140	GRN 291	GRN 385	JECFA, 2006	Current notice
	Crystalline lutein m.w. 568.88	Crystalline lutein m.w. 568.88	Crystalline lutein m.w. 568.88	Lutein	Crystalline lutein m.w. 568.88
Lutein content	76% (>74%)	>74%	>67%	Not reported	≥70.0% wt
Z content	7% (2-9%)	<8%	>13.5%; Z:MZ=1:1	Not reported	≥4.0% wt
Intended use	Expanded from GRN 110	Same as GRN 140	Same as GRN 140	Not reported	Expanded from GRN 140
EDI of lutein (mg/person/day) at 90th percentile	13.4* (or 0.28 mg/kg BW/day)	13.4* (or 0.28 mg/kg BW/day)	13.4* (or 0.28 mg/kg BW/day)	Not reported	16.7** (or 0.291 mg/kg BW/day)
Cumulative EDI of lutein (mg/person/day) at 90th percentile	Not reported	Not reported	Not reported	Not reported	19.1 (16.7 under the intended uses + 2.4 from regular diets)
EDI of Z (mg/person/day) at 90th percentile	1.2 mg (or 0.03 mg/kg BW/day)	1.2 mg (or 0.03 mg/kg BW/day)	Z-2.4 (3R,3'R-1.2; MZ, 1.2)	Not reported	1.3 (Current use, 1.2 + 0.08 from the newly added products)
ADI of lutein	Not reported	Not reported	Not reported	Up to 120 mg/day	Not reported
Safety determination	Totality of evidence	Totality of evidence	Totality of evidence	NOAEL values from animal studies and human clinical studies	Totality of evidence

Z=zeaxanthin; MZ=meso-zeaxanthin; *EDI of GRN 140 is based on the CSFII 1994-1996, 1998 database;
**EDI of the current notice is based on the NHANES 2009-2010 database.

IV.B. Review of safety data

As noted above, the FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. For each of these notices, after FDA's review, the agency issued letters indicating that it had no questions concerning the safety of lutein and its esters. As the lutein in this GRAS determination is similar in specifications compared to the other luteins in the FDA GRAS Notices, it is recognized that the information and data in the other GRAS Notices is pertinent to the safety of the lutein in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRNs 110, 140, 221, 291, 385, 390, and 432. Additionally, this notice discusses additional studies that have been published since the FDA's last reviews of 2011-2012. The subject of present GRAS assessment is lutein.

IV.B.1. Metabolism of lutein

Oral ingestions of lutein and zeaxanthin are likely to follow the same digestion and intestinal absorption pathways as dietary fat (Borel et al., 2007). Upon absorption, both lutein and zeaxanthin are incorporated into chylomicrons, and are approximately evenly distributed between high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in circulation (Goulinet and Chapman, 1997). This is in contrast with hydrocarbon carotenoids (lycopene, α- and β-carotene) which are transported in LDL (45% and 76%, respectively). Lutein appears to be distributed to tissue following the interaction of lipoprotein particles with receptors, and degradation of lipoproteins by extra hepatic enzymes such as lipoprotein lipase. Uptake and transport of lutein and zeaxanthin (xanthophyll carotenoids) from the bloodstream into the human macula are mediated by specific xanthophyll-binding proteins and human retinal lutein-binding protein may facilitate lutein's localization to human macula (Bhosale et al., 2009). Leo et al. (1995) suggested that most of lutein is likely to undergo biliary excretion into the feces in humans, that biliary concentrations reflect plasma levels in both normal and pathologic states, and that decreased biliary excretion of carotenoids does not increase plasma concentrations.

IV.B.2. Mutagenicity and Genotoxicity studies

In the previous GRAS Notices to the FDA, the safety of lutein has been established in mutagenicity and genotoxicity studies. Since the last review of 2012, no new mutagenicity studies have been published. Thus, this GRAS Notice summarizes the studies already reviewed in previous GRAS notices. As shown in Table 7, lutein was not mutagenic or clastogenic (Kruger et al., 2002; Kuttan and Sabu, 2004; Ravikrishan et al., 2011; Wang et al., 2006). Also, an *in vivo* rat study showed that lutein acted as an inhibitor during promotion, but not initiation of hepatocarcinogenesis (Moreno et al., 2007).

A recent mutagenicity test by Ravikkrishan et al. (2011) employed *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537) with and without metabolic activation (S9). Lutein/zeaxanthin concentrate (67% lutein +13.5% 1:1 mixture of zeaxanthin and meso-zeaxanthin) was dissolved in dimethyl sulfoxide (DMSO) and was tested in two independent experiments at concentrations of 31, 63, 125, 250 and 500 ug/plate. No substantial increases in the revertant colony count in any of the five strains were reported at any of the test concentrations in the presence or absence of metabolic activation (S9 mix). Positive controls resulted in significant increases in the revertant count. The spontaneous reversion rates in the negative and positive control were within the range of historical data. In the second experiment, no biologically relevant increase in the revertant counts was observed in any of the five tester

strains pre-incubated with the test item. The results of these investigations suggest that under the experimental conditions, lutein/zeaxanthin concentrate did not induce gene mutation.

However, an *in vitro* comet assay showed conflicting results. Santocono et al. (2006) determined whether these compounds were capable of minimizing DNA damage and influencing the kinetics of DNA repair. DNA damage was assessed using the Comet assay to detect primary DNA damage in individual cells. The addition of zeaxanthin (after 60 min incubation) and lutein (after 180 min incubation) to rat epithelial cells after 2 min of UVA exposition showed a genotoxic effect. The addition of carotenoids to neuroblastoma cells after 30 min UVA exposition positively influenced the kinetics of DNA repair in the first 15min of incubation. At longer exposition times, while the behaviour measured was not constant, a genotoxic effect was not observed. Another Comet assay of serum lymphocytes from humans did not show any DNA damage in response to lutein intakes at a dose of 15 mg/day for 12 weeks (Collins et al., 1998). In this study, comet assay (single cell alkaline gel electrophoresis) was employed to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes from 40 volunteers supplemented with alpha/beta-carotene, lutein, lycopene or placebo. There was a significant negative correlation between basal concentrations of lutein and oxidized pyrimidines. Overall, there is no evidence that lutein is mutagenic, genotoxic, or clastogenic.

Table 7. Summary of mutagenicity and genotoxicity studies

Test	Study condition	Results	Reference
Ames test; *in vitro* mutagenic potential	Lutein at conc. of 334, 668 and 1335 ug/plate; (*Salmonella typhimurium* strains TA97, TA98, TA100 and TA102) in the presence and absence of S9 mix.	Dose-related anti-mutagenic effect	Wang et al. 2006
Ames test; *in vitro* mutagenic potential	Crystalline lutein (75% purity) at conc. of 31-500 ug/plate; (*Salmonella typhimurium* strains TA98 andTA100) in the presence and absence of S9 mix.	Not mutagenic	Ravikrishan et al., 2011
Ames test; *in vitro* mutagenic potential	*Salmonella typhimurium* strains (TA98, TA100 and TA1535) in the presence and absence of S9 mix.	Dose-related anti-mutagenic effect	Kuttan and Sabu, 2004; cited in GRN 385
Ames test; *in vitro* mutagenic potential	Crystalline lutein 10% beadlet at conc. of 100-5,000 ug/plate; (*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA 1535) in the presence and absence of S9 mix.	Not mutagenic	Kruger et al., 2002
In vitro clastogenic potential	Standard chromosome aberration test using Chinese hamster ovary (CHO) cells; lutein (66.8, 133.5 and 267.0 mg/L	Not clastogenic	Wang et al., 2006
In vitro comet assay assessing DNA damage	UVA (2 min)-induced DNA damage in rat tracheal epithelial cells and in human neuroblastoma cells The presence of carotenoids reduced DNA damage when rat epithelial cells were exposed to UVA radiation for 2min.	Lutein (after 180 min incubation) showed a genotoxic effect. At longer exposure times, a genotoxic effect was not observed.	Santocono et al., 2006
Comet assay of lymphocytes from humans	Consume 15 mg/day lutein for 12 weeks and then comet assay of blood lymphocytes to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes	Lutein has anti-genotoxic effects (decreased oxidized pyrimidines)	Collins et al., 1998

IV.B.3. Animal Toxicity Studies

Previous seven GRAS notifications on lutein and its esters (GRN 140, 221, 385, 291, 390 110, 432) extensively reviewed animal toxicity data for lutein and its esters. This GRAS notice

included an additional study (Nidhi and Baskaran, 2013) since the submission of GRN 385 and 432. As shown in Table 8, the safety of lutein has been proven in various animal models.

IV.B.3.1. Acute and subacute toxicity studies

Harikumar et al. (2008) reported that the oral LD_{50} of lutein (85% purity in sunflower oil) isolated from marigold was >4,000 mg/kg BW in female Wistar rats. The oral LD_{50} for a purified plant extract containing 70 to 85% lutein was determined to be >2,000 mg/kg BW in male and female Wistar rats (JECFA, 2006; Ravikkrishan et al., 2011). In a preliminary acute toxicity study of Nidhi and Baskaran (2013), LD_{50} of lutein from *Tagetes erecta* in lutein-deficient male mice was determined to be higher than 10,000 mg/kg BW, the highest dose tested.

In a 4 week subacute toxicity study, the administration of lutein via the diet or by gavage was not associated with toxicologically-significant adverse effects at doses up to 639 mg/kg BW/day for lutein in rats, the highest level tested (Kruger et al., 2002). The study of Harikuma et al. (2008) reported the no-observed-adverse-effect level (NOAEL) for lutein as 400 mg/kg BW/day, the highest dose tested in this rat study. In a recent subacute study of Nidhi and Baskaran (2013), male mice were gavaged with 0, 100, 1,000 mg/kg BW/day for a period of 4 weeks. Plasma lutein levels increased dose dependently ($P < 0.01$) after acute and subacute feeding of lutein in lutein-deficient mice. Compared to the control (peanut oil without lutein) group, no treatment-related toxicologically significant effects of lutein were prominent in clinical observation, ophthalmic examinations, body and organ weights as well as hematological, histopathological, and other clinical chemistry parameters. The authors concluded that the NOAEL for lutein in lutein-deficient mice was determined as 1,000 mg/kg BW/day, the highest dose tested.

Thus, it is summarized that LD_{50} of lutein is higher than 10,000 mg/kg BW in lutein-deficient male mice (Nidhi and Baskaran, 2013) and the NOAEL for lutein exceeds 1,000 mg/kg BW/day in male mice (Nidhi and Baskaran, 2013), the highest dose tested. In rats, the NOAEL for lutein exceeds 639 mg/kg BW/day, the highest dose tested (Kruger et al., 2002).

IV.B.3.2. Subchronic toxicity studies

In 13 week subchronic toxicity studies, the administration of lutein via the diet or by gavage was not associated with toxicologically-significant adverse effects at doses up to 400 mg/kg BW/day in rats, the highest level tested (Harikumar et al., 2008; Kruger et al., 2002; Ravikrishan et al., 2011). The 13 week rat study of Kruger et al. (2002) reported the NOAEL of 260 mg/kg BW/day, the highest dose tested. In 90 day subchronic rat studies of Harikumar et al., (2008) and Ravikrishan et al. (2011), the NOAEL was determined to be 400 mg/kg BW/day, the highest level tested. Thus, it is summarized that the NOAEL for lutein exceeds 400 mg/kg BW/day, the highest level tested in rats (Harikumar et al., 2008; Ravikrishan et al., 2011).

In addition, consumption of lutein at a dose of 9.3 mg/kg BW/day for 1 year did not result in adverse effects in monkeys (Khachik et al., 2006).

IV.B.3.3. 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 BW/day on gestation days 6 to 20. No maternal toxicity or embryotoxicity/teratogenicity reported (Edwards et al., 2002; cited in GRN 390).

Table 8. Summary of animal toxicity studies of lutein

	Daily dose, mg/kg BW	Duration	NOAEL, mg/kg BW/day	Reference
13 Rhesus monkeys	Lutein 9.34 +Z 0.66 mg/kg BW; Z, 10 mg/kg BW; Lutein+Z, 0.5 mg/kg BW each; or control	12 mo	NOAEL- lutein, 9.34 mg/kg BW; Z, 10 mg/kg BW, the highest levels tested	Khachik et al., 2006
70 young adult female Wistar rats	Lutein* (85% purity in sunflower oil); 1,000, 2,000, or 4,000 mg/kg BW	Single dose; observed 2 wk	No mortality at 4,000 mg/kg BW	Harikuma et al., 2008
6 young (9-10 wk old) female Wistar rats	Lutein* (crystalline; >67% purity+13.5% Z); 2,000 mg/kg BW	Single dose; observed 2 wk	No mortality at 2,000 mg/kg BW	Ravikrisha n et al., 2011
50 lutein-deficient mice	0.57 (physiological dose), 100, 1,000, and 10,000 mg/kg BW	Single dose; observed 2 wk	$LD_{50,}$ >10,000 mg/kg BW, the highest dose tested	Nidhi and Baskaran, 2013
84 male and female Wistar rats	2.6, 7.7, 26.0, 77.3, 260, or 773.2 mg crystalline lutein+Z /kg BW (>77% purity; >75% lutein +>2% Z); corresponding to 2.1, 6.4, 21.3, 63.9, 213 or 638.7 mg/kg BW for pure lutein+Z	4 wk	NOAEL- 773 mg/kg BW for crystalline lutein+Z with >77% purity (>75% lutein+>2% Z); or 638.7 mg/kg BW for pure lutein+Z	Kruger et al., 2002
30 male mice	100, or 1,000 mg/kg BW	4 wk	NOAEL- 1,000 mg/kg BW/day	Nidhi and Baskaran, 2013
50 young (~4 wk) male and female Wistar rats	2.6, 26, or 260 mg/kg BW for crystalline lutein+Z (>77% purity; >75% lutein +>2% Z); corresponding to 2.1, 22.4, or 208 pure lutein+Z	13 wk	NOAEL- 260 mg/kg BW for crystalline lutein+Z with >77% purity (>75% lutein+>2% Z); or 208 mg/kg BW for pure lutein+Z	Kruger et al., 2002
80 young (6-9 wk old) male and female Wistar rats	4, 40, and 400 mg/kg BW crystalline lutein (>67% lutein +13.5% Z with both Z isomers)	90 d	NOAEL- 400 mg/kg BW/day	Ravikrisha n et al., 2011
70 young adult male and female Wistar rats	Lutein (85% purity in sunflower oil); 4, 40, and 400 mg/kg BW	4 wk	NOAEL- 400 mg/kg BW/day	Harikumar et al., 2008
70 young adult male and female Wistar rats	Lutein (85% purity in sunflower oil); 4, 40, and 400 mg/kg BW	13 wk	NOAEL- 400 mg/kg BW/day	Harikumar et al., 2008

Z=zeaxanthin

IV.B.4. Human clinical studies

This notice incorporates by reference the safety and metabolism studies discussed in GRNs 385 and 432. This notice discusses additional studies that have been published since the submission of GRN 385 and 432. The notified substance in GRN 432 was lutein ester, but it included the safety studies of lutein as the basis of discussing the safety of lutein. As shown in Table 9, all of the 24 studies (26 articles) published since 2011 reported no adverse effects of lutein and no studies reported results inconsistent with the FDA's reviews of 2011-2012.

In these recent studies, doses tested for lutein were up to 21 mg/day and the duration of the studies were up to 12 months (AREDS Research group, 2013, 2014; Berrow et al., 2013; Connolly et al., 2011; Evans et al., 2013; Garcia-Layana et al., 2013; Graydon et al., 2012; Hammond et al, 2012; Hu et al., 2011; Huang et al., 2013; Ma et al., 2012a, 2012b; Murray et al., 2013; Nolan et al., 2011, 2012; Richer et al., 2011; Sasamoto et al., 2011; Tanito et al., 2012; Thomson et al., 2014; Tian et al., 2013; Wang et al., 2013; Weigert et al., 2011; Xu et al., 2013; Yao et al., 2013; Zeimer et al., 2012; Zou et al., 2014). No adverse effects were observed in any of the clinical trials. Repeated daily doses of 20-21 mg lutein were not associated with adverse effects (Huang et al., 2012; Ma et al., 2012a, 2012b; Nolan et al., 2012; Thompson et al., 2014; Wang et al., 2013; Weigert et al., 2011; Xu et al., 2013; Yao et al., 2013; Zou et al., 2014). In particular, longer term administration of 20 mg lutein/day for 1 year resulted in no adverse effects (Yao et al., 2013; Zhou et al., 2014).

In addition, meta analysis of 6 prospective cohort studies involving 4,416 cases and 41,999 participants revealed that dietary lutein and zeaxanthin intake had a significant inverse association with nuclear cataract (Relative risk=0.75; 95 % CI: 0.65, 0.85), but not with cortical cataract and posterior subcapsular cataract (Ma et al., 2014). Dose-response analysis showed that every 300 μg/day increment in dietary lutein and zeaxanthin intake was associated with a 3 %, 1 %, or 3 % reduction in the risk of nuclear cataract, cortical cataract, or posterior subcapsular cataract, respectively. No adverse effects of lutein and zeaxanthin were found. The absence of any adverse effects in these clinical trials provides support for the safety of lutein. The AREDS 2 Research Group (2013, 2014) also reported that intake of 10 mg lutein plus 2 mg zeaxanthin for an average of 4.8 years did not result in any adverse effects.

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
14 subjects with early AMD (8 participants aged 56-81 years in the treated group and 6 participants aged 61-83 years in the non-treated group)	Supplement (12 mg L+0.6 mg Z+ 150 mg Vit C +15 mg Vit E+ 1080 mg omega 3 FA); or no supplement (control group)	40 weeks. At 60 weeks, the treated group attended a fourth visit following 20 weeks of supplement withdrawal	Retinal and visual function in people with early stages of AMD	No differences between the treated and control groups during supplementation. Multifocal electroretinography (mfERG) ring 3 N2 latency (P=0.041) and ring 4 P1 latency (P=0.016) increased, and a trend for reduction of mfERG amplitudes was observed in rings 1, 3 and 4 on supplement withdrawal, suggesting a potentially beneficial effect of lutein based supplementation in AMD-affected eyes.	Berrow et al., 2013
144 subjects with subclinical atherosclerosis aged 45-68 years	20 mg L (n=48); 20 mg L+20 mg lycopene (n=48); or placebo	12 months, P	Carotid artery intima-media thickness (CAIMT)	Serum lutein concentrations increased significantly from 0.34 to 1.96 µmol/l in the lutein group (P< 0.001) and from 0.35 to 1.66 µmol/l in the combination group (P< 0·001). The mean values of CAIMT decreased significantly by 0.035 mm (P= 0.042) and 0.073 mm (P< 0.001) in the lutein and combination groups, respectively.	Zou et al., 2014
39 older adults (BMI, 25.3; mean age, 68.8 year) not meeting Australian physical activity guidelines	21 mg L; or placebo	4 weeks, P	Physical activity	Plasma lutein were inversely associated with absolute (r = -0.36, p = 0.03) and percentage changes (r = -0.39, p = 0.02) in sedentary time and positively associated with the percentage change in average daily activity counts (r = 0.36, p = 0.03).	Thomson et al., 2014

AUC=Area Under the Curve; AREDS2=Age-Related Eye Disease Study 2; BMI=body mass index; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; L=lutein; mfERG = multifocal electroretinography; MP =Macular pigment; MZ=meso-zeaxanthin; P=parallel design; VA=visual acuity; X=cross over design; Z=zeaxanthin.

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
126 patients with AMD (AREDS [Age-related Eye Disease Study] stages 2, 3, and 4)	1 to 3-20 mg L in 1-3 months and 10 mg L in 4 - 6 months; or placebo	6 months, P	MPOD and visual acuity (VA)	Lutein significantly increased MPOD by 27.9% ± 2.9% ($p < 0.001$ vs. placebo). No significant effect of lutein supplementation on mean differential light threshold (MDLT) or VA was seen, although a tendency toward an increase was seen for both parameters (MDLT, $p = 0.096$ vs. placebo; VA, $p = 0.070$ vs. placebo). However, a significant correlation was found between the increase in MPOD and the increase in MDLT after 6 months ($r = 0.25$, $p = 0.027$), as well as between the increase in MPOD after 6 months and the increase in VA after 6 months ($r = 0.27$, $p = 0.013$).	Weigert et al., 2011
65 early athero-sclerosis patients	20 mg L (n= 34); or placebo (n= 31)	3 months, P	Serum cytokines, apoE and lipoprotein profiles	Lutein supplements significantly decreased in serum interleukin-6 and monocyte chemoattractant protein-1 (MCP-1) at 3 month ($p<0.05$). Intragroup comparison revealed a significant difference in changes of serum MCP-1 between the 2 groups ($p= 0.021$). The serum LDL and triglycerides (TG) significantly decreased in lutein group ($p<0.05$).	Xu et al., 2013
48 healthy volunteers	Single dose of 20 mg of lutein as either a starch-matrix (lutein 5 %); or as a cross-linked alginate-matrix beadlet (lutein 20 %) formulation.	Up to 672 h, X	Plasma kinetics of lutein and Z provided as two different beadlet formulations	By 14 h post-dose, total plasma lutein increased by 7 % with alginate matrix beadlets and by 126% with starch-matrix. Total lutein AUC (0-72h) and AUC (0-672h) were 1.8-fold and 1.3-fold higher, respectively, for starch-matrix compared to alginate-matrix beadlet. Both formulations were well tolerated by subjects in this study.	Evans et al., 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Daily dose of L and/or Z	Duration	Measured Outcomes	Results	Reference
117 healthy nonsmokers	10 L; 20 mg L; or placebo	12 weeks, P	Levels of plasma carotenoid, total antioxidant capacity (TAOC), lipoprotein profile, and antioxidant enzymes activities	Plasma lutein and TAOC significantly increased in both active treatment groups. A significant reduction was found in malondialdehyde in the 20 mg lutein group.	Wang et al., 2013
108 subjects with early AMD	10 mg L; 20 mg L; 10 mg L+10 mg Z; or placebo	48 weeks, P	Retinal function using mfERG or MPOD	The negative trough and first positive peak (N1P1) response densities significantly increased in ring 1 for the 20 mg lutein group and for the lutein and Z group, and in ring 2 for the 20 mg lutein group, indicating improvement in renal function. The increases in MPOD related positively to the increases in N1P1 response density in ring 1 and ring 2 for nearly all active treatment groups. N1P1 response densities in ring 3 to ring 6 or P1 peak latencies in all rings did not change significantly in any group.	Ma et al., 2012a, 2012b
108 patients with early AMD (aged >50 years)	10 mg L; 20 mg L; 10 mg L+10 mg Z; or placebo	48 weeks, P	Serum and macular (in vivo measured MPOD) responses	Serum lutein levels in the all lutein and L+Z groups increased 4.24-, 4.66-, and 6.23-fold during the trial, respectively (all $P < 0.001$). MPOD and serum lutein levels increased linearly with the dosage and their increasing rates were statistically correlated (all $P < 0.05$). No notable changes were detected in the placebo group for MPOD and serum concentrations.	Huang et al, 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
120 healthy drivers with long-term light exposure (drivers).	20 mg L; or placebo	1year, P	VA, MPOD, and visual performance	Significant increase in serum lutein and central MPOD in the treatment group; no change was observed in the placebo group. Significant improvements in the score of the National Eye Institute 25-Item Visual Functioning Questionnaire driving subscale in the a treatment group.	Yao et al., 2013
31 healthy subjects with such atypical MP spatial profiles	20 mg L + 2 mg Z; 10 mg MZ+ 10 mg L+ 2 mg Z; 17 mg MZ+ 3 mg L+ 2 mg Z; or placebo	8 weeks, P	MP in subjects where the profile does not exhibit the typical central peak (i.e. peaked MP at foveal epicentre).	No significant increase in MP at any eccentricity in the 20 mg L + 2 mg Z group. There was a trend towards an increase in MP at all eccentricities in, with a significant increase found at 0.25° and 0.50° 10 mg in the MZ+ 10 mg L+ 2 mg Z group. A significant increase in MP at 0.25° in the 17 mg MZ+ 3 mg L+ 2 mg Z group 3, but at no other eccentricity. The typical central peak of MP can be realised in subjects with atypical spatial profiles, following supplementation with all three macular carotenoids, but not with a supplement lacking MZ.	Nolan et al., 2012
322 healthy female twin volunteers, aged 16–50 yr	18 mg L + 2.4 mg Z; or placebo	6 months, P	Heritability of MP augmentation in response to supplemental L and Z	Serum L and Z levels were raised significantly from baseline following 3 months' supplementation (mean increase 223% and 633%, respectively), with no MPOD increase. After 6 months' supplementation, a small increase in MPOD was seen (mean increase 0.025, P = 0.02). Genetic factors explained 27% (95% confidence interval [CI] 7–45) of the variation in MPOD response. Distribution profiles of MP did not change in response to supplementation.	Hammond et al, 2012

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
44 patients with AMD	12 mg L+ 280 mg DHA daily; or placebo	1 year, P	MPOD	At baseline, the MPOD in placebo was 0.286 meanwhile it was 0.291 in the supplemented group. One year later, the mean MPOD was increased by 0.059 in the placebo group and by 0.162 in patients receiving lutein + DHA ($p < 0.05$).	Garcia-Layana et al., 2013
97 subjects	12 mg L + 1 mg Z; no control group	6-mo intervention, 3 mo FU	MPOD, a ring structure	L and Z intake led to a general shift toward higher MP values in eyes without ring structure. Ring structures were neither attenuated nor generated de novo following supplementation.	Zeimer et al., 2012
4203 elderly at risk for developing late AMD 50 -85 y	10 mg L +2 mg Z; 1.0 g omega-3 FA; the combination; or placebo	median of 4.8 years, P	development of late AMD	In exploratory analysis of lutein/zeaxanthin vs no lutein/zeaxanthin, the hazard ratio of the development of late AMD was 0.90 (95% CI, 0.82-0.99; P = 0.04). No differences in adverse events or serious adverse event among the groups.	AREDS 2 Research Group, 2013, 2014
72 patients with early AMD (mean age 70.5 ± 8.7)	10 mg L ester; or a placebo	12 months; P	MPOD and VA	At the end of the trial, an overall increase in the mean MPOD level was found for the L group from 0.38 ± 0.19 to 0.53 ± 0.22 optical density units. No change in MPOD was found for the control group. In the L subgroup (n = 19),there was a mean improvement in VA from 0.23 ± 0.12 at baseline to 0.16 ± 0.10 at visit 4 ($P < 0.05$). In the placebo subgroup (n = 14), there was a small deterioration from 0.18 ± 0.13 to 0.19 ± 0.12 (P = 0.70).	Murray et al., 2013

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Dose	Duration	Measured Outcomes	Results	Reference
44 healthy subjects	10.6 mg MZ; 5.9 mg L+ 1.2 mg Z; or placebo	6 months, P	The spatial profile of MPOD, and serum conc. of L and Z	The test group had significantly increased serum concentrations of L and Z central MPOD (0.25°: P = 0.001; 0.5°: P = 0.001), with no significant change in the control group. Clinical pathology analysis confirmed that all variables remained within the normal reference range.	Connolly et al., 2011
22 healthy volunteers without high myopia	10 mg L; or 10 mg Z	3 months, P	MPOD	MPOD resonance Raman spectrophotometry (RRS) and MPOD autofluorescence imaging (AFI) levels increased >20 % from baseline at 2 and 3 months after lutein supplementation. In the Z group, MPOD (RRS) levels remained unchanged in those with and without high myopia. The authors concluded that lutein supplementation increased MPOD levels within the fovea more effectively than did zeaxanthin.	Tanito et al., 2012
72 subjects with signs of early AMD	10 mg L; or placebo	12 months, P	Complement factor D (CFD) and its products C5a and C3d	Lutein supplementation markedly decreases circulating levels of the complement factors CFD, C5a and C3d levels, suggesting that lutein supplementation may be a powerful tool in the prevention and treatment of AMD.	Tian et al., 2013
43 eyes of 43 subjects, with central serous chorioretinopathy (CSC), and 33 fellow eyes with AMD	6 mg L (no control group)	1 year	1-year changes in MPOD and visual function	Daily supplementation with 6 mg of lutein did not affect the MPOD level for 1 year, but supplementation seems to improve visual functions such as contrast sensitivity and retinal sensitivity.	Sasamoto et al., 2011

Table 9. Human clinical studies reporting no adverse effects of lutein: summary of articles published since 2011, continued

Subjects	Daily dose	Duration	Measured Outcomes	Results	Reference
121 normal subjects (18-41 years)	12 mg L+ 1mg Z	1year, P	Visual performance	Supplementation of L+Z improved MPOD.	Nolan et al., 2011
60 subjects with mild-to moderate AMD (57 men, 3 women; mean 74.9 years)	8 mg Z; 8 mg Z +9 mg L; or 9 mg L- Faux placebo- control group	1year, P	MPOD	There were no intergroup differences in 3 major AMD risk factors (age, smoking, and body mass index as well as disease duration and Visual Function Questionnaire 25 composite score differences) and MPOD.	Richer et al., 2011
Study 1) 52 healthy subjects Study 2) 75 healthy subjects	Either carrot juice (a carotene-rich food) or spinach powder (L+Z-rich food); supplements containing L + Z, β-carotene; or placebo	Two 8-week studies, P	MP level, serological markers of endothelial activation, and inflammation and oxidation	No effects on MP level or markers of endothelial activation, inflammation or oxidation were observed. However, the change in serum lutein and Z was associated or tended to be associated with the change in MP levels in those receiving lutein rich foods (L: r=0.40, P = 0.05; Z: r=0.30, P = 0.14) or the lutein and Z supplement (L; r=0.43, P = 0.03; Z: r=0.22, P = 0.28).	Graydon et al., 2012
90 nonproliferative diabetic retinopathy (NDR) patients divided evenly into 3 groups	6mg L+ 0.5 mg Z; No L/Z supplementation (NDR control group and control group).	3 months, P	Serum L/Z concentrations visual function between patients with NDR and normal subjects	Serum L/Z concentrations of NDR group increased significantly by 2 times (P=0.000) and VA improved significantly after supplementation. Average contrast sensitivity (CS) values after 3 months increased significantly and the foveal thickness decreased (P =0.05).	Hu et al., 2011

AUC=Area Under the Curve; AREDS2=Age-Related Eye Disease Study 2; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; L=lutein; MPOD= macular pigment optical density; MZ=meso-zeaxanthin; MP =Macular pigment; VA=visual acuity; Z=zeaxanthin

V. SAFETY DETERMINATION

Numerous human and animal studies have reported health benefits of lutein with no major adverse effects. INNOBIO® uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of lutein and its ester. This GRAS determination is based on the data and information generally available and consented opinion about the safety of lutein. The literature indicates that lutein offers consumers health benefits without serious adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of lutein as well as appropriate corroborative data.

1. INNOBIO's lutein will be used as a food ingredient (nutrient) at concentrations of up to 0.3 to 3 mg/serving (reference amounts customarily consumed, 21CFR 101.12) in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; dairy product analogs; egg substitute 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; soups and soup mixes. Intended use excludes food categories such as egg products and soup and soup mixes that come under USDA. Compositional analysis of INNOBIO lutein demonstrated that product primarily contains ≥70% lutein with small amount of ≥4% zeaxanthin, carbohydrate, and protein. The intended use of lutein in the specified food categories will result in cumulative 90th percentile EDIs (intakes from regular diets, current exposure, and newly added food categories) of 19.1 mg lutein/person/day and 1.3 mg zeaxanthin/person/day, respectively.

2. These exposure estimates (EDI under the untended uses, and/or cumulative exposure) are far below the ADI of up to 1 mg/kg BW/day or 60 mg/person/day established by The Panel on Food Additives and Nutrient Sources added to Food of EFSA as well as ADI of up to 2 mg/kg BW/day or 120 mg/person/day determined by JECFA, based on animal and human studies. In addition, these exposure estimates are below the upper limit of Observed Safe Level (OSL; 20 mg/person/day) established by Shao and Hathcock (2006), based on a review of 30 human clinical studies.

3. In response to several GRAS notifications on lutein (crystalline lutein - GRN 140, 291, 385; suspended lutein - GRN 221, 390; lutein ester - GRN 110 and 432), the FDA did not question the safety of lutein and lutein esters for the specified food uses. The FDA responses to GRAS notifications on lutein indicate that the FDA is satisfied with the safety-in-use of the lutein, as long as consumption is below 20 mg/person/day.

4. In the previous GRAS notices to the FDA, the safety of lutein has been established in toxicological studies in animals, mutagenicity studies, and is further supported by clinical studies in human. Additionally, the safety is corroborated by additional studies conducted with various sources of lutein, lutein-rich foods, and lutein supplements. Furthermore, historical consumption of eggs and fruits and vegetables where these carotenoids predominate (e.g., green leafy vegetables, such as spinach and kale) support the safety of

lutein and zeaxanthin. Additional studies published subsequent to the FDA GRAS notices continue to support safety of lutein as a food ingredient.

VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF LUTEIN (CRYSTALLINE, SUSPENDED OIL, POWDER, AND BEADLETS)

The intended uses of Lutein (crystalline, suspended oil, powder, and beadlets) have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

On behalf of INNOBIO® Limited (INNOBIO®), we, the undersigned expert panel members, Susan Cho, Ph.D., Robert Martin, Ph.D., Madhusudan Soni, Ph.D., have independently evaluated the materials summarized in the lutein Generally Recognized as Safe (GRAS) report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that lutein, produced consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of lutein are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

_____ _____

Susan Cho, Ph.D. Date
NutraSource, Inc.

_____ _____

Robert Martin, Ph.D. Date

_____ _____

Madhusudan Soni, Ph.D. Date
Soni and Associates

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APPENDIX A. CERTIFICATE OF ANALYSIS

INNOBIO® Crystalline Lutein

Lot No.:30201-B318F1 Date of Manufacture: 06/18/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	77.1
Lutein (%)	70.0 Min.	USP 32/NF 27	70.9
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.5
Moisture (%)	1.0 Max.	USP 731	0.04
Particle Size	95% through 40 mesh	USP 786	96.5
Lead (ppm)	1.0 Max.	EN ISO 17294	0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	43.1
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:_____ (b) (6) Date: 07/01/2013

INNOBIO® Crystalline Lutein

Lot No.:30201-B324F1 Date of Manufacture: 06/24/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	77.9
Lutein (%)	70.0 Min.	USP 32/NF 27	71.7
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.6
Moisture (%)	1.0 Max.	USP 731	0.1
Particle Size	95% through 40 mesh	USP 786	95.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.01
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.05
Mercury (ppm)	0.1 Max.	BS EN 13806	<10
Hexane (ppm)	10.0 Max.	USP 467	3.9
Ethanol (ppm)	50.0 Max.	USP 467	33.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:_____ (b) (6) Date: 07/03/2013

INNOBIO® Crystalline Lutein

Lot No.:30201-B325F1 Date of Manufacture: 06/25/2013

Description: INNOBIO® Crystalline Lutein is a reddish orange colored powder with characteristic odor. It is naturally derived from marigold flowers (*Tagetes erecta*).

Test	Limits	Methods	Results
Appearance	Reddish-orange powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	75.0 Min.	USP 32/NF 27	78.2
Lutein (%)	70.0 Min.	USP 32/NF 27	71.9
Zeaxanthin (%)	4.0 Min.	USP 32/NF 27	4.7
Moisture (%)	1.0 Max.	USP 731	0.1
Particle Size	95% through 40 mesh	USP 786	95.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.1
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.01
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.05
Mercury (ppm)	0.1 Max.	BS EN 13806	<10
Hexane (ppm)	10.0 Max.	USP 467	5.7
Ethanol (ppm)	50.0 Max.	USP 467	36.3
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under vacuum, packed in corrugated paper cartons.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below -10°C.
Shelf Life: One year in the original package according to storage conditions.

Approval:_____ (b) (6) Date: 07/03/2013

INNOBIO® Lutein 20% Oil Suspension
Lot No.: 30215-D313F1 Date of Manufacture: 06/13/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.6
Lutein (%)	20.0 Min.	USP 32/NF 27	20.4
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.4
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	10.8
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

Approval: (b) (6) Date: 06/24/2013

INNOBIO® Lutein 20% Oil Suspension

Lot No.: 30215-D315F1 Date of Manufacture: 06/15/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.9
Lutein (%)	20.0 Min.	USP 32/NF 27	20.6
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.5
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.3
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.8
Ethanol (ppm)	50.0 Max.	USP 467	17.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	50
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

Approval: (b) (6) Date: 06/24/2013

INNOBIO® Lutein 20% Oil Suspension
Lot No.: 30215-D317F1 Date of Manufacture: 06/17/2013

Description: INNOBIO® Lutein 20% Oil Suspension is micronized crystalline lutein suspended in the vegetable oil (sunflower oil, or safflower oil, or corn oil). The crystalline lutein is naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red oil suspension.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	22.1
Lutein (%)	20.0 Min.	USP 32/NF 27	20.3
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	1.0 Max.	AOCS Td 2-64	0.1
Lead (ppm)	1.0 Max.	EN ISO 17294	0.2
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.1
Ethanol (ppm)	50.0 Max.	USP 467	27.3
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: 20kg per aluminum drum purged with nitrogen, and then packed in cartons.
Storage and Handling: Store in a dry and cool place, protected from light, heat and oxygen. The preferred temperature is below 30°C.
Shelf Life: This product is stable for at least three years in the unopened original package. Upon opening, the product should be warmed and mixed thoroughly before sampling or using.

Approval: (b) (6) Date: 06/26/2013

INNOBIO® Lutein 20% CWD Powder

Code: 30202-20
Lot No.: 30202-318D1 Date of Manufacture: 04/18/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	23.9
Lutein (%)	20.0 Min.	USP 32/NF 27	22.0
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	5.0 Max.	USP 731	2.1
Particle Size	95% through 40 mesh	USP 786	98.5
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.3
Ethanol (ppm)	50.0 Max.	USP 467	10.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 04/25/2013

INNOBIO® Lutein 20% CWD Powder

Code: 30202-20
Lot No.: 30202-328A1 Date of Manufacture: 01/28/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	24.2
Lutein (%)	20.0 Min.	USP 32/NF 27	22.3
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.4
Moisture (%)	5.0 Max.	USP 731	2.2
Particle Size	95% through 40 mesh	USP 786	98.2
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.9
Ethanol (ppm)	50.0 Max.	USP 467	20.4
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	50
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 02/25/2013

INNOBIO® Lutein 20% CWD Powder

Lot No.: 30202-D307K1 Date of Manufacture: 11/07/2013

Description: INNOBIO® Lutein 20% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	21.5 Min.	USP 32/NF 27	24.6
Lutein (%)	20.0 Min.	USP 32/NF 27	22.5
Zeaxanthin (%)	1.0 Min.	USP 32/NF 27	1.5
Moisture (%)	5.0 Max.	USP 731	2.5
Particle Size	95% through 40 mesh	USP 786	97.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.8
Ethanol (ppm)	50.0 Max.	USP 467	12.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	60
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 11/18/2013

INNOBIO® Lutein 10% CWD Powder

Lot No.: 13050901 Date of Manufacture: 05/09/2013

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.9
Lutein (%)	10.0 Min.	USP 32/NF 27	10.9
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.9
Particle Size	95% through 40 mesh	USP 786	96.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.4
Ethanol (ppm)	50.0 Max.	USP 467	11.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 05/19/2013

INNOBIO® Lutein 10% CWD Powder

Lot No.: 30202-B108C1 Date of Manufacture: 03/08/2011

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.4
Lutein (%)	10.0 Min.	USP 32/NF 27	10.5
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.3
Particle Size	95% through 40 mesh	USP 786	96.2
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.9
Ethanol (ppm)	50.0 Max.	USP 467	10.9
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 03/15/2011

INNOBIO® Lutein 10% CWD Powder

Lot No.: 30202-326D1 Date of Manufacture: 04/26/2013

Description: INNOBIO® Lutein 10% CWD Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.5
Lutein (%)	10.0 Min.	USP 32/NF 27	10.6
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	5.0 Max.	USP 731	1.1
Particle Size	95% through 40 mesh	USP 786	97.1
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.7
Ethanol (ppm)	50.0 Max.	USP 467	8.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 05/03/2013

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-215E1 Date of Manufacture:05/15/2012

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.3
Lutein (%)	10.0 Min.	USP 32/NF 27	10.4
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.68
Moisture (%)	7.0 Max.	USP 731	0.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.9
-through sieve No.100	15.0 Max.	USP 786	18.6
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.6
Ethanol (ppm)	50.0 Max.	USP 467	19.5
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approva (b) (6) Date: 06/20/2012

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-303D1 Date of Manufacture: 04/03/2013

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conform
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.5
Lutein (%)	10.0 Min.	USP 32/NF 27	10.6
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.69
Moisture (%)	7.0 Max.	USP 731	3.1
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	99.5
-through sieve No.100	15.0 Max.	USP 786	17.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.0
Ethanol (ppm)	50.0 Max.	USP 467	12.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 04/08/2013

INNOBIO® Lutein 10% Beadlets

Lot No.: 30213-327C1 Date of Manufacture: 03/27/2013

Description: INNOBIO® Lutein 10% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	orange-red, free-flowing beadlets.	Visual	Conform
Total carotenoids (including lutein and zeaxanthin), %	10.8 Min.	USP 32/NF 27	11.8
Lutein (%)	10.0 Min.	USP 32/NF 27	10.9
Zeaxanthin (%)	0.5 Min.	USP 32/NF 27	0.7
Moisture (%)	7.0 Max.	USP 731	3.2
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.5
-through sieve No.100	15.0 Max.	USP 786	18.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	4.2
Ethanol (ppm)	50.0 Max.	USP 467	9.8
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:_____ (b) (6) Date: 04/03/2013

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-215G1 Date of Manufacture: 07/15/2012

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.6
Lutein (%)	5.0 Min.	USP 32/NF 27	5.1
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.33
Moisture (%)	5.0 Max.	USP 731	3.9
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.4
-through sieve No.100	15.0 Max.	USP 786	19.7
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	3.6
Ethanol (ppm)	50.0 Max.	USP 467	10.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___ (b) (6) Date: 07/25/2012

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-311C1 Date of Manufacture: 03/11/2013

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.7
Lutein (%)	5.0 Min.	USP 32/NF 27	5.2
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.34
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.2
-through sieve No.100	15.0 Max.	USP 786	19.3
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	2.4
Ethanol (ppm)	50.0 Max.	USP 467	8.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___(b) (6) Date: 03/27/2013

INNOBIO® Lutein 5% Beadlets

Lot No.: 30213-311C1 Date of Manufacture: 03/11/2013

Description: INNOBIO® Lutein 5% Beadlets are tiny, free flowing spherical particles of double shell structures manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*). The product may contain few tiny white starch particles.

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.7
Lutein (%)	5.0 Min.	USP 32/NF 27	5.2
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.33
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size (%)			
-through sieve No.20	100	USP 786	100
-through sieve No.40	85.0 Min.	USP 786	98.5
-through sieve No.100	15.0 Max.	USP 786	18.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.9
Ethanol (ppm)	50.0 Max.	USP 467	9.0
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___(b) (6) Date: 03/27/2013

INNOBIO® Lutein 5% CWS Powder

Lot No.: 30202-224B1 Date of Manufacture: 02/24/2012

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	6.2
Lutein (%)	5.0 Min.	USP 32/NF 27	5.7
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.37
Moisture (%)	5.0 Max.	USP 731	1.1
Particle Size	95% through 40 mesh	USP 786	99.4
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.5
Ethanol (ppm)	50.0 Max.	USP 467	6.2
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	80
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval:___ (b) (6) Date: 03/09/2012

INNOBIO® Lutein 5% CWS Powder

Code: 30202-05
Lot No.: 30202-D311F1 Date of Manufacture: 06/11/2013

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.9
Lutein (%)	5.0 Min.	USP 32/NF 27	5.4
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.35
Moisture (%)	5.0 Max.	USP 731	2.4
Particle Size	95% through 40 mesh	USP 786	99.9
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	0.7
Ethanol (ppm)	50.0 Max.	USP 467	9.6
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 06/21/2013

INNOBIO® Lutein 5% CWS Powder

Code: 30202-05
Lot No.: 30202-213F1 Date of Manufacture: 06/13/2013

Description: INNOBIO® Lutein 5% CWS Powder is a free flowing powder manufactured by the microencapsulation technology. It contains lutein naturally derived from marigold flowers (*Tagetes erecta*).

Tests	Limits	Methods	Results
Appearance	Orange-red, free-flowing powder	Visual	Conforms
Total carotenoids (including lutein and zeaxanthin), %	5.4 Min.	USP 32/NF 27	5.8
Lutein (%)	5.0 Min.	USP 32/NF 27	5.4
Zeaxanthin (%)	0.25 Min.	USP 32/NF 27	0.34
Moisture (%)	5.0 Max.	USP 731	2.0
Particle Size	95% through 40 mesh	USP 786	98.8
Lead (ppm)	1.0 Max.	EN ISO 17294	<0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294/ AOAC 2013.06	<0.1
Cadmium (ppm)	1.0 Max.	EN ISO 17294	<0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	<0.05
Hexane (ppm)	10.0 Max.	USP 467	1.2
Ethanol (ppm)	50.0 Max.	USP 467	3.7
Total Plate Count (CFU/g)	1,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	100 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in 5kg net aluminum foil bags under nitrogen, and five bags per cardboard drum.
Storage and Handling: Store in a cool dry place, protected from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire content after opening.

Approval: (b) (6) Date: 06/27/2013