ORIGINAL SUBMISSION

NutraSource, Inc.
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October 7, 2014

Dr. Antonia Mattia
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS notice for Meso-Zeaxanthin

Dear Dr. Antonia Mattia:

On behalf of INNOBIO® Limited, we are submitting for FDA review a GRAS notification for meso-zeaxanthin. 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)

10/7/2014

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

enclosure

# GRAS EXEMPTION CLAIM for INNOBIO® MESO-ZEAXANTHIN

Prepared by: NutraSource, Inc.
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GRN 000550

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## GRAS EXEMPTION CLAIM

### A. MESO-ZEAXANTHIN: 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 meso-zeaxanthin (MZ) (crystalline, oil suspension, powder, and beadlet forms) is 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 MZ is exempt from the requirement of premarket approval.

Signed
(b) (6)

10/5/2014

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 meso-zeaxanthin.

Trade names are:
INNOBIO® Crystalline Meso-zeaxanthin,
INNOBIO® Meso-zeaxanthin 20 Oil Suspension,
INNOBIO® Meso-zeaxanthin 20 Cold Water Dispersible (CWD) Powder,
INNOBIO® Meso-zeaxanthin 10 CWD Powder,
INNOBIO® Meso-zeaxanthin 10 Beadlets,

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Agent for
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INNOBIO® Meso-zeaxanthin 10 CWD Powder,
INNOBIO® Meso-zeaxanthin 10 Beadlets,

INNOBIO® Meso-zeaxanthin 5 Beadlets, and
INNOBIO® Meso-zeaxanthin 5 Cold Water Soluble (CWS) Powder.

**D. Product Description**

INNOBIO® MZ preparations are derived from purified natural extracts from the marigold *(Tagetes erecta)* flower. The INNOBIO® Crystalline MZ contains ≥60% MZ, ≤7% R'R-zeaxanthin and ≥3.5% lutein. INNOBIO® MZ 20 Oil Suspension and INNOBIO® MZ 20 CWD Powder are diluted from INNOBIO® Crystalline MZ to contain ≥18% MZ, ≤2% R'R-zeaxanthin and ≥1% lutein. INNOBIO® MZ 10 CWD Powder INNOBIO® MZ 10 Beadlets are diluted from INNOBIO® Crystalline MZ to contain ≥9% MZ, ≤1% R'R-zeaxanthin and ≥0.5% lutein. INNOBIO® MZ 5 Beadlets and INNOBIO® MZ 5 CWS Powder are diluted from INNOBIO® Crystalline MZ to contain ≥4.5% MZ, ≤0.5% R'R-zeaxanthin and ≥0.25% lutein.

The common structure in all carotenoids, MZ, R'R-zeaxanthin, and lutein, is a linear, conjugated chain of double bonds that is responsible for the brilliant red, orange and yellow pigments found in nature. Structurally, lutein and zeaxanthin have identical chemical formulas and are isomers, but they are not stereoisomers. The main difference between them is in the location of a double bond in one of the end rings. While lutein is present as a single stereoisomer, zeaxanthin occurs primarily as a mixture of [(3R,3'R)-β, β–carotene-3,3'-diol] and [(3R,3'S)- β, β-carotene-3,3'-diol], with a much smaller amount of [(3S,3'S)- β, β-carotene-3,3'-diol]. The first two predominant zeaxanthin isomers are referred to as zeaxanthin and MZ, respectively. The absolute configuration of the hydroxyl groups located on the 3 and 3' carbon atoms of the carotenoid endgroups is identical in the lutein and MZ molecules. Thus, the conversion of lutein into MZ involves a shift of one carbon-carbon double bond in the ∑-ring of lutein. An alternative mechanism for the formation of MZ posits that the metabolite, dehydrolutein, gives rise to MZ through an enzymatic reduction pathway (Landrum and Bone, 2004).

Zeaxanthin is the structure found in dietary sources of zeaxanthin, such as corn and eggs (Bone et al., 2007; Thurnham et al., 2008, 2013). Although MZ is naturally present in human foods (Nolan et al., 2014), commercially available MZ (sometime referred to as "zeaxanthin isomers") cannot be extracted from plant materials; it is synthesized by exposing lutein to heat and alkaline conditions. Meso-zeaxanthin, being a chemical alteration of lutein, is considered semi-synthetic in the published literature.

**E. Applicable Conditions for Use of the Notified Substance**

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

A recent GRAS notice for MZ preparation containing 60% MZ, 5% zeaxanthin and 15% lutein received a "no questions" letter from FDA (GRN 481; FDA, 2014). In this GRN notice, the 90 percentile intake of MZ and zeaxanthin will be 10.58 and 0.88 mg/person/day, respectively. Also, the 90 percentile intake of lutein was estimated at 2.57 mg/person/day. The FDA did not question the acceptability and suitability of the studies to establish the safety of MZ for the proposed food uses.

In a recent scientific opinion on the substantiation of health claims related to MZ, the European Food Safety Authority (EFSA) Panel addressed the scientific substantiation of health claims in relation to MZ and maintenance of vision to conclude that MZ is sufficiently characterized (EFSA, 2010a). The EFSA (2010a) document on Scientific Opinion on the substantiation of health claims related to MZ and maintenance of vision stated, "In the human clinical trials, a dosage of 14.9 mg per day of MZ has been administered for 120 days, without any adverse effects. Rat studies with more than 200 mg per kg per day have shown no-observed-adverse-effect (NOAEL). Ames tests have shown that MZ is anti-mutagenic". The EFSA Panel also did not raise the safety concerns on intakes of 0.75 mg/kg BW/day for synthetic zeaxanthin, corresponding to a daily intake of 53 mg for a person with a body weight of 70 kg (EFSA, 2012).

Since INNOBIO® MZ preparations contain lutein and MZ and lutein follow the same metabolic pathways, additional information on GRAS notices of lutein can be referenced. The FDA has received seven GRAS Notices related to food uses of lutein and lutein esters: 3 separate GRAS notifications on crystalline lutein 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). 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, 2010b) 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 an Adequate Daily Intake (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**

The INNOBIO® intended use of MZ preparations has been expanded from food products listed in the GRN 481 (FDA, 2014) by adding protein bars, low sodium tortillas, and vitamin water at a level of 1.2 mg MZ/serving (RACC; reference amounts customarily consumed, 21 CFR 101.12). As shown in Table 1, the INNOBIO® MZ preparations are intended for use as food ingredients 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 0.18 to 1.8 mg MZ per serving (corresponding to 0.3-3.0 mg INNOBIO® Crystalline MZ). INNOBIO® does not intend to use MZ as a component of infant formula and other infant and toddler foods or in foods under USDA's jurisdiction such as meat, poultry and egg products.

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

Using food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES; 2009-2010), the mean intakes of MZ under the intended uses were estimated to be 5.2 mg/person/day for all population and 5.3 mg/person/day for all-users,

while the 90th percentile intakes were determined as 9.9 and 10.0 mg/person/day (or 0.173 and 0.175 mg/kg BW/day), respectively. The 90th percentile intake of zeaxanthin and the sum of both forms of zeaxanthin from intended uses is estimated to be 0.83 and 10.83 mg/person/day (or 0.015 and 0.190 mg/kg BW/day) in all-users, respectively. These levels are far below the safe intake level of 0.75 mg/kg BW/day for synthetic zeaxanthin (or 53 mg for a person with a BW of 70 kg) established by a Panel of Food of European Food Safety Authority (EFSA, 2012).

The 90th percentile intake of all 3 carotenoids under the intended use of the current notice is estimated to be 11.4 mg/person/day (or 0.20 mg/kg BW/day) in all-users.

Table 1. Food Categories and Intended Use Levels of MZ in the US.

| Food Category | Proposed Food | Use Levels, mg/RACC[1,2] | | | | |
|---|---|---|---|---|---|---|
| | | MZ | INNOBIO® crystalline MZ (≥60%) | MZ 20 (≥18%) | MZ 10 (≥9%) | MZ 5 (≥4.5%) |
| Baked Goods and Baking Mixes | Protein bars, cereal bars, and energy bars | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | crackers and crisp-breads | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Tortillas, low sodium | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Beverages and Beverage Bases | Vitamin water | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Bottled water | 0.3 | 0.5 | 1.7 | 3.3 | 6.7 |
| | Carbonated beverages; Meal replacements | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Tea, ready-to-drink | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |
| Breakfast Cereals | Instant and regular hot cereals | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Ready-to-eat cereals | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Chewing Gum | Chewing gum | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Dairy Product Analogs | Imitation milks | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Soy milks | 0.9 | 1.5 | 7.5 | 15.0 | |
| Egg Products | Liquid, frozen or dried egg substitutes | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Fats and Oils | Margarine-like spreads, salad dressings | 0.9 | 1.5 | 7.5 | 15.0 | 30.0 |
| Frozen Dairy Desserts and Mixes | Frozen yogurt | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Gravies and Sauces | Tomato based sauces | 0.18 | 0.3 | 1.0 | 2.0 | 4.0 |
| Hard Candy | Hard candy | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Infant and Toddler Foods | Junior, strained and toddler-type baby foods | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Milk Products | Dry milk flavored milk and milk drinks | 1.8 | 3.0 | 10.0 | 20.0 | 40.0 |
| | Fermented milk beverages | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |
| | Yogurt | 1.8 | 3.0 | 10.0 | 20.0 | 40.0 |
| | Milk based meal replacement | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Processed Fruits and Fruit Juices | Energy, sport and isotonic drinks | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Fruit-flavored drinks, fruit juice, nectars | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Vegetable Juice | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Soft Candy | Chewy and nougat candy; Fruit snacks | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Soup and soup mixes | Canned soup | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |

[1]Use levels are the same as those described in GRN 481 except 3 newly added items.
[2]MZ 20 series indicates INNOBIO MZ 20 Oil Suspension and 20 CWD Powder; MZ series 10= INNOBIO® MZ 10 CWD Powder and INNOBIO® MZ 10 Beadlets; MZ 5 series= INNOBIO® MZ 5 Beadlets and INNOBIO® MZ 5 CWS Powder.

**E.4. Scientific Basis for the GRAS Determination**

A recent GRAS notice for MZ preparation containing 60% MZ, 5% zeaxanthin and 15% lutein received a "no questions" letter from FDA (GRN 481; FDA, 2014). As the MZ in this GRAS determination is similar in specifications compared to another MZ in the FDA GRAS Notice, it is recognized that the information and data in the other GRAS Notices is pertinent to the safety of the MZ in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 481. Additionally, this notice discusses additional studies that have been published since the FDA's last reviews of 2013-2014. The subject of present GRAS assessment is MZ.

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

1. MZ will be used as a dietary ingredient at concentrations of up to 0.18 to 1.8 mg/serving (corresponding to 0.3-3.0 mg INNOBIO® Crystalline MZ per serving; reference amounts customarily consumed, 21CFR 101.12) in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes. Intended use excludes food categories such as egg products and soup and soup mixes that come under USDA. Under the intended use, the 90 percentile intakes of MZ and zeaxanthin are estimated to be 10.00 and 0.83 mg/person/day, respectively. INNOBIO® Crystalline MZ also contains 3.5% lutein; therefore, the 90th percentile lutein intake from the proposed uses will be 0.56 mg/person/day. The $90^{th}$ percentile intake of all 3 carotenoids will be 11.4 mg/person/day.

2. The NOAEL for MZ was determined to be 300 mg/kg BW/day, the highest level tested in animals (Xu et al., 2013). Intended use of INNOBIO® MZ results in EDI which is much lower than the NOAEL found in animal studies. In humans, consumption of MZ at daily doses up to 17 mg for up to 12 months did not show any adverse effects. For these 'pivotal' studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints. A recent GRAS notice for MZ preparation containing 60% MZ, 5% zeaxanthin and 15% lutein received a "no questions" letter from FDA (GRN 481; FDA, 2014).

3. INNOBIO® MZ preparations contain small amounts of lutein. In addition, meso-zeaxanthin is a metabolite of lutein. Thus, the safety of lutein may be considered for the safety assessment of MZ. FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. In response to these 7 separate GRAS notifications on lutein, FDA did not question the safety of lutein and its esters for the specified food uses. In particular, FDA had no question on the safety of lutein concentrate which included 6.75% MZ (GRN 385).

4. The safety of MZ has been established in toxicological studies in animals, mutagenicity studies, and is further supported by other animal studies (Thurnham and Howard, 2013; Xu et al., 2013) and human clinical studies (Bone et al., 2007; Connolly et al., 2010, 2011;

Loughman et al., 2012; Meagher et al., 2013; Nolan et al., 2012; Sabour-Pickett et al., 2014; Thurnham et al, 2008). The compositional data and product properties are consistent with carefully controlled current Good Manufacturing Practice (cGMP) production and purification. INNOBIO® MZ preparations contain no impurities or contaminants of concern for human health. Several reviews by experts in the field also have documented the safety of MZ.

Pursuant to 21 CFR 9170.35, INNOBIO® MZ (crystalline, suspended oil, powder, and beadlets) has 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 MZ as a component of food. Expert Panel members, Susan S. Cho, Ph.D., David Thurnham, 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 cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Expert Panel further unanimously concludes that the intended use of MZ is 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**: 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.

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# EXPERT PANEL STATEMENT
# DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEASO-ZEAXANTHIN AS A FOOD INGREDIENT

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# I. INTRODUCTION

INNOBIO® Limited (Dalian, Liaoning, China) intends to market meso-zeaxanthin (MZ) derived from marigold *(Tagetes erecta)* flower as a nutritional food ingredient in conventional foods. INNOBIO® has determined through scientific procedures that the use of MZ as a food ingredient (nutritional supplement) in conventional foods, as described herein, is Generally Recognized as Safe (GRAS). The purpose of this dossier is to (1) Outline the identity and composition of MZ preparations, (2) Estimate exposure under the intended condition of use, (3) Document the literature pertaining to the safety of MZ preparations, and (4) Assemble an independent panel of recognized experts (hereinafter referred to as the Expert Panel). The data and information summarized in this dossier demonstrate that the intended use of MZ preparations, produced using current Good Manufacturing Practices (cGMP) and meeting food-grade specifications, is GRAS, based on scientific procedures, as described herein.

## I.A. Background

Macular pigment (MP) was first identified in the mid-1980s as a combination of lutein and zeaxanthin (Bone et al., 1988). It has been demonstrated that MP is composed mainly of three isometric carotenoids, lutein, R,R-zeaxanthin and MZ, present in an approximate ratio of 2:1:1 in the retina. These three pigments represented 72% of the total carotenoid content in the eye (Thurnham, 2007). While lutein is present as a single stereoisomer, zeaxanthin occurs primarily as a mixture of [(3R,3'R)-β, β–carotene-3,3'-diol] and [(3R,3'S)- β, β-carotene-3,3'-diol], with a much smaller amount of [(3S,3'S)- β, β-carotene-3,3'-diol]. The first two predominant zeaxanthin isomers are referred to as zeaxanthin and MZ, respectively. The absolute configuration of the hydroxyl groups located on the 3 and 3' carbon atoms of the carotenoid endgroups is identical in the lutein and MZ molecules. Thus, the conversion of lutein into MZ involves a shift of one carbon-carbon double bond in the ∑-ring of lutein. An alternative mechanism for the formation of MZ posits that the metabolite, dehydrolutein, gives rise to MZ through an enzymatic reduction pathway (Landrum and Bone, 2004).

All three carotenoids occur in MP and dietary intake of lutein and zeaxanthin is related to reduced risk of cataracts and age-related macular degeneration (AMD). MZ resides directly over the centre of the macula, where light is focused and where the strongest need for hazardous actinic blue light protection exists (Landrum et al., 1999). Persons with AMD have low concentrations of the MP in the fovea. MZ may be of specific importance as it has also been found to be concentrated centrally in the macula (Bone et al., 1997) and persons whose MP concentration was low at the centre was found to benefit from supplements containing MZ (Nolan et al., 2012). It has been also reported that MZ provides slightly better protection against lipid membrane oxidation than zeaxanthin (Bhosale and Bernstein, 2005) and that all three carotenoids have the synergistic antioxidant properties. It is also known that supplementation with

MP's constituent carotenoids can enhance visual performance in non-diseased and diseased eyes, with best results following supplementation with all three of MP's constituent carotenoids (MZ, lutein and zeaxanthin in a mg ratio of 10:10:2) (Loskutova et al., 2013). Thus, it may be advantageous to the macula to increase the ratio of total zeaxanthin (zeaxanthin + MZ) to lutein. INNOBIO® intends to supply a blend of MZ, R,R-zeaxanthin and lutein for use as a dietary ingredient in selected foods.

## II. INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

### II.A. Common or Trade Name

The common name of the notified substance is meso-zeaxanthin. Trade names are:
INNOBIO® Crystalline Meso-Zeaxanthin,
INNOBIO® Meso-Zeaxanthin 20 Oil Suspension,
INNOBIO® Meso-Zeaxanthin 20 Cold Water Dispersible (CWD) Powder,
INNOBIO® Meso-Zeaxanthin 10 CWD Powder,
INNOBIO® Meso-Zeaxanthin 10 Beadlets,
INNOBIO® Meso-Zeaxanthin 5 Beadlets, and
INNOBIO® Meso-Zeaxanthin 5 Cold Water Soluble (CWS) Powder.

### II.B. Chemistry, Physico-Chemical Properties, and Chemical Description

INNOBIO® MZ preparations are derived from purified extracts of the marigold *(Tagetes erecta)* flower. The INNOBIO® Crystalline MZ contains ⩾60% MZ, ⩽7% R'R-zeaxanthin and ⩾3.5% lutein. INNOBIO® MZ 20 Oil Suspension and INNOBIO® MZ 20 CWD Powder are diluted from INNOBIO® Crystalline MZ to contain ⩾18% MZ, ⩽2% R'R-zeaxanthin and ⩾1% lutein. INNOBIO® MZ 10 CWD Powder and INNOBIO® MZ 10 Beadlets are diluted from INNOBIO® Crystalline MZ to contain ⩾9% MZ, ⩽1% R'R-zeaxanthin and ⩾0.5% lutein. INNOBIO® MZ 5% Beadlets and INNOBIO® MZ 5% CWS Powder are diluted from INNOBIO® Crystalline MZ to contain ⩾4.5% MZ, ⩽0.5% R'R-zeaxanthin and ⩾0.25% lutein.

The common structure in all carotenoids is a linear, conjugated chain of double bonds that is responsible for the brilliant red, orange and yellow pigments found in nature (Bone et al., 2011). Structurally, lutein and zeaxanthin have identical chemical formulas and are isomers, but they are not stereoisomers. Lutein and zeaxanthin consist of the basic C40 isoprenoid structure common to all carotenoids along with two cyclic end groups, one β - and one α-ionone ring (Bone et al., 2011). The main difference between them is in the location of a double bond in one of the end rings. Zeaxanthin and its stereoisomers have identical chemical formulas to lutein but their absorption spectrum is shifted noticeably to the red. Table 1 presents chemical and physical characteristics of zeaxanthin and lutein.

MZ and R'R-zeaxanthin differ in their biochemical structure as shown in Figure 1. Zeaxanthin refers to (3R, 3'R)-zeaxanthin, which is the structure found in dietary sources of zeaxanthin, such as corn and eggs (Bone et al., 2007). Commercially available MZ (sometime referred to as "zeaxanthin isomers") cannot be extracted from plant materials; it is synthesized by exposing lutein to heat and alkaline conditions (Bone et al., 2007). Meso-zeaxanthin, being a chemical alteration of lutein, is considered semi-synthetic in the published literature (Bone et al., 2007).

Table 1. Chemical and physical characteristics of zeaxanthin and lutein

| **Property/Parameter** | **Zeaxanthin** | **Lutein** |
|---|---|---|
| CAS Registry No. | 144-68-3 | 127-40-2 |
| Chemical Names | β, β-carotene-3,3'-diol | β, ε-carotene-3,3'-diol |
| Chemical isomers | 1.(3R,3'R)- β, β-carotene-3,3'-diol;<br>2. (3R,3'S)- β, β-carotene-3,3'-diol (meso);<br>3. (3S,3'S)- β, β-carotene-3,3'-diol | No stereoisomers |
| Empirical formula | $C_{40}H_{56}0_2$ | $C_{40}H_{56}0_2$ |
| Molecular weight | 568.88 | 568.88 |
| Melting point | 207-215.5°C | 177-178°C |
| Density | 0.38-0.41 g/mL | 0.35-0.40 g/mL |

*Based on information provided by INNOBIO® and other publicly available sources.

Figure 1. Structure of MZ, zeaxanthin, and lutein.

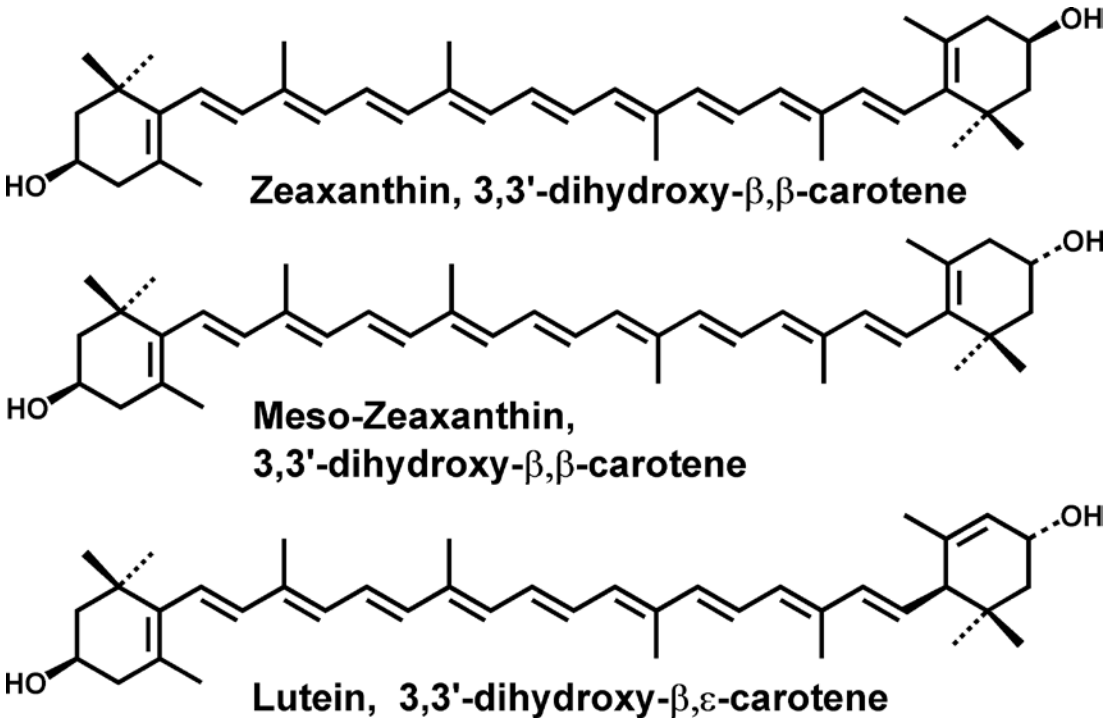


Table 2 compares the composition of MZ prepared by INNOBIO® and that described in GRN 481. The ratio of MZ:Z:lutein is approximately 17:2:1 for INNOBIO's preparations and 4:0.3:1 for GRN 481.

Table 2. Principal constituents of crystalline MZ

| GRAS Notice | Current notice | GRN 481 |
|---|---|---|
| Principal Constituent (% Weight) | | |
| Total carotenoids | ⩾70 | ⩾80.0 |
| Total Zeaxanthin (both MZ and 3R,3R'-Zeaxanthin) | ⩾65 | ⩾65 |
| MZ | ⩾60 | ⩾60 |
| 3R,3R'-Zeaxanthin | ⩽7.0 | ⩽5.0 |
| Lutein | ⩾3.5 | ⩾15 |
| Total Fat | ＜6.0 | 9.51 |
| Waxes | ＜16.0 | 1.77 |
| Total Carbohydrate | ＜3.5 | 3.22 |
| Protein | 0 | 0.00 |
| Moisture | 5.0 | 5.00 |
| Ash | <1.0 | 0.50 |

**II.C. Manufacturing Process of INNOBIO® MZ Preparations**

INNOBIO® MZ preparations are naturally derived from marigold flower (*Tagetes erecta*) and manufactured through catalytic isomerization (with excessive solid sodium hydroxide and the additives such as formic acid, acetic acid, or oxalic acid, etc.). Manufacturing processes of INNOBIO® MZ products are described as follows.

General Description of INNOBIO 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 manufacturing process of INNOBIO® products. Processing aids, such as solvents and water, buffer salts and other ingredients used in the manufacturing process are food grades.

Manufacturing Process of Crystalline MZ

The manufacturing process of INNOBIO® Crystalline MZ starts from fresh marigold flowers that are dried to prepare meal. Marigold oleoresin is obtained from the marigold meal by hexane extraction. The extraction process 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 like heavy metals and pesticide residues. Second, microfiltration is applied to eliminate impurities introduced from hexane extraction, including black specks, plant fibers, pollen and others. Finally, marigold oleoresin is obtained by reduced pressure distillation to avoid degradation of carotenoids. The oleoresin is then subjected to saponification and catalytic isomerization (translocation of the double bond) as described below to obtain the free crystalline MZ with defined qualities.

The saponification and isomerization process consists of admixing the marigold oleoresin with the alcohol solvent (ethanol, isopropyl alcohol, methanol or a mixture), excessive solid sodium hydroxide and the additive (formic acid, or acetic acid, or oxalic acid, etc.) under the pressure of 0-4MPa at 90-130℃ for 12-36 hours. More crystalline MZ is precipitated by decreasing the reactor temperature to 40-80℃ and diluting the reaction mixture with water at 30-50℃. The excessive sodium hydroxide in the mixture is neutralized with the acetic acid solution to get the final pH of 7.5-9.0. The MZ wet cake obtained by filtration is purified by washing with the alcohol/water solution (V/V 40-70%) and alcohol. After adding a small quantity of antioxidants (tocopherols and ascorbyl palmitate), the wet crystalline MZ is dried at 30-80℃ under vacuum to remove the alcohol solvent and water. Crystalline MZ is sieved with the 40 mesh and then packaged in a 5kg net aluminum foil bag under vacuum.

Manufacturing Process of MZ 20 Oil Suspension:

INNOBIO® MZ 20 Oil Suspension is made by suspending crystalline MZ in vegetable oils (e.g., sunflower oil, safflower oil or corn oil) to a final concentration of at least 19% MZ or 20% of total zeaxanthin. MZ oil suspension is milled to the D50 diameter of 80

μm or less, and then packaged in a 20 kg net aluminum drum under nitrogen.

Manufacturing Process of MZ 5 Cold Water Soluble (CWS) Powder, MZ 10 Cold Water Dispersible (CWD) Powder and MZ 20 CWD Powder

INNOBIO® MZ 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 4.5% of free MZ or 5% of total zeaxanthin. INNOBIO® MZ 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 9% and 18% of MZ or 10% and 20% of total zeaxanthin, respectively.

The CWS and the CWD powders have different application directions but share an identical manufacturing process: The concentrations of modified starch and gum arabic vary: 20-30% for CWS and >30% for CWD preparations. The process starts from admixing coating materials and antioxidants (mixture of 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 MZ is then added to the mixture, which is stirred to form emulsion. The emulsion of MZ is milled and then spray dried at the temperature range of 140-200℃ to form powder. After sieving and mixing, the MZ powder is packaged in a 5kg net aluminum foil bag under nitrogen to obtain the final powder product of MZ.

Manufacturing Process of MZ 5 Beadlets and MZ 10 Beadlets:

INNOBIO® MZ 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 4.5% and 9% of free MZ or at least 5% and 10% of total zeaxanthin, respectively.

The production starts from admixing coating materials and antioxidants (mixture of gelatin, sucrose, modified starch, gum arabic, tocopherols, ascorbyl palmitate, and corn starch) with water at the temperature range of 40-80°C for complete dissolution. Crystalline MZ is then added to the mixture, which is stirred to form emulsion. The emulsion of MZ is milled and then spray dried at the temperature range of 80-180℃ to form powder, which goes through fluidized corn starch to form MZ beadlets. The beadlets are then dried at the temperature range of 10-60℃ to remove the residual water. After sieving, the MZ beadlets are packaged in a 5kg net aluminum foil bag under nitrogen to obtain the final beadlets product of MZ.

**II.D. Specifications of MZ preparations**

Specifications for INNOBIO® MZ preparations are listed in Table 3. Analytical results from 3 nonconsecutive lots are provided in Appendix A to demonstrate that MZ preparations are consistently manufactured to meet the specifications.

Table 3-1. Specifications for INNOBIO® Crystalline MZ

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Reddish-orange powder | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 65.0 Min. | USP 32/NF 27, modified* |
| MZ, % | 60.0 Min. | |
| Lutein, % | 3.5 Min. | USP 32/NF 27 |
| Moisture, % | 1.0 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 |
| Methanol, 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 |

*Details of method are shown in Appendix C.

Table 3-2. Specifications for INNOBIO® MZ 20 Oil Suspension

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Orange-red oil suspension. | Visual |
| Zeaxanthin (MZ +R,R' zeaxanthin), % | 20.0 Min. | USP 32/NF 27, modified |
| MZ, % | 18.0 Min. | |
| Lutein, % | 1.0 Min. | USP 32/NF 27 |
| Moisture, % | 1.0 Max. | USP 731 |
| 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 |
| Methanol, 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 3-3. Specifications for INNOBIO® MZ 20 CWD Powder

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 20.0 Min. | USP 32/NF 27, modified |
| MZ, % | 18.0 Min. | |
| Lutein, % | 1.0 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 |
| Methanol, 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 3-4. Specifications for INNOBIO® MZ 10 CWD Powder

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 10.0 Min. | USP 32/NF 27, modified |
| MZ, % | 9.0 Min. | |
| Lutein, % | 0.5 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 |
| Methanol, 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 3-5. Specifications for INNOBIO® MZ 10 Beadlets

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 10.0 Min. | USP 32/NF 27, modified |
| MZ, % | 9.0 Min. | |
| Lutein, % | 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 |
| Methanol, 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 3-6. Specifications for INNOBIO® MZ 5 Beadlets

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 5.0 Min. | USP 32/NF 27, modified |
| MZ, % | 4.5 Min. | |
| Lutein, % | 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 |
| Methanol, 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 3-7. Specifications for INNOBIO® MZ 5 CWS Powder

| Parameter | Specification | Method |
|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual |
| Zeaxanthin (MZ+R,R' Zeaxanthin), % | 5.0 Min. | USP 32/NF 27, modified |
| MZ, % | 4.5 Min. | |
| Lutein, % | 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 |
| Methanol, 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 MZ

Maoka et al. (1986) first reported that MZ was found in human foods such as shrimp, carapace, depot fat deposits in turtles, tilapia, catfish, halibut, Alaska pollock, and other species of fishes. Recently, Nolan et al. (2014) found that MZ was naturally present in salmon skin, sardine skin, and trout flesh and skin. MZ is also present in significant quantities in commercially produced chickens and eggs in Mexico where it is commonly added for desirable coloration in these products (Bone et al., 2007; Thurnham, 2007). As a result of this, MZ is found in significant quantities in the resultant poultry products, which are used as human foods.

Lutein and zeaxanthin are widely distributed in the human diet, in particular dark green leafy vegetables, in a ratio of approximately 5-7:1, respectively (Sommerburg et al., 1998). Lutein and zeaxanthin also occur in high concentrations in chicken egg yolk (Handelman et al., 1999).

### III.B. Intended Use

The INNOBIO® intended use of MZ preparations has been expanded from food products listed in the GRN 481 (FDA, 2014) by adding protein bars, low sodium tortillas, and vitamin water at a level of 1.2 mg MZ/serving (RACC; reference amounts customarily consumed, 21 CFR 101.12). Table 4 shows the maximum use levels for MZ under the intended use. The INNOBIO® MZ 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 0.18 to 1.8 mg MZ per serving (corresponding to 0.3-3.0 mg INNOBIO® Crystalline MZ). 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. INNOBIO® does not intend to use MZ as a component of infant formula or in foods under USDA's jurisdiction such as meat, poultry and egg products.

Table 4. Food categories and intended use levels of MZ in the US.

| Food Category | Proposed Food | Use Levels, mg/RACC[1,2] | | | | |
|---|---|---|---|---|---|---|
| | | MZ | INNOBIO® crystalline MZ (≥60%) | MZ 20 series (≥18%) | MZ 10 series (≥9%) | MZ 5 series (≥4.5%) |
| Baked Goods and Baking Mixes | Protein bars, cereal bars, and energy bars | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | crackers and crisp-breads | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Tortillas, low sodium | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Beverages and Beverage Bases | Vitamin water | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Bottled water | 0.3 | 0.5 | 1.7 | 3.3 | 6.7 |
| | Carbonated beverages; Meal replacements | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Tea, ready-to-drink | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |
| Breakfast Cereals | Instant and regular hot cereals | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Ready-to-eat cereals | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Chewing Gum | Chewing gum | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Dairy Product Analogs | Imitation milks | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Soy milks | 0.9 | 1.5 | 7.5 | 15.0 | |
| Egg Substitute Products | Liquid, frozen or dried egg substitutes | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Fats and Oils | Margarine-like spreads, salad dressings | 0.9 | 1.5 | 7.5 | 15.0 | 30.0 |
| Frozen Dairy Desserts and Mixes | Frozen yogurt | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Gravies and Sauces | Tomato based sauces | 0.18 | 0.3 | 1.0 | 2.0 | 4.0 |
| Hard Candy | Hard candy | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Infant and Toddler Foods | Junior, strained and toddler-type baby foods | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Milk Products | Dry milk flavored milk and milk drinks | 1.8 | 3.0 | 10.0 | 20.0 | 40.0 |
| | Fermented milk beverages | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |
| | Yogurt | 1.8 | 3.0 | 10.0 | 20.0 | 40.0 |
| | Milk based meal replacement | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Processed Fruits and Fruit Juices | Energy, sport and isotonic drinks | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Fruit-flavored drinks, fruit juice, nectars | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| | Vegetable Juice | 1.2 | 2.0 | 6.7 | 13.3 | 26.7 |
| Soft Candy | Chewy and nougat candy; Fruit snacks | 0.6 | 1.0 | 3.3 | 6.7 | 13.4 |
| Soup and soup mixes | Canned soup | 0.36 | 0.6 | 2.0 | 4.0 | 8.0 |

[1]Use levels are the same as those described in GRN 481 except 3 newly added items.
[2]MZ 20 series indicates INNOBIO MZ 20 Oil Suspension and 20 CWD Powder; MZ series 10= INNOBIO® MZ 10 CWD Powder and INNOBIO® MZ 10 Beadlets; MZ 5 series= INNOBIO® MZ 5 Beadlets and INNOBIO® MZ 5 CWS Powder.

### III.C.2. 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 MZ 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. The usual intake estimation procedure requires at least 2 days of nutrient intake data for at least a representative subsample of the individuals in the sample in order to estimate variances (Goldman, 2005). In each of the two survey years, data were collected from a nationally representative sample of individuals of all ages. The food and dietary supplement record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. Table 5 shows EDIs under the intended use.

Table 5. EDIs under the intended use

| Population | Sample N | EDIs | | | | |
|---|---|---|---|---|---|---|
| | | Unit | Mean | SE | P90 | SE |
| MZ | | | | | | |
| All population, aged 2+y | 9,042 | mg | 5.22 | 0.072 | 9.92 | 0.154 |
| All population, aged 2+y | 8,971 | mg/kg bw | 0.086 | 0.002 | 0.173 | 0.004 |
| All-users, aged 2+y | 8,848 | mg | 5.32 | 0.068 | 10.00 | 0.151 |
| All-users, aged 2+y | 8,780 | mg/kg bw | 0.088 | 0.0016 | 0.175 | 0.004 |
| Zeaxanthin | | | | | | |
| All population, aged 2+y | 9,042 | mg | 0.44 | 0.006 | 0.83 | 0.013 |
| All population, aged 2+y | 8,971 | mg/kg bw | 0.007 | 0.0001 | 0.014 | 0.0003 |
| All-users, aged 2+y | 8,848 | mg | 0.44 | 0.056 | 0.83 | 0.012 |
| All-users, aged 2+y | 8,780 | mg/kg bw | 0.073 | 0.0001 | 0.015 | 0.0003 |
| Lutein | | | | | | |
| All population, aged 2+y | 9,042 | mg | 0.30 | 0.004 | 0.58 | 0.009 |
| All population, aged 2+y | 8,971 | mg/kg bw | 0.005 | 0.0001 | 0.010 | 0.0002 |
| All-users, aged 2+y | 8,848 | mg | 0.31 | 0.004 | 0.56 | 0.009 |
| All-users, aged 2+y | 8,780 | mg/kg bw | 0.005 | 0.0001 | 0.010 | 0.0002 |
| Sum of all 3 carotenoids | | | | | | |
| All population, aged 2+y | 9,042 | mg | 5.96 | 0.088 | 11.33 | 0.175 |
| All population, aged 2+y | 8,971 | mg/kg bw | 0.098 | 0.0018 | 0.197 | 0.0044 |
| All-users, aged 2+y | 8,848 | mg | 6.07 | 0.077 | 11.39 | 0.172 |
| All-users, aged 2+y | 8,780 | mg/kg bw | 0.100 | 0.0018 | 0.200 | 0.0043 |

P90=90th percentile; SE=standard error.

The mean intakes of MZ under the intended uses were estimated to be 5.2 mg/person/day for all population and 5.3 mg/person/day for all-users, while the 90th percentile intakes were determined as 9.9 and 10.0 mg/person/day (or 0.173 and 0.175 mg/kg BW/day), respectively. The 90th percentile intakes of zeaxanthin and the sum of both forms of zeaxanthin from intended uses are estimated to be 0.83 and 10.83 mg/person/day (or 0.015 and 0.190 mg/kg BW/day) in all-users, respectively. These levels are far below the safe intake level of 0.75 mg/kg BW/day for synthetic

zeaxanthin, (or 53 mg for a person with a BW of 70 kg) established by a Panel of Food of European Food Safety Authority (EFSA, 2012).

The 90th percentile intake of all 3 carotenoids under the intended use of the current notice is estimated to be 11.4 mg/person/day (or 0.200 mg/kg BW/day) in all-users.

The 90th percentile intake of lutein from intended uses was estimated to be 0.56 mg/person/day (0.010 mg/kg BW/day) in all-users. Our recent GRAS notification of lutein (submitted in September, 2014; expanded from GRN 140 by adding 3 new food products) would result in the 90th percentile intakes of 16.7 mg/person/day (0.251 mg/kg BW/day) and estimated cumulative exposure of 19.1 mg/person/day (intakes from regular diets, 2.4 mg/day; and expanded list of food categories from the lutein GRAS notice, 16.7 mg/person/day). Even if INNOBIO® Lutein and INNOBIO® MZ preparations are used together, cumulative exposure of lutein (from 2 GRAS notices) will not exceed 19.7 mg. These EDIs are based on the assumption that 100% of the food products listed in GRN 140 and 3 added food categories 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 European Food Safety Authority (EFSA, 2010) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), based on animal and human studies (JECFA, 2006).

## IV. Basis for GRAS determination

### IV.A. Current Regulatory Status

A recent GRAS notice for MZ preparation containing 60% MZ, 5% zeaxanthin, and 15% lutein received a "no questions" letter from FDA (GRN 481; FDA, 2014). In the GRN 481, EDI calculations were based on the food consumption data reported in the United States Department of Agriculture's (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII 1994-1 996**)** and the 1998 Supplemental Children's Survey (CSFII 1998**)**. In GRN 481, the 90 percentile intake of MZ and zeaxanthin were estimated to be 10.58 and 0.88 mg/person/day, respectively. Also, the 90 percentile intake of lutein was estimated at 2.57 mg/person/day. Overall, the 90 percentile intake of 3 carotenoids was estimated at 14.1 mg/person/day. As the MZ in this GRAS determination is similar in specifications compared to another MZ in the FDA GRAS Notice (GRN 481), it is recognized that the information and data in GRN 481 is pertinent to the safety of the MZ in this GRAS determination.

Another GRAS notice for a lutein blend containing 13.5% zeaxanthin isomers, R,R- and meso-, at a 50:50 ratio also received a "no questions" letter from FDA (GRN 385; FDA, 2011). Daily estimated intake of MZ was estimated at 1.2 mg/person/day. The pertinent information is available as indicated below:
GRN 481:
http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm381221.pdf
GRN 385: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=385.

In addition, a New Dietary Ingredient Notification (NDI) of zeaxanthin containing

approximately 2 % MZ in beadlet form was accepted and filed by FDA with no objection (2001).

In a recent scientific opinion on the substantiation of health claims related to MZ, the European Food Safety Authority (EFSA) Panel addressed the scientific substantiation of health claims in relation to MZ and maintenance of vision to conclude that MZ is sufficiently characterized (EFSA, 2010). The EFSA (2010) stated, "In the human clinical trials, a dosage of 14.9 mg per day of MZ has been administered for 120 days, without any adverse effects. Rat studies with more than 200 mg per kg per day have shown no-observed-adverse-effect (NOAEL). Ames tests have shown that MZ is anti-mutagenic". The EFSA Panel also did not raise the safety concerns on intakes of 0.75 mg/kg BW/day for synthetic zeaxanthin, corresponding to a daily intake of 53 mg for a person with a BW of 70 kg (EFSA, 2012).

Since MZ and lutein follow the same metabolic pathways, additional information on GRAS determination of lutein can be referenced as follows:
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 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, based on animal and human studies, the Panel on Food Additives and Nutrient Sources added to Food of EFSA (2010) has established an ADI of 1 mg/kg bw/day or 60 mg/day for a 60 kg adult, while JECFA established an ADI for lutein from *Tagetes erecta* L. and synthetic zeaxanthin) of up to 2 mg/kg body weight/day or up to 120 mg/person (JECFA, 2006).

**IV.B. Intended Technical Effects**

INNOBIO® MZ preparations derived from Marigold flowers (*T erecta* L) are intended to be used as a nutritional ingredient to provide a supplementary source of MZ in consumers' diets.

MZ 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....*" . Thus, MZ is exempt from the definition of "color additive" under 21 CFR 70.3(g).

**IV.C. Review of Safety Data**

As noted above, FDA has received 1 GRAS Notice (GRN 481) related to food uses of MZ and 7 notices for lutein. For each notice, after FDA's review, the agency issued letters indicating that it had no questions concerning the safety of MZ and other MP carotenoids. As the MZ in this GRAS determination is similar in specifications compared to another MZ in the previous FDA GRAS Notice (GRN 481), it is recognized that the information and data in another GRAS Notice is pertinent to the safety of the MZ in this GRAS determination. Therefore, this notice incorporates by reference the safety and metabolism studies discussed in GRN 481. Additionally, this notice discusses additional studies of MZ that have been published since the FDA's

last reviews of 2013-2014. The subject of present GRAS assessment is MZ containing small amounts of zeaxanthin and lutein.

This notice also incorporates by reference the safety and metabolism studies of lutein (containing zeaxanthin) discussed in GRN 110, 140, 221, 291, 385, 390, and 432. This notice discusses additional studies of lutein (containing zeaxanthin) that have been published since the FDA's last reviews of 2011-2012. Since the FDA's last review of MZ in 2014 (GRN 481; U.S. FDA, 2014) and lutein in 2011-2012 (GRN 432), no literature contradicting the agency's prior decision has been published.

**IV.C.1. Metabolic Fate of MZ**

Of the 42 dietary carotenoids, 14 are absorbed and used by the human body, yet only lutein, zeaxanthin and MZ are found at the macula (Loskutova et al., 2013). It appears that humans ingest relatively low levels of MZ. Despite its low concentrations in a normal diet, MZ accounts for about one third of total MP at the macula. MZ is known to be produced in the macula following isomerization of retinal lutein at the macula (Johnson et al., 2005) and deposited in the macula along with lutein and zeaxanthin as part of the MP (Bone et al., 1985; Landrum and Bone 2001). The absolute configuration of the hydroxyl groups located on the 3 and 3' carbon atoms of the carotenoid end groups is identical in the lutein and MZ molecules. The conversion of lutein into MZ involves a shift of one carbon-carbon double bond in the ∑-ring of lutein. Since MZ is a metabolite of lutein, it is expected that MZ follows a similar metabolic pathway to that of lutein.

**IV.C.2. Mutagenicity/Genotoxicity Studies of MZ**

Xu et al. (2013) reported that the testing results for Ames test, mice bone marrow erythrocyte micronucleus and mice sperm abnormality were all negative. The study demonstrated that MZ is neither cytotoxic nor mutagenic at 5 mg/plate and other diluted dosage levels for *Salmonella typhimurium* TA97, TA98, TA100, and TA102 in the absence and presence of a microsomal metabolizing system. The trials of the *in vivo* bone marrow erythrocyte micronucleus and sperm abnormality of mice were performed and found that at 5.0 g/kg, MZ did not increase the numbers of micronucleated polychromatic erythrocytes (PCEs) and abnormal sperms under the experiment conditions, and did not affect the proportion of PCEs to total erythrocytes. Based on the negative results in the two *in vivo* studies and the negative Ames test results, it can be concluded that MZ has no genotoxicity under the testing conditions.

**IV.C.3. Animal Acute/Subacute Toxicity Studies of MZ**

In an acute oral toxicity tests by Xu et al (2013a), the maximum tolerable dose was more than 10.0 g/kg BW (the highest dose tested) in Sprague Dawley (SD) rats and ICR mice; a single dose of 10.0 g/kg BW showed no toxicological signs during the 2 week observation period.

**IV.C.4. Animal Subchronic Toxicity Studies of MZ**

A few animal studies reported that the "No-Observed-Adverse-Effect-Level" (NOAEL) for MZ was in excess of 200-300 mg/kg BW/day, far greater than the EDI in this notice (0.175 -0.19 mg/kg BW/day) or doses used in dietary supplements, which are typically less than 0.5 mg/kg BW/day (Thurnham and Howard, 2013; Xu et al., 2013a).

Thurnham and Howard (2013) examined the potential toxicity of MZ in rats at dose levels of 0, 2, 20 and 200 mg/kg BW/day by oral gavage (n=10/sex/group) over a 13 week period. No treatment related abnormalities were found in animal behavior, clinical chemistry, ophthalmological and clinical pathology, and hematology in both control and test groups. In the recovery data, there were significantly higher serum sodium, total protein and globulin concentrations in the 200 mg/kg BW/day group than in the male control rates. Prothrombin time was also significantly higher for the 200 mg/kg BW/day female rats than respective controls. Differences were minor and not considered pathologically important. Otherwise, no treatment-related clinical, biochemical or pathological signs or symptoms were noted and the NOAEL of MZ was determined to be greater than 200 mg/kg BW/day.

In a 90-day subchronic toxicity study, Xu and colleagues (2013) randomized 80 SD rats into 4 groups; 0 (conrol), 300 (low dose group), 600 (intermediate dose group), and 1,200 mg/kg BW/day (high dose group). The body weight, food consumption, food utilization index, midway (day-42) and termination (day-90) hematological parameters, and blood chemistry parameters were not statistically significantly different among the groups. However, the blood AST levels of the 1,200 mg/kg male rat groups had significant increase (by 64%; $P < 0.05$) compared with the negative control group at midway of the experiment although no differences were found among the groups at the end of 13 weeks. Necropsy and pathological examination showed case of illness with histological results; liver cells vacuolar degeneration increased significantly in 1,200 mg/kg and 600 mg/kg dose groups. Spotted liver cell necrosis with inflammatory cell infiltration increased significantly in the 1,200 mg/kg dose group compared with the negative control group. Other indexes had no significant toxicity in all the tested level. The data suggested that MZ administered at the tested dosage within the range of 600-1,200 mg/kg BW/day for 90 days had hepatotoxicity. The daily oral administration of MZ at dose of 300 mg/kg BW/day was well tolerated in rats. Furthermore, gross examination of internal organs like liver, kidney, spleen, testes, ovaries, and organ-to-body weight ratios were also found normal. The NOAEL of MZ in rats was determined to be 300 mg/kg BW/day.

The findings of Xu et al. (2013) are consistent with the study of Thurnham and Howard (2013), in which the NOAEL of MZ was >200 mg/kg/day. After the application of a 100-fold safety factor, the authors suggested the ADI value of 3 mg/kg BW/day, which corresponds to 210 mg/person/day for a 70 kg adult.

Ravikrishnan et al. (2011) assessed subchronic effects of a lutein and zeaxanthin concentrate, which contains 6.75% MZ, in Wistar rats. In this subchronic study, Wistar rats (10/sex/group) were administered by gavage lutein/zeaxanthin concentrate at doses of 0, 4, 40, or 400 mg/kg BW/day for 90 days. Compared with the control group, administration of lutein/zeaxanthin concentrate did not result in any toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weights, feed consumption, and organ weights. No toxicological relevant findings were noted in urinalysis, hematology or clinical biochemistry parameters at the end of the treatment or recovery period. Terminal necropsy did not reveal any treatment-related gross or histopathology findings. Authors suggested a NOAEL for lutein/zeaxanthin concentrate as 400 mg/kg BW/day, the highest dose tested. The NOAEL for MZ was found to be 27 mg/kg BW/day, the maximum dose tested.

Table 6. Mutagenicity/genotoxicity studies reporting no adverse effects of MZ

| Organism | Dose | Duration | Outcomes tested | Reference |
|---|---|---|---|---|
| *S. typhimurium* (TA97, TA98, TA100, TA102) | 5000, 1000, 200, and 40 µg/dish | N/A | Ames test with and without metabolic activation (S9) | Xu et al., 2013 |
| Mice, ICR (n=50, 50%M, 25-30 g) | 1.25, 2.5, and 5.0 g/kg BW MZ; positive control = cyclophosphamide; negative control= distilled water. | Intragastrically administration twice at 24 hr intervals, execution conducted 6 hr after the last dose | Bone marrow erythrocyte micronucleus – total PCE and PCE/RBC | Xu et al., 2013a |
| Mice, ICR (n=35, 100%M, 25-30 g) | 1.25, 2.5, 5.0 g/kg BW MZ; positive control= mitomycin C; negative control = distilled water. | Daily intubations for 5 days, execution at 35 days after first dose administration. | Total sperm count, sperm morphology, and total abnormal sperm | Xu et al., 2013a |

PCE=polychromatic erythrocytes; RBC= red blood cells.

Table 7. Acute animal toxicity studies of MZ

| Animal | Dose | Duration | Outcomes tested | NOAEL | Reference |
|---|---|---|---|---|---|
| Mice, ICR (n=20, 50%M, 18-22 g) | 10 g/kg BW for each oral/intragastric gavage administration | MZ was administered twice at 4 h intervals, mice were monitored for 2 weeks | Maximum tolerable dose test, toxicological signs, and morbidity | 10 g/kg BW | Xu et al., 2013 |
| Rats, Sprague Dawley (n=20, 50%M, 180-220 g) | 5 g MZ/kg BW for each oral/intragastric gavage administration | MZ was administered twice at 4 h intervals, mice were monitored for 2 weeks | Maximum tolerable dose test, toxicological signs, and morbidity | 5 g/kg BW | Xu et al., 2013a |

Table 8. Subchronic animal toxicity studies of MZ

| Animal | Daily Dose of MZ, mg/kg/day | Duration | Outcomes tested | NOAEL, mg/kg BW/day | Reference |
|---|---|---|---|---|---|
| Rat, Han Wistar (n=100, 50% Male, 7-8 weeks old) | 0, 2, 20 or 200 mg/kg/day; or corn oil (control); | 13 weeks followed by a 4 week recovery period. | Clinical observations, BW, organ weight, feed consumption ophthalmology, hematology, blood biochemistry, necropsy and histopathology. | >200 | Thurnham and Howard, 2013 |
| Rats, SD (n=80, 50%M) | 300, 600, or 1,200 mg/kg BW/day; or water (control) | 90 days | Behavior, toxicological signs, BW, feed consumption, physical examination, hematology, ophthalmology, blood biochemistry, and histopathology | 300 | Xu et al., 2013a |
| Rats, Wistar (n=80, 50%M) | 0, 0.27, 2.7, or 27 mg/kg BW/day | 90 days | Behavior, toxicological signs, BW, feed consumption, physical examination, hematology, ophthalmology, blood biochemistry, and histopathology | 27 | Ravikrish-nan et al., 2011 |

BW=body weght; M=male; MZ= meso-zeaxanthin; SD= Sprague Dawley.

**IV.C.5. Additional Animal Studies of MZ**

Firdous et al. (2010) evaluated the effect of MZ on nitroso diethyl amine (NDEA)-induced hepatocelluar carcinoma in male Wistar rats. Treatment of NDEA 5 days per week for 20 weeks induced hepatocellular carcinoma in all of the control group animals. The number of tumors and percentage of incidence were reduced significantly in animals administered with 50 and 250 mg/kg BW of MZ. In the NDEA alone treated animals there was a significant elevation in the levels of hepatic marker enzymes, including gamma-glutamyl transpeptidase (GGT), alanine aminotransaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatise (ALP) in both serum and liver tissue in a dose dependent manner. In the NDEA plus MZ treated groups these levels were closer to those observed in the non-NDEA treated rats. The data indicated a dose-dependent ability of MZ to inhibit NDEA induced hepatocellular carcinoma. No adverse effects of MZ were reported.

Firdous and Kuttan (2012a) investigated the chemo-protective potential of MZ in 30 male albino mice equally divided into 5 groups: 1) untreated (negative control), 2) cisplatin alone (positive control), 3) cisplatin + sunflower oil (vehicle control), 4) cisplatin + MZ (50 mg/kg BW), and 5) cisplatin + MZ (250 mg/kg BW). Oral

administration began 5 days prior to cisplatin injection and was continued till the animals were killed 72 hours after cisplatin treatment. Administration of MZ significantly reversed the myelosuppression produced by cisplatin in a dose-dependent manner, as indicated via significantly increased levels of total white blood cell count, hemoglobin content, bone marrow cellularity and α-esterase cells. Increased levels of urea and creatinine with cisplatin were significantly decreased with MZ treatment. MZ significantly enhanced the antioxidant enzymes (such as superoxide dismutase, catalase, and glutathione peroxidase) and decreased markers of oxidative stress (lipid peroxidation, tissue hydroperoxides, and conjugated dienes) compared to cisplatin control groups in the kidney. Histopathology of kidney of the positive control group animals showed many atrophied glomeruli. In addition, Bowman's capsule appear widened, renal tubules showed atrophy in many areas, and intestinal tissue also showed scattered lymphocytes and plasma cells. Such histopathological changes were found to be minimal in the MZ treated animals. The authors suggest a protective role of MZ. No adverse effects of MZ were reported.

An analysis of MZ against doxorubicin (DOX)-induced cardiotoxicity in Wistar rats indicated a potential cardio-protective role of this carotenoid (Firdous and Kuttan, 2012b). Evaluation of serum markers of cardiac injury including lactate dehydrogenase, creatine phosphokinase, ALT and AST, which were significantly increased by DOX treatment, were decreased to normal levels by MZ pretreatment. Cardiac activities of superoxide dismutase, catalase and glutathione peroxidase, as well as glutathione were reduced by DOX, but were significantly mitigated via MZ pretreatment. MZ significantly diminished the DOX-induced oxidative stress markers (lipid peroxidation, tissue hydroperoxides and conjugated dienes) in cardiac tissues. Furthermore, DOX-induced myocardial injury was reversed significantly by MZ pretreatment. Histopathological analysis of heart tissue further showed a potential dose-dependent protective effect of MZ.

Firdous and colleagues (2011) studied the effects of MZ in three different models of hepatotoxicity (paracetamol-, $CCl_4$-, and ethanol- induced hepatotoxicity) in male Wistar rats. MZ administration significantly decreased levels of marker enzymes of heptatic injury (serum ALT, AST, and ALP and serum bilirubin) in a dose-dependent manner, despite being elevated by hepato-toxins. Oxidative stress markers (tissue lipid peroxidation, conjugated dienes and tissue hydroperoxides) were high in the paracetamol treated control group animals, but were lowered by MZ administration. Levels of glutathione and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in liver tissue were increased by MZ pretreatment compared to control group during alcohol and $CCl_4$ induced hepatotoxicity. Hydroxyproline, an indicator of fibrosis in liver tissue, decreased remarkably by MZ administration despite its notable elevation in ethanol treated rats. Histopathological analysis of liver tissue showed the hepatoprotective potential of MZ.

Table 9. Animal studies showing no adverse effects of MZ

| Animal | Dose | Duration | Outcomes tested | Results | Reference |
|---|---|---|---|---|---|
| Mice, Swiss Albino (N=30, 100% Male, 22.6+1.5 g) | 5 Groups:<br>1) normal<br>2) Cisplatin alone (Cisplatin control)<br>3) Cisplatin + sunflower oil (Vehicle control)<br>4) Cisplatin + MZ (50 mg/kg BW)<br>5) Cisplatin + MZ (250 mg/kg BW)<br>NB: Groups 2-5 a single dose of cisplatin (16 mg/kg BW ip) | 8 days: oral administration of MZ started 5 days prior to cisplatin injection and was continued till the animals were killed after 3 days. | Hematology, bone marrow cellularity, number of maturing monocytes, α-esterase activity, serum creatinine, serum urea, oxidative stress and antioxidant activity, and kidney histopathological analysis. | No adverse effects of MZ were found. MZ was protective against cisplatin -induced myelo -suppression. | Firdous and Kuttan, 2012a |
| Rat, Wistar (n=30, 100% male, 200-250 g) | 5% suspension of MZ was prepared in sunflower oil.<br>5 Groups:<br>1) untreated (Normal)<br>2) Doxorubicin alone (DOX control)<br>3) DOX and sunflower oil (Vehicle control)<br>4) DOX+ MZ 100 mg/kg BW<br>5) DOX + MZ 250 mg/kg BW | Oral administration of MZ was started 15 days prior to DOX injection. Animals in all groups were sacrificed 24 hours after DOX administration. | Serum markers of cardiac injury (lactate dehydrogenase, creatine phosphokinase, ALT and AST), markers of oxidative damage (lipid peroxidation, tissue hydroperoxides and conjugated dienes), antioxidant enzymes (superoxide dismutase, catalase activity, glutathione peroxidase), glutathione, ECG, and histopathology | No adverse effects of MZ were found. MZ was protective against DOX-induced cardiac injury. | Firdous and Kuttan, 2012b |

Table 9. Animal studies showing no adverse effects of MZ, continued

| Animal | Dose | Duration | Outcomes tested | Results | Reference |
|---|---|---|---|---|---|
| Rat, Wistar (n=38, 100% Male, 160-170 g, with nitroso diethly amine (NDEA) induced hepatocellular carcinoma) | 5% MZ suspension in sunflower oil.<br>5 Groups:<br>1) Untreated<br>2) NDEA control<br>3) NDEA+vehicle<br>4) NDEA+ 50 mg MZ/kg BW, or<br>5) NDEA+250mg MZ/kg BW | 5 day/week for 20 consecutive weeks | Survival rate, liver weight, tumor incidence, histopathological analysis, and marker enzymes of hepatic damage including GGT, ALT, AST, and ALP in both serum and liver tissue. | No adverse effects of MZ were found. MZ was protective against NDEA-induced hepato-toxicity. | Firdous et al., 2010 |
| Rat, Wistar (Paracetamol induced hepatic injury n= 36, $CCl_4$ induced hepatotoxicity n=30, Ethanol induced hepatic damage n=32; 100% male, 150-200 g) | <u>Paracetamol induced hepatic injury,</u><br>5 groups:1) Untreated;<br>2) paracetamol + sunflower oil (vehicle control);<br>3)-5) paracetamol + 50, 100 or 250 mg/kg BW MZ, or 6) paracetamol + 100 mg/kg BW silymarin (standard liver protectant).<br>Dose of paracetamol (3 g/kg BW, 2 ml, oral)<br><u>CCL4 induced hepatotoxicity,</u><br>4 groups: 1) Untreated;<br>2) $CCL_4$ + sunflower oil (vehicle control);<br>3)-5) CCL4 + 50, 100 or 250 mg/kg BW MZ;<br>CCL4 dose (2.5 ml/kg BW, ip).<br><u>Ethanol induced hepatic damage,</u><br>4 groups: 1) Untreated;<br>2) ethanol + sunflower oil (vehicle control);<br>3)-4) ethanol + 100 or 250 mg/kg BW MZ.<br>Dose of ethanol (7.5 g/kg BW, oral) | Paracetamol induced hepatic injury: MZ and silymarin treatment (oral) were started 7 days prior to paracetamol administration until 24 h after paracetamol administration.<br>$CCl_4$ induced hepatotoxicity: MZ treatment was started 7 days prior to $CCl_4$ injection. until 24 h after 2 $CCl_4$ administration.<br>Ethanol induced hepatic damage: 6 months | Hepatic injury marker enzymes, markers of oxidative stress, antioxidant profile of liver tissue, hydroxyproline content in liver, and histopathological analysis of liver. | No adverse effects of MZ were found. MZ was protective against chemical-induced hepato-toxicity. | Firdous et al., 2011 |

ALP=alkaline phosphatise; ALT= alanine aminotransaminase; AST= aspartate aminotransferase; BW= body weight; DOX=doxorubicin; ECG = electrocardiography; GGT=gamma-glutamyl transpeptidase; ip= intraperitoneally; NDEA=nitroso diethly amine; SOD=superoxide dismutase.

### IV.C.6. Animal Toxicity Studies of R'R-zeaxanthin

Since INNOBIO® Crystalline MZ contains less than 7% R'R-zeaxanthin, toxicity studies of zeaxanthin are reviewed in this notice. Ravi et al. (2014) investigated potential adverse effects of RR-zeaxanthin (65%) enriched product (obtained from paprika; Capsicum annum fruits) in subchronic toxicity and mutagenicity studies. The oral $LD_{50}$ of zeaxanthin concentrate (65% zeaxanthin) in rats was greater than 2,000 mg/kg BW. For the subchronic toxicity study, Wistar rats (10/sex/group) were gavaged daily with zeaxanthin concentrate at doses of 0, 4, 40 and 400 mg/kg BW/day for 90-days. No treatment-related clinical signs and mortalities were observed. Similarly, no treatment-related toxicologically significant changes in body weight, feed consumption; ophthalmoscopic examination, neurological examination, hematology, urine analysis and organ weights were observed. Statistically significant changes observed in some clinical chemistry parameters were considered toxicologically and biologically insignificant and non-adverse. Macroscopic and microscopic examinations did not reveal treatment-related abnormalities. The results of mutagenicity testing using *Salmonella typhimurium* did not reveal any genotoxicity. The NOAEL for zeaxanthin concentrate (65% zeaxanthin) was determined to be 400 mg/kg BW/day, the highest dose tested.

### IV.C.7. Animal Toxicity Studies of Lutein

Since MZ is a metabolite of lutein and INNOBIO® Crystalline MZ contains 3.5% lutein, we have briefly summarized animal toxicity studies of lutein.

Lutein was found to be not mutagenic or clastogenic (Kruger et al., 2002; Ravikrishan et al., 2011; Wang et al., 2006). Also, *in vivo* rat study showed that lutein acted as an inhibitor during promotion, but not initiation of hepatocarcinogenesis (Moreno et al., 2007).

Subchronic toxicity studies showed that the NOAEL for lutein was over 400 mg/kg BW/day (Harikumar et al., 2008; Kruger et al., 2002; Ravikrishan et al., 2011) (Table 10). In addition, in a developmental toxicity study, female SD rats were orally administered doses of up to 1,118 mg lutein/kg BW/day on gestation days 6 to 20 without showing maternal toxicity or embryotoxicity/teratogenicity (Edwards et al., 2002; cited in GRN 390).

Table 10. Animal toxicity studies of lutein

| | Daily dose, mg/kg BW | Duration | NOAEL, mg/kg BW/day | Reference |
|---|---|---|---|---|
| Crystalline form | | | | |
| 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 g/kg BW | Harikumar 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 g/kg BW | Ravikri-shan et al., 2011 |
| 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 |
| 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, or 400 mg/kg BW crystalline lutein (>67% lutein +13.5% Z with both Z isomers) | 90 d | NOAEL- 400 mg/kg BW/day | Ravikri-shan et al., 2011 |
| Suspended in Oil | | | | |
| 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 |
| 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 |

BW= Body weight; Z=zeaxanthin.

## IV.D. Human Clinical Studies

### IV.D.1. Human Clinical Studies of MZ

Table 11 summarizes human clinical studies showing no adverse effects of MZ.

Consumption of MZ at daily doses of up to 17 mg for up to 12 months did not show any adverse effects as evidenced by serum analysis, renal and liver function, as well as lipid profile, hematological parameters, and indicators of inflammation, are unaffected by such supplementation (Bone et al., 2007; Connolly et al., 2010, 2011; Loughman et al., 2012; Meagher et al., 2013; Nolan et al., 2012; Sabour-Pickett et al., 2014; Thurnham et al, 2008). For these 'pivotal' studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints.

Table 11. Human clinical studies showing no adverse effects of MZ

| Subjects | N | L (mg) | Z (mg) | MZ (mg) | Duration (weeks) | Outcome measures | Study |
|---|---|---|---|---|---|---|---|
| 52 early AMD | 17 | 20 | 2 | 0 | 52 | Visual performance | Sabour-Pickett et al., 2014 |
| | 21 | 10 | 2 | 10 | | | |
| | 14 | 3 | 2 | 17 | | | |
| 19 Healthy | 10 | 5.5 | 1.4 | 14.9 | 17 | Serum response, MPOD | Bone et al., 2007 |
| | 9 | 0 | 0 | 0 | | | |
| 19 Healthy | 19 | 10.8 | 1.2 | 8 | 3 | Serum response | Thurnham et al, 2008 |
| 10 Healthy/ Early AMD | 10 | 3.7 | 0.8 | 7.3 | 8 | Serum response, MPOD | Connolly et al., 2010 |
| 44 Healthy | 22 (18) | 5.9 | 1.2 | 10.6 | 24 | Serum response (including lipid profiles), MPOD, pathological exam | Connolly et al., 2011 |
| | 22 (17) | 0 | 0 | 0 | | | |
| 30 Healthy | 10 | 20 | 2 | 0 | 8 | MPOD | Nolan et al., 2012 |
| | 10 | 10 | 2 | 10 | | | |
| | 10 | 3 | 2 | 17 | | | |
| 54 Healthy/ AMD | 21 | 20 | 2 | 0 | 8 | Serum response | Meagher et al., 2013 |
| | 20 | 10 | 2 | 10 | | | |
| | 13 | 3 | 2 | 17 | | | |
| 36 Healthy | 11 | 20 | 2 | 0 | 26 | Serum response, MPOD, visual performance | Loughman et al., 2012 |
| | 11 | 10 | 2 | 10 | | | |
| | 10 | 0 | 0 | 0 | | | |

AMD=age-related macular degeneration; L=lutein; MPOD= macular pigment optical density; MZ=meso-zeaxanthin; Z=zeaxanthin.

Sabour-Pickett et al. (2014) investigated the impact of three different macular carotenoid formulations on MPOD and visual performance in subjects with early AMD. In this study, 52 subjects were supplemented and followed for 12 months, 17 of them were in intervention Group 1 (20 mg/day lutein plus 2 mg/day zeaxanthin); 21 in Group 2 (10 mg/day MZ, 10 mg/day lutein plus, 2 mg/day zeaxanthin); and 14 in Group 3 (17 mg/day MZ, 3 mg/day lutein, plus 2 mg/day zeaxanthin). A

statistically significant increase in the MPOD was observed at all measured eccentricities in Group 2 ($P \leq 0.005$) and in Group 3 ($P < 0.05$, for all), but only at 1.75° in Group 1 ($P = 0.018$). Statistically significant ($P < 0.05$) improvements in letter contrast sensitivity were seen at all spatial frequencies (except 1.2 cycles per degree) in Group 3, and at low spatial frequencies in Groups 1 and 2. Authors concluded that augmentation of the MPOD across its spatial profile and enhancements in contrast sensitivity were best achieved after supplementation with a formulation containing high doses of MZ in combination with lutein and zeaxanthin. No adverse effects of all 3 carotenoids were reported.

Meagher et al. (2013) evaluated 4-week and 8-week serum responses to three different MP carotenoids supplements in normal subjects (n=27) and in subjects with AMD (n=27). Subjects were randomly assigned to: Group 1 (20 mg L plus 2 mg zeaxanthin), Group 2 (10 mg L, 2mg zeaxanthin and 10 mg MZ) or Group 3 (3 mg lutein, 2 mg zeaxanthin and 17 mg MZ). Serum carotenoids were quantified at baseline, and at 4 and 8 weeks using HPLC. Response data for normal and AMD subjects were comparable and therefore combined for analysis. Serum MZ increased significantly in Group 1 (0·009 umol/l (absolute value); P=0·015), Group 2 (0·063 umol/l; P<0.001) and Group 3 (0·073 umol/l; P<0.001). Serum lutein increased significantly in Group 1 (269%; P<0.001) and Group 2 (340%; P<0.001), with no significant change in Group 3. Serum zeaxanthin increased significantly in Group 1 (69 %; P<0.001) and Group 2 (75 %; P<0.001), with no significant change in Group 3 (-6 %; NS). The formulation containing all three macular carotenoids (Group 2 supplement) was the most efficacious in terms of achieving the highest combined concentration of the three MP constituent carotenoids in serum, thereby potentially optimising the bioavailability of these compounds for capture by the target tissue (retina). No adverse effects of all 3 carotenoids were reported.

Bone et al. (2007) reported that supplementation of unesterified carotenoids (14.9 mg of MZ, 5.5 mg of lutein, and 1.4 mg of zeaxanthin as a suspension in soybean oil) for 120 days increased MPOD, measured at 460 nm, at an average rate of 0.59 +/- 0.79 milli-absorbance unit/day in the 10 supplemented subjects. Prior to supplementation, the average serum concentrations for the 10 subjects were 30.5 ± 12.5nmol/dL for lutein, and 9.7 ± 4.8 nmol/dL for zeaxanthin + MZ. From week 6 to the end of supplementation, these values rose to 38.0 ± 12.0 nmol/dL for lutein, and 26.4 ± 6.5 nmol/dL for zeaxanthin + MZ. The presence in the serum of MZ, in addition to lutein and zeaxanthin, resulting from supplementation was confirmed in the two subjects, A and B, who participated in this part of the study. Prior to supplementation, the serum concentrations of lutein, zeaxanthin, and MZ were 22.9, 4.4, and 0.0 nmol/dL, and 14.8, 2.1, and 0.0 nmol/dL, for subjects A and B, respectively. After 6 weeks of supplementation, these values were 23.9, 8.7, and 4.4 nmol/dL for subject A, and 45.4, 15.4, and 14.5 nmol/dL for subject B. This study showed that MZ is absorbed into the serum following ingestion. The data indicate that a supplement containing predominantly MZ is generally effective at raising MPOD, and may turn out to be a useful addition to the defenses against AMD. No adverse effects of MZ were reported.

Thurnham et al. (2008) measured the bioavailability of MZ using 19 healthy volunteers by administering a capsule of Lutein Plus (containing 8.0 mg MZ, 10.8 mg lutein, and 1.2mg zeaxanthin) everyday for 3 weeks. Blood was taken at baseline, day 10 and day 22. Plasma concentrations per mg dose at day 22 suggested that

zeaxanthin was about 50% more actively retained by the body than lutein (although the difference was not significant in women) and 2.5-3.0 times more than MZ. Concentrations of MZ at day 22 were 2.5 times higher in women than men. By comparing with literature values, it appears that uptake of lutein and zeaxanthin into plasma appeared to be slightly depressed by the presence of MZ. No adverse effects of MZ were reported.

Connolly et al. (2010) designed a study to measure serum and macular responses to a macular carotenoid formulation. Ten subjects with early AMD were instructed to consume a formulation containing 7.3 mg of MZ, 3.7 mg of lutein, and 0.8 mg of zeaxanthin everyday for 8 weeks. There was a significant increase in serum concentrations of MZ and lutein after two weeks of supplementation ($p < 0.05$). Baseline serum carotenoid analysis detected a small peak eluting at the same time as MZ in all subjects, with a mean ± SD of 0.02 ± 0.01 μmol/L. Authors reported significant increases in MPOD at 0.25°, 0.5°, 1°, and average MPOD across its spatial profile after just two weeks of supplementation ($p < 0.05$, for all). No adverse effects of MZ were reported.

In 2011, Connolly et al. investigated serum and macular response to, and safety of supplementation with MZ, lutein, and zeaxanthin. Forty-four healthy subjects consumed one tablet per day containing 10.6 mg MZ, 5.9 mg L, and 1.2 mg zeaxanthin or placebo (control group) for 6 months. Serum concentrations of lutein and zeaxanthin increased significantly in the carotenoids group ($P = 0.001$ and 0.003, respectively) and remained stable in the control group ($P > 0.05$). There was a significant increase in central MPOD in the carotenoids group (0.25°: $P = 0.001$; 0.5°: $P = 0.001$), with no significant change in the placebo group ($P > 0.05$). Clinical pathology analysis confirmed that all variables remained within the normal reference range, with the exception of total cholesterol (TC) and low-density lipoprotein (LDL-C), which exhibited baseline values outside the accepted normal reference range before supplementation. Pathology analysis suggested no adverse clinical implications of consuming these carotenoids. Authors concluded that subjects supplemented with MZ, lutein, and zeaxanthin exhibited significant increases in serum concentrations of these carotenoids and a subsequent increase in central MPOD. Pathology analysis suggested no adverse clinical implications of consuming these carotenoids.

Nolan et al. (2012) investigated the impact of macular carotenoid supplementation on the spatial profile of MP in subjects where the profile does not exhibit the typical central peak (i.e. peaked MP at foveal epicentre). Thirty one healthy subjects with such atypical MP spatial profiles were assigned to one of three intervention groups: Group 1: (n =10), 20 mg/day lutein+ 2 mg/day zeaxanthin; Group 2: (n =10), 10 mg/day MZ + 10 mg/day lutein + 2 mg/day zeaxanthin; Group 3: (n =10), 17 mg/day MZ + 3 mg/day lutein + 2 mg/day zeaxanthin. Subjects were instructed to take one capsule daily over an 8-week period. MP at 0.25°, 0.5°, 1°, 1.75° and 3° was measured using customized-heterochromatic flicker photometry at baseline, 4 weeks and 8 weeks. Over the study period, authors reported no statistically significant increase in MP at any eccentricity in Group 1 ($p > 0.05$, for all eccentricities). There was a trend towards an increase in MP at all eccentricities in Group 2, with a significant increase found at 0.25° and 0.50° ($p= 0.000$ and $p= 0.016$, respectively). There was a statistically significant increase in MP at 0.25° in Group 3 ($p < 0.005$),

but at no other eccentricity ($p > 0.05$, for all other). Authors reported that the typical central peak of MP can be realized in subjects with atypical spatial profiles, following supplementation with a preparation containing all three macular carotenoids, but not with a supplement lacking MZ. No adverse effects of MZ were reported.

Loughman et al. (2012) investigated changes in MPOD and visual performance following supplementation with different macular carotenoid formulations. Thirty-six subjects (19 male, 17 female; mean ± SD, age 51 ± 13 years) were randomly assigned to one of the following three intervention (supplementation) groups: (1) Group 1 (20 mg lutein and 2 mg zeaxanthin); (2) Group 2 (10 mg lutein, 2 mg zeaxanthin, and 10 mg MZ); and Group 3 (placebo). Outcomes measures included visual performance and MPOD response. Data were collected at baseline, at 3 months, and at 6 months. At 3 and 6 months, a statistically significant increase in MPOD was found at all eccentricities (other than the most peripheral $3^{o}$ location) in Group 2 with 10 mg MZ ($P < 0.05$ for all), whereas no significant increase in MPOD was found at any eccentricity for subjects in Groups 1 and 3 (no MZ). Statistically significant improvements in visual performance measures including visual acuity and contrast sensitivity with and without glare were observed for Group 2 only. No improvements in any parameters of visual performance were observed for subjects supplemented with placebo ($P > 0.05$ for all). No adverse effects of MZ were reported.

**IV.D. 2. Human Clinical Studies of Lutein**

Since INNOBIO® Crystalline MZ contains >3.5% lutein and MZ is a metabolite of lutein, we have summarized human clinical studies of lutein (Table 12). Our summary is limited to the studies published since 2012 (AREDS Research group, 2013, 2014; Berrow et al., 2013; Evans et al., 2013; Garcia-Layana et al., 2013; Graydon et al., 2012; Hammond et al, 2012; Huang et al., 2013; Landrum et al., 2012; Ma et al., 2012a, 2012b; Murray et al., 2013; Nolan et al., 2012; Tanito et al., 2012; Thomson et al., 2014; Tian et al., 2013; Wang et al., 2013; Xu et al., 2013; Yao et al., 2013; Zeimer et al., 2012; Zou et al., 2014). As shown in Table 12, daily consumption of 20-21 mg lutein for 1 year did not cause any adverse effects. Our review supports the upper limit of Observed Safe Level (OSL) as 20 mg/person/day established by Shao and Hathcock (2006) from a previous review of 30 human clinical studies. This OSL method provided strong evidence supporting the safety of lutein intake at levels up to 20 mg/day.

Table 12. Human clinical studies reporting no adverse effects of lutein

| Subjects | Daily dose | Duration | Measured Outcomes | Reference |
|---|---|---|---|---|
| 14 participants | Lutein based oral supplement; or no supplement (control group) | 40 weeks. Observed another 20 weeks after supplement withdrawal | Retinal and visual function in people with early stages of ARMD (age-related maculopathy, ARM) | Berrow et al., 2013 |
| 144 subjects with subclinical atherosclerosis | 20 mg lutein (n 48); 20 mg lutein +20 mg lycopene (n 48); or placebo | 12 months | Carotid artery intima-media thickness | Zou et al., 2014 |
| older adults (BMI, 25.3) not meeting Australian physical activity guidelines | 21 mg of lutein; or placebo with 250 mL of full-cream milk | 4 weeks | Physical activity | Thomson et al., 2014 |
| 65 Early atherosclerosis patients | 20 mg lutein; or placebo | 3 months | Serum cytokines, apoE and lipoprotein profiles in early atherosclerosis population. | Xu et al., 2013b |
| 48 healthy volunteers | Single dose of 20 mg of lutein | 672 hours | Plasma kinetics of lutein and Z provided as two different beadlet formulations | Evans et al., 2013 |
| 117 healthy nonsmokers | 10 or 20 mg of lutein; or placebo | 12 weeks | Levels of plasma carotenoid, TAOC, lipoprotein profile, and antioxidant enzymes activities | Wang et al., 2013 |
| 108 subjects with early AMD | 10 mg lutein; 20 mg lutein; 10 mg lutein plus 10 mg Z; or placebo | 48 weeks | Retinal function using multifocal electroretinograms (mfERG) | Ma et al., 2012a |
| 120 healthy drivers with long-term light exposure (drivers). | 20 mg of lutein; or placebo | 1year | Visual function | Yao et al., 2013 |
| 108 subjects with early AMD, 50 to 79 years of age | 4 groups-1) 10 mg lutein; 2) 20 mg lutein; 3)10 mg lutein +10 mg Z; or 4) placebo | 48 weeks | Macular pigment and visual function | Ma et al., 2012b |
| 108 patients with early AMD aged > 50 year | low lutein (LL; 10 mg); high lutein (HL; 20 mg); lutein plus zeaxanthin (LZ; each 10 mg); or placebo | 48 weeks | Serum and macular (*in vivo* measured MPOD) responses | Huang et al, 2013 |
| 322 healthy female twin volunteers, aged 16–50 years | 18 mg L +2.4 mg Z | 6 months | Heritability of MP augmentation | Hammond et al, 2012 |

Table 12. Human clinical studies reporting no adverse effects of lutein, continued

| Subjects | Daily Dose | Duration | Measured Outcomes | Reference |
|---|---|---|---|---|
| Thirty one healthy subjects with such atypical MP spatial profiles were assigned to one of three intervention groups | Group 1) 20 mg lutein + 2 mg Z; Group 2) 10 mg MZ+10 mg L+ 2 mg Z; Group 3) 17 mg MZ +3 mg L + 2 mg Z. | 8 weeks | MP in subjects where the profile does not exhibit the typical central peak (i.e. peaked MP at foveal epicentre). | Nolan et al., 2012 |
| 30 healthy volunteers | 20 mg lutein diacetate supplement; 20 mg crystalline lutein; or a placebo | 24 weeks | Effects on serum and macular pigment | Landrum et al., 2012 |
| 4203 elderly at risk for developing late AMD 50 -85 y | 10 mg/2 mg lutein/zeaxanthin; 1.0 g omega-3 LC PUFA; the combination or placebo. | median of 4.8 years | Development of late AMD | AREDS2) Research Group, 2013, 2014 |
| 44 patients with AMD | 12 mg of lutein + 280 mg of DHA; or placebo | 1 year | MPOD | Garcia-Layana et al., 2013 |
| 72 patients with early AMD (mean age 70.5 ± 8.7) | Lutein capsules (10 mg ester); or a placebo | 12 months | MPOD and visual acuity | Murray et al., 2013 |
| 97 subjects | 12 mg L and 1 mg Z | 6-month | A ring structure | Zeimer et al., 2012 |
| 22 healthy volunteers without high myopia | 10 mg lutein; or 10 mg Z | 3 months | MPOD | Tanito et al., 2012 |
| 72 subjects with signs of early AMD | 10 mg lutein; or a placebo | 12 months | Complement factor D (CFD) and its products C5a and C3d | Tian et al., 2013 |
| Study 1. 52 Subjects<br><br>Study 2. 75 Subjects | 1) Either carrot juice (a carotene-rich food) or spinach powder (a lutein- and Z-rich food)<br>2) supplements containing lutein and Z, β-carotene; or 3) placebo | two 8-week studies | MP level and serological markers of endothelial activation, inflammation and oxidation | Graydon et al., 2012 |

AREDS2=Age-Related Eye Disease Study 2; AUC=Area Under the Curve; BMI=body mass index; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; LC-PUFA= long chain polyunsaturated fatty acids; MP=macular pigments; MPOD=macular pigment optical density; MZ =meso-zeaxanthin; Z= zeaxanthin.

## V. SAFETY DETERMINTATION

Numerous human and animal studies have reported health benefits of MZ 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 MZ. This GRAS determination is based on the data and information generally available and consented opinion about the safety of MZ. The literature indicates that MZ offers consumers health benefits without serious adverse effects.

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

1. MZ will be used as a food ingredient at concentrations of up to 0.18 to 1.8 mg/serving (or 0.3-3.0 mg INNOBIO® Crystalline MZ per 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, and soups and soup mixes. Intended use excludes food categories such as egg products and soup and soup mixes that come under USDA. The 90 percentile intakes of MZ and zeaxanthin are estimated to be 10.00 and 0.83 mg/person/day, respectively. INNOBIO® Crystalline MZ also contains 3.5% lutein; therefore, the 90th percentile lutein intake from the proposed uses will be 0.56 mg/person/day. The 90$^{th}$ percentile intake of all 3 carotenoids is estimated to be 11.4 mg/person/day (or 0.200 mg/kg BW/day).

2. The NOAEL for MZ was determined to be 300 mg/kg BW/day, the highest level tested in animals. Intended use of INNOBIO® MZ results in EDI which is much lower than the NOAEL found in animal studies. In humans, consumption of MZ at daily doses of 17 mg for up to 12 months did not show any adverse effects. For these ‘pivotal’ studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints. A recent GRAS notice for MZ preparation containing 60% MZ, 5% zeaxanthin and 15% lutein received a "no questions" letter from FDA (GRN 481; FDA, 2014).

3. INNOBIO® MZ preparations contain small amounts of lutein. In addition, mso-zeaxanthin is a metabolite of lutein. Thus, the safety of lutein may be considered for the safety assessment of MZ. FDA has received seven GRAS Notices related to food uses of lutein and lutein esters. In response to these 7 separate GRAS notifications on lutein, FDA did not question the safety of lutein and its esters for the specified food uses. In particular, FDA had no question on the safety of lutein concentrate which included 6.75% MZ (GRN 385).

4. The safety of MZ has been established in toxicological studies in animals and mutagenicity studies, and is further supported by other animal studies and human clinical studies. Additional studies published subsequent to the FDA

GRAS notices continue to support the safety of MZ as a food ingredient. The compositional data and product properties are consistent with carefully controlled cGMP production and purification. INNOBIO® MZ preparations contain no impurities or contaminants of concern for human health. Several reviews by experts in the field also have documented the safety of MZ.

## VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF MESO-ZEAXANTHIN

The intended use of INNOBIO® Meso-Zeaxanthin (crystalline, powders, beadlets, and suspended oil) has 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., David Thurnham, Ph.D., Madhusudan Soni, Ph.D., have independently evaluated the materials summarized in the Meso-Zeaxanthin 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 meso-zeaxanthin which contains small amounts of lutein and zeaxanthin, 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 meso-zeaxanthin is 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.

_____ _____

David Thurnham, Ph.D. Date
Independent nutrition consultant and
Emeritus Professor of the University of Ulster, U.K.

_____ _____

Madhusudan Soni, Ph.D. Date
Soni and Associates

## VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF MESO-ZEAXANTHIN

The intended use of INNOBIO® Meso-Zeaxanthin (crystalline, powders, beadlets, and suspended oil) has 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., David Thurnham, Ph.D., Madhusudan Soni, Ph.D., have independently evaluated the materials summarized in the Meso-Zeaxanthin 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 meso-zeaxanthin which contains small amounts of lutein and zeaxanthin, 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 meso-zeaxanthin is GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

(b) (6)

10/1/2014

Susan Cho, Ph.D.
NutraSource, Inc.

Date

(b) (6)

22nd September 2014

David Thurnham, Ph.D.
Independent nutrition consultant and
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Date

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30 Sept. 2014

Madhusudan Soni, Ph.D.
Soni and Associates

Date

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## Appendix A. Certificate of Analysis

INNOBIO® Crystalline MZ

Code: 30101

Lot No.: (b) (6) Date of Manufacture: 01/04/2013

Description: INNOBIO® Crystalline MZ 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 |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 65.0 Min. | USP32/NF27, modified | 67.1 |
| MZ (%) | 60.0 Min. | | 61.4 |
| Lutein (%) | 3.5 Min. | USP 32/NF 27 | 3.7 |
| Moisture (%) | 1.0 Max. | USP 731 | 0.1 |
| Particle Size | 95% through 40 mesh | USP 786 | 96.6 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 30.3 |
| 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 |

Approval: (b) (6) Date: 01/16/2013

INNOBIO® Crystalline MZ

Code: 30101

Lot No.: (b) (6) Date of Manufacture: 01/06/2013

Description: INNOBIO® Crystalline MZ 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 |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 65.0 Min. | USP32/NF27, modified | 68.4 |
| MZ (%) | 60.0 Min. | | 62.6 |
| Lutein (%) | 3.5 Min. | USP 32/NF 27 | 3.8 |
| Moisture (%) | 1.0 Max. | USP 731 | 0.2 |
| Particle Size | 95% through 40 mesh | USP 786 | 95.8 |
| Lead (ppm) | 1.0 Max. | EN ISO 17294 | 0.2 |
| 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 | 2.0 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 43.3 |
| 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 |

Approval: (b) (6) Date: 01/21/2013

INNOBIO® Crystalline MZ

Code: 30101

Lot No.(b) (6) Date of Manufacture: 01/08/2013

Description: INNOBIO® Crystalline MZ 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 |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 65.0 Min. | USP32/NF27, modified | 67.8 |
| MZ (%) | 60.0 Min. | | 61.7 |
| Lutein (%) | 3.5 Min. | USP 32/NF 27 | 3.7 |
| Moisture (%) | 1.0 Max. | USP 731 | 0.2 |
| Particle Size | 95% through 40 mesh | USP 786 | 96.4 |
| 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 | 5.5 |
| Methanol (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 |

Approval:____(b) (6)_____ Date: ___01/21/2013____

INNOBIO® MZ 20% Oil Suspension

Code: 30105-20

Lot No.: (b) (6) Date of Manufacture: 03/19/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red oil suspension | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 20.5 |
| MZ (%) | 18.0 Min. | | 18.9 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.2 |
| Moisture (%) | 1.0 Max. | AOCS Td 2-64 | 0.2 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 4.0 |
| 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 |

Approval:_____ (b) (6) _____ Date: 03/25/2012

INNOBIO® MZ 20% Oil Suspension

Code: 30115-20

Lot No.: (b) (6) Date of Manufacture: 03/26/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red oil suspension | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 20.6 |
| MZ (%) | 18.0 Min. | | 18.7 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.1 |
| Moisture (%) | 1.0 Max. | AOCS Td 2-64 | 0.01 |
| 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 | 1.6 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 3.8 |
| 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 |

Approval: (b) (6) Date: 04/10/2012

INNOBIO® MZ 20% Oil Suspension

Code: 30125-20

Lot No.: (b) (6) Date of Manufacture: 10/19/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red oil suspension | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 20.8 |
| MZ (%) | 18.0 Min. | | 19.4 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.3 |
| Moisture (%) | 1.0 Max. | AOCS Td 2-64 | 0.01 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 3.0 |
| 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 |

Approval:_____ (b) (6) _____ Date: 11/04/2013

INNOBIO® MZ 20% CWD Powder

Code: 30102-20
Lot No.: (b) (6) Date of Manufacture: 01/05/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 21.2 |
| MZ (%) | 18.0 Min. | | 19.5 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.3 |
| Moisture (%) | 5.0 Max. | USP 731 | 1.1 |
| 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 | 2.0 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 8.2 |
| 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 |

Approval: (b) (6) Date: 01/14/2012

INNOBIO® MZ 20% CWD Powder

Code: 30102-20

Lot No.: (b) (6) Date of Manufacture: 01/07/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 20.5 |
| MZ (%) | 18.0 Min. | | 18.9 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.3 |
| Moisture (%) | 5.0 Max. | USP 731 | 1.5 |
| Particle Size | 95% through 40 mesh | USP 786 | 97.8 |
| 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 | Negative |
| Methanol (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 |

Approval: (b) (6) Date: 01/13/2012

INNOBIO® MZ 20% CWD Powder

Code: 30102-20
Lot No.(b) (6) Date of Manufacture: 01/16/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 20.0 Min. | USP32/NF27, modified | 20.7 |
| MZ (%) | 18.0 Min. | | 18.8 |
| Lutein (%) | 1.0 Min. | USP 32/NF 27 | 1.3 |
| Moisture (%) | 5.0 Max. | USP 731 | 1.4 |
| Particle Size | 95% through 40 mesh | USP 786 | 97.5 |
| 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 | 1.2 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 4.5 |
| 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 |

Approval:_____(b) (6)_____ Date: _____01/23/2012_____

INNOBIO® MZ 10% CWD Powder

Code: 30102-10
Lot No.:(b) (6) Date of Manufacture: 04/07/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP32/NF27, modified | 10.6 |
| MZ (%) | 9.0 Min. | | 9.6 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 5.0 Max. | USP 731 | 1.1 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 5.6 |
| 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 |

Approval:___(b) (6)_____ Date: 04/15/2013

INNOBIO® MZ 10% CWD Powder

Code: 30102-10
Lot No.: (b) (6) [redacted] Date of Manufacture: 03/08/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP32/NF27, modified | 10.9 |
| MZ (%) | 9.0 Min. | | 9.8 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 5.0 Max. | USP 731 | 1.2 |
| 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 | 1.2 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 4.1 |
| 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 |

Approval: [redacted] Date: 03/18/2013

INNOBIO® MZ 10% CWD Powder

Code: 30102-10
Lot No.: (b) (6) Date of Manufacture: 07/08/2010

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP32/NF27, modified | 10.3 |
| MZ (%) | 9.0 Min. | | 9.5 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 5.0 Max. | USP 731 | 0.9 |
| Particle Size | 95% through 40 mesh | USP 786 | 97.2 |
| 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 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 3.4 |
| 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 |

Approval: (b) (6) Date: 07/15/2010

INNOBIO® MZ 10% Beadlets

Code: 30113-10
Lot No.: (b) (6) [redacted] Date of Manufacture: 04/08/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP32/NF27, modified | 10.4 |
| MZ (%) | 9.0 Min. | | 9.3 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.2 |
| 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 | 18.4 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 2.2 |
| 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 |

Approval: [redacted] Date: 04/17/2013

INNOBIO® MZ 10% Beadlets

Code: 30113-10
Lot No.(b) (6) Date of Manufacture: 05/08/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP 32/NF 27, modified | 10.4 |
| MZ (%) | 9.0 Min. | USP32/NF27, modified | 9.7 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.0 |
| Particle Size (%) | | | |
| -through sieve No.20 | 100 | USP 786 | 100 |
| -through sieve No.40 | 85.0 Min. | USP 786 | 97.9 |
| -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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 2.0 |
| 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 |

Approval:_____ Date: 05/21/2013

INNOBIO® MZ 10% Beadlets

Code: 30113-10
Lot No.: (b) (6) Date of Manufacture: 06/24/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 10.0 Min. | USP 32/NF 27, modified | 10.3 |
| MZ (%) | 9.0 Min. | USP 32/NF 27, modified | 9.6 |
| Lutein (%) | 0.5 Min. | USP 32/NF 27 | 0.6 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.8 |
| 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 | 18.5 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 1.9 |
| 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 |

Approval:_____ Date: 07/01/2013

INNOBIO® MZ 5% Beadlets

Code: 30113-05

Lot No.:(b) (6) Date of Manufacture: 03/15/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.3 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 4.8 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.29 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.3 |
| 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 | 18.5 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 4.7 |
| 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 |

Approval:_____ Date: 03/22/2012

INNOBIO® MZ 5% Beadlets

Code: 30113-05
Lot No.: Date of Manufacture: 02/21/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.5 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 4.9 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.30 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.5 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 3.5 |
| 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 |

Approval:_____ Date: 02/28/2012

INNOBIO® MZ 5% Beadlets
Code: 30113-05
Lot No.: (b) (6) Date of Manufacture: 04/15/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Reddish-brown, free-flowing beadlets | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.8 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 5.3 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.33 |
| Moisture (%) | 7.0 Max. | USP 731 | 1.9 |
| Particle Size (%) | | | |
| -through sieve No.20 | 100 | USP 786 | 100 |
| -through sieve No.40 | 85.0 Min. | USP 786 | 97.5 |
| -through sieve No.100 | 15.0 Max. | USP 786 | 19.4 |
| 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 | 0.7 |
| Methanol (ppm) | 50.0 Max. | USP 467 | 1.9 |
| 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 |

Approval:_____ Date: 04/27/2013

INNOBIO® MZ 5% CWS Powder

Code: 30102-05
Lot No.: (b) (6) Date of Manufacture: 10/28/2013

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.8 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 5.3 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.33 |
| Moisture (%) | 5.0 Max. | USP 731 | 4.2 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 3.3 |
| Total Plate Count (CFU/g) | 1,000 Max. | ISO 4833 | 90 |
| 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 |

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

INNOBIO® MZ 5% CWS Powder
Code: 30102-05
Lot No.: [redacted] Date of Manufacture: 03/29/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.4 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 4.8 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.29 |
| Moisture (%) | 5.0 Max. | USP 731 | 0.7 |
| Particle Size | 95% through 40 mesh | USP 786 | 96.4 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 1.5 |
| Total Plate Count (CFU/g) | 1,000 Max. | ISO 4833 | 40 |
| 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 |

Approval: (b) (6) Date: 04/10/2012

INNOBIO® MZ 5% CWS Powder

Code: 30102-05
Lot No.: [redacted] Date of Manufacture: 03/31/2012

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

| Tests | Limits | Methods | Results |
|---|---|---|---|
| Appearance | Orange-red, free-flowing powder | Visual | Conforms |
| Zeaxanthin (MZ +R,R' Zeaxanthin) (%) | 5.0 Min. | USP 32/NF 27, modified | 5.5 |
| MZ (%) | 4.5 Min. | USP 32/NF 27, modified | 5.0 |
| Lutein (%) | 0.25 Min. | USP 32/NF 27 | 0.30 |
| Moisture (%) | 5.0 Max. | USP 731 | 0.9 |
| Particle Size | 95% through 40 mesh | USP 786 | 97.4 |
| 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 | Negative |
| Methanol (ppm) | 50.0 Max. | USP 467 | 4.7 |
| 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 |

Approval: (b) (6) [redacted] Date: 04/10/2012

**APPENDIX B. Flow Diagram of Manufacturing Process**

Figure 1. Manufacturing process of INNOBIO® Crystalline MZ

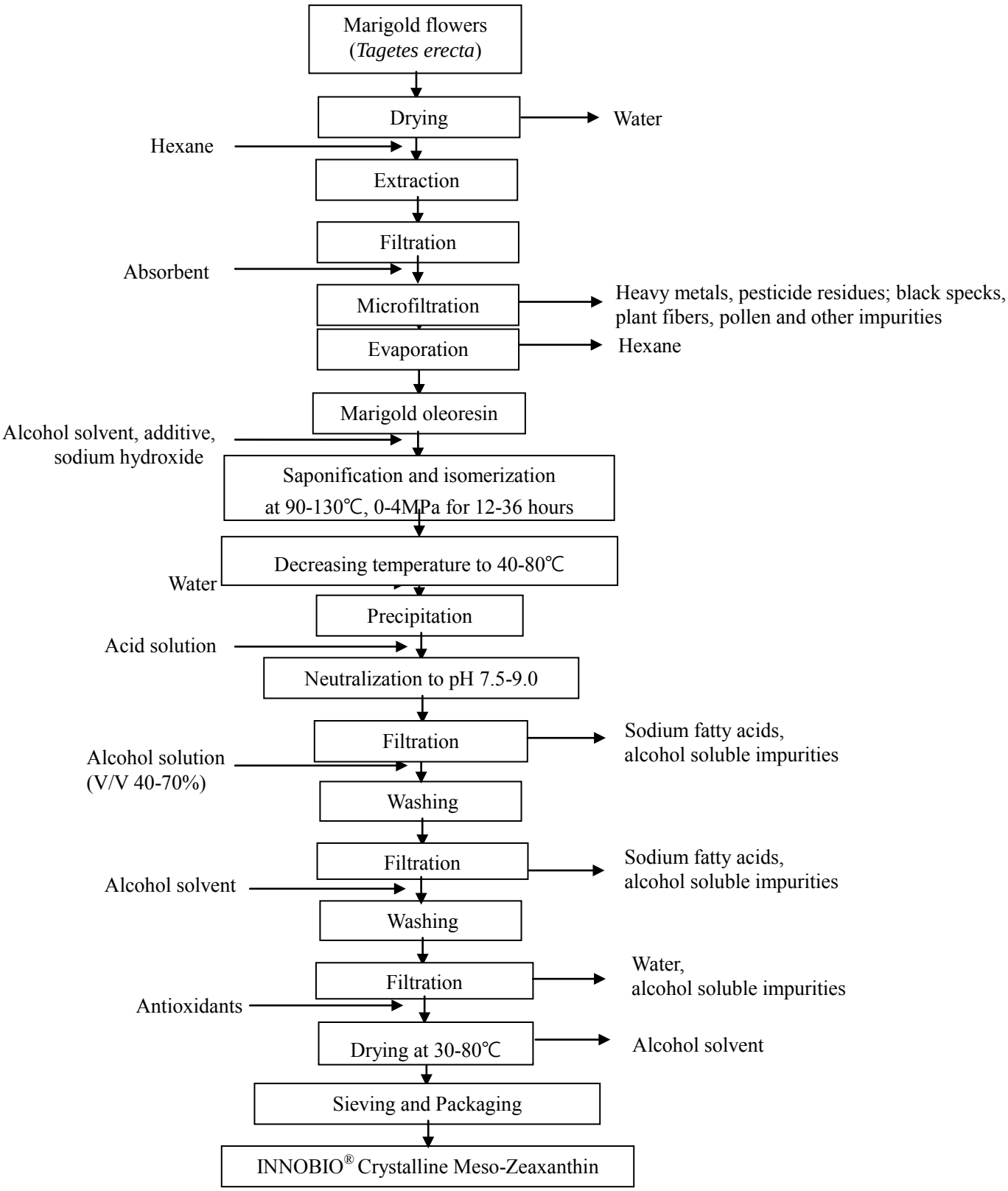


Figure 2. Manufacturing process of INNOBIO® MZ 20% Oil Suspension

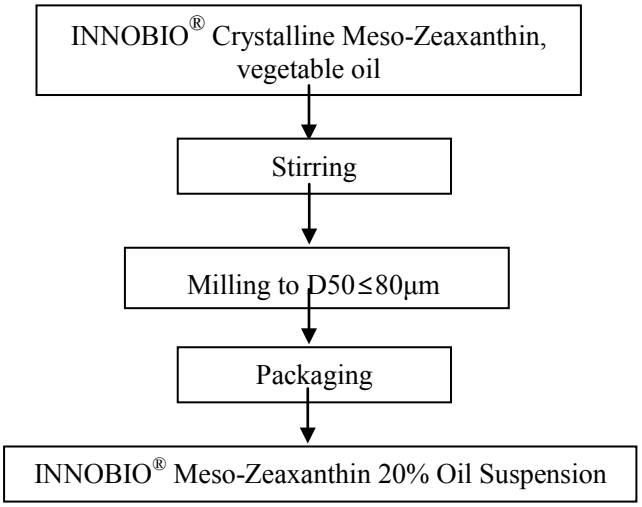


Figure 3. Manufacturing process of INNOBIO® MZ 5% CWS Powder

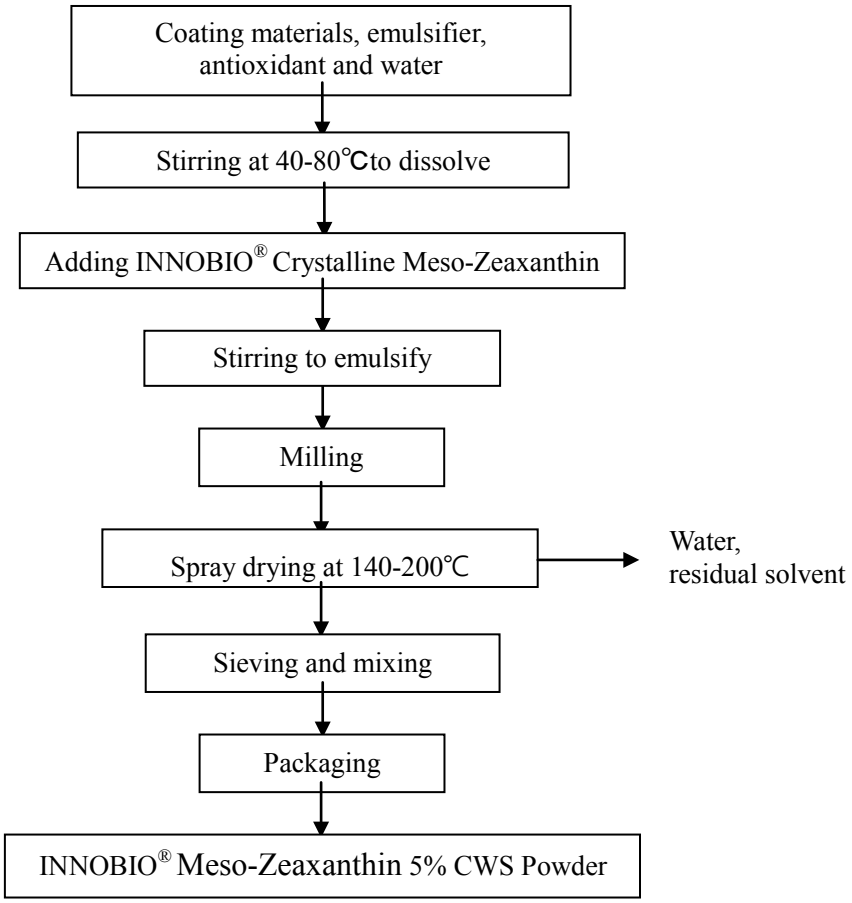


Figure 4. Manufacturing process of INNOBIO® MZ 10% CWD Powder

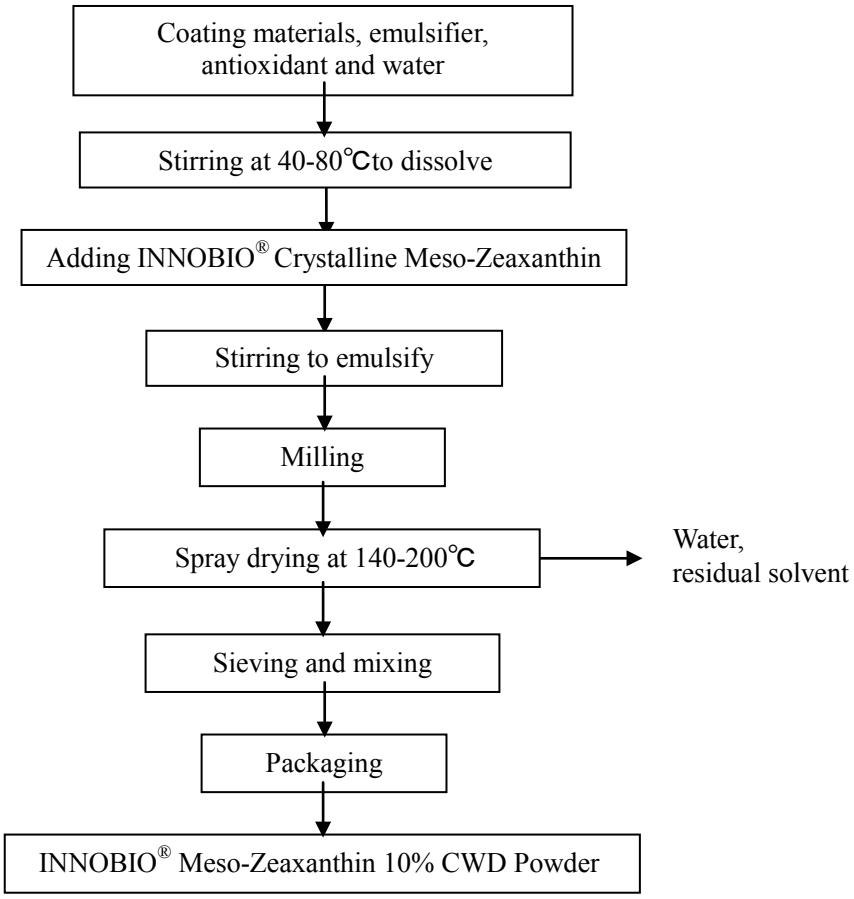


Figure 5. Manufacturing process of INNOBIO® MZ 20% CWD Powder

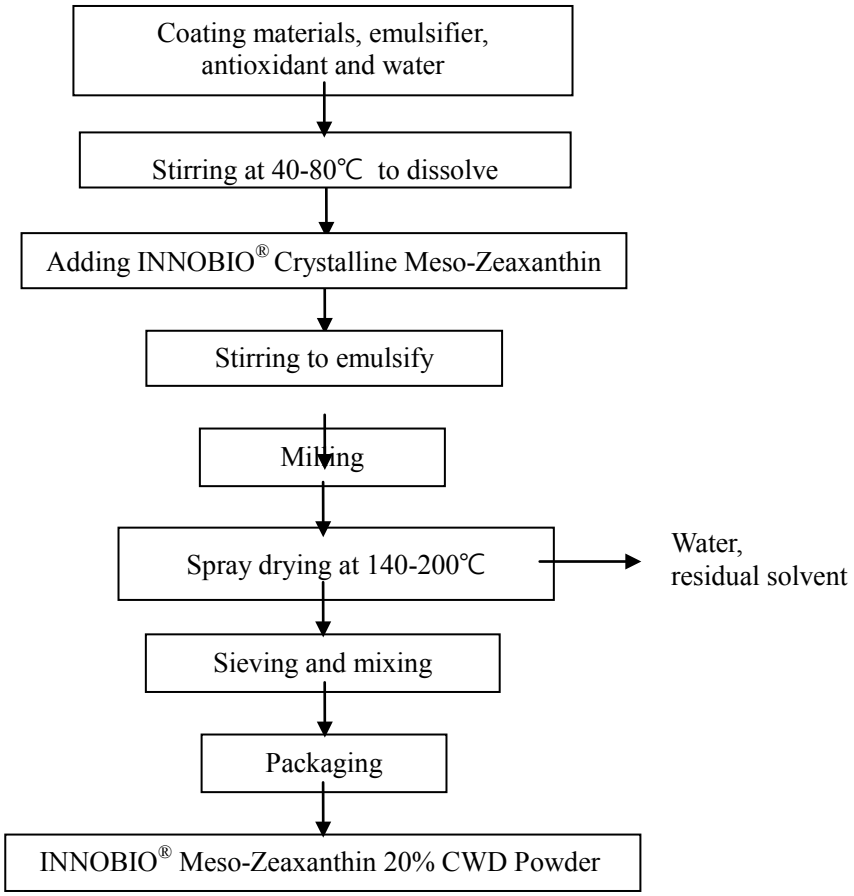


Figure 6. Manufacturing process of INNOBIO® MZ 5% Beadlets

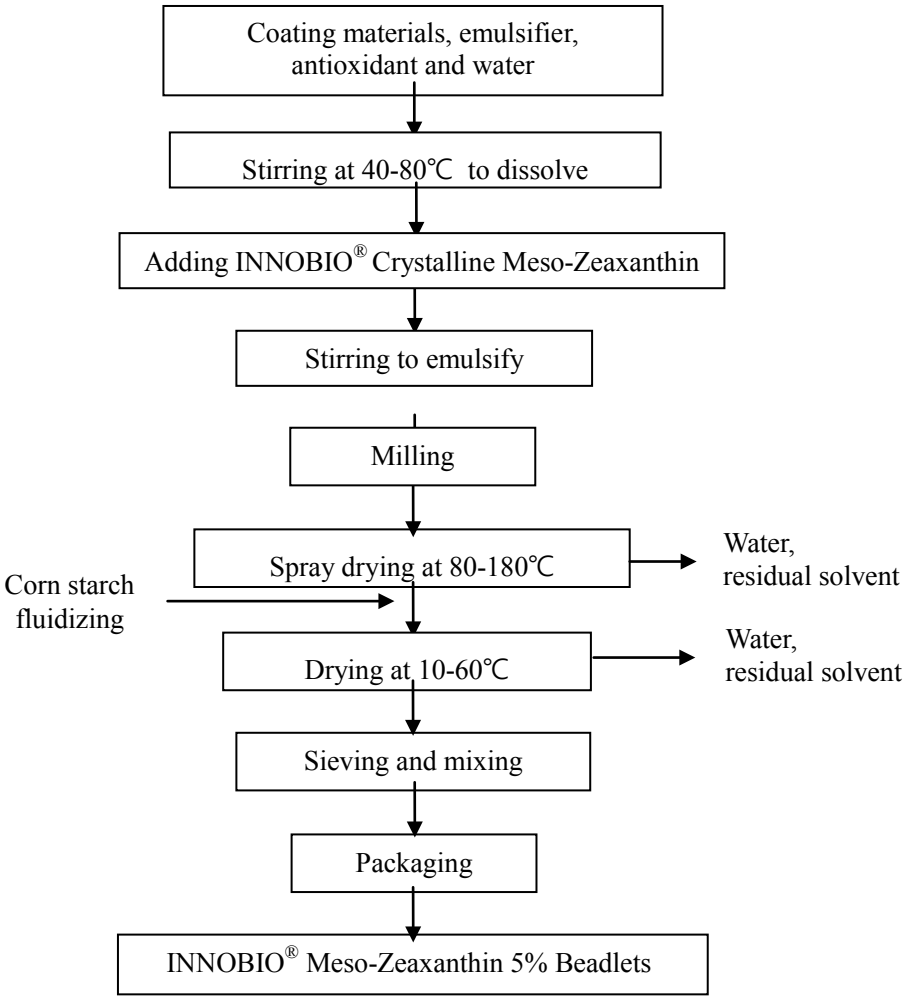
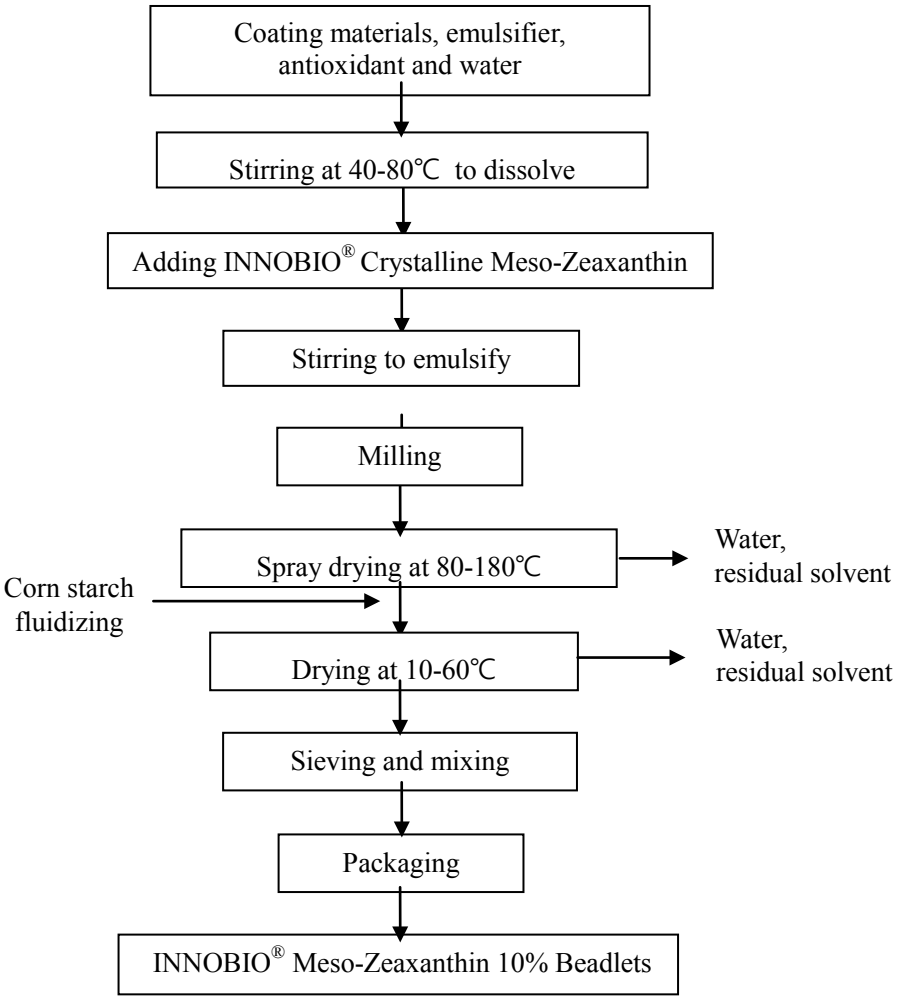


Figure 7. Manufacturing process of INNOBIO® MZ 10% Beadlets



**Relative Measurement of Zeaxanthin Stereoisomers by Chiral HPLC**

## Principle

To measure the relative percentages of the (3R,3'R), (3R,3'S) and (3S,3'S) – stereoisomers of zeaxanthin in dietary ingredient and finished product forms of dietary supplements. Extracted samples are injected and separated on a chiral carbamate-modified amylose HPLC column and detected by absorbance at 450 nm.

## Reagents and Supplies

20 mL glass vials with screw top caps
50 and 100 mL volumetric flasks
5 and 10 mL pipets
0.10-1.00 mL micropipet
3 mL disposable syringes and 0.45 micron PTFE syringe filters
HPLC sample vials (if using an autosampler)
Solvents
- HPLC: hexane, isopropranol
- Extraction: HPLC grade water and HEAT (hexane/ethanol/acetone/toluene) or dichloromethane

Standards
- (3R,3'R) – and *meso*-(3R,3'S) – all-E-zeaxanthin (>90% pure)

Saponification materials (optional)
- 30% methanolic KOH
- 35% (saturated) aqueous sodium chloride solution
- 15 mL culture tubes with screw top caps
- vortex mixer

Alginate digestion solution (see footnote 3)

## Instruments and settings

Analytical balance (± 0.1 mg)
Ultrasonic bath with heating to 50ºC
High performance liquid chromatography system with UV/vis diode array detector
- Column: Chiralpak AD-H (Chiral Technologies, Inc., Westchester, PA, USA), 250 x 4.6 mm (5 µm particle size)

Mobile phase table

| Time (min) | Flow rate (ml/min) | % n-Hexane | % Isopropanol | Gradient |
|---|---|---|---|---|
| 0 | 0.8 | 95 | 5 | |
| 50 | 0.8 | 95 | 5 | |
| 55 | 0.8 | 50 | 50 | linear |
| 63 | 0.8 | 50 | 50 | |
| 65 | 0.8 | 95 | 5 | linear |
| 75 | 0.8 | 95 | 5 | |

Column temperature: Room temperature (approx. 23°C)
Injection volume: 20 µl
Detection: absorbance at 450 nm

**Preparation of Sample**

Note: All preparations are scaled to provide nominal concentrations of less than 10 ppm and preferably between 1-6 ppm of zeaxanthin in the injection solution. Higher concentrations could lead to errors due to solubility limitations of zeaxanthin in the injection solvent.

(1) Powder concentrates (typically 20-50 wt% zeaxanthin)

Use ultrasonic agitation to dissolve 30-50 mg of sample in 50 mL HEAT[1] or dichloromethane. Add 0.10 mL of this solution to an empty glass vial with at least 15 mL capacity and evaporate residual solvent under nitrogen flow.[2] Add 10 mL of 5:95 isopropanol:hexane to the vial, cap and mix thoroughly to dissolve. Filter this final solution through 0.45 micron PTFE syringe filter and inject into the HPLC.

(2) Beadlets (about 5-20 wt% zea) and tablets/capsules (about 2-8 mg zea per tablet)

In a 100 mL volumetric flask, combine 50-100 mg of beadlets or one-two pulverized tablets (to provide 4-8 mg of zeaxanthin) with 5 mL water[3] and agitate with ultrasonication for 30 min at 50ºC. Alternatively, one can grind the sample and water together in a mortar and pestle and transfer the mixture to a 100 mL volumetric flask using HEAT or dichloromethane, if necessary, to rinse the mortar and complete the transfer of solids to the flask. Fill the flask to the mark with one of the following solvent blends: (a) HEAT[1] or (b) 40 mL ethanol and dichloromethane as needed. Sonicate the resulting mixture for 10 min with occasional hand mixing. If using HEAT, allow any undissolved aqueous phase to separate to the bottom of the flask. Dilute 0.50 mL of

[1] HEAT is hexane, ethanol, acetone, and toluene combined in 10:6:7:7 volumetric ratio.

[2] The manufacturer of the Chiralpak AD-H column advises against injection of methylene chloride or acetone, even in trace quantities. To ensure best column longevity, it is advisable to evaporate the residual solvent from the first dilution prior to addition of the injection solvent.

[3] Samples that include alginate-encapsulated zeaxanthin might require more aggressive treatment. For more complete release of zeaxanthin from samples such as these, replace water with the following extraction buffer. To prepare, dissolve 6.8 g potassium dihydrogenphosphate and 1.0 g $Na_2EDTA$ dihydrate in approximately 90 mL water in an Erlenmeyer flask. Add approximately 1.8 g of sodium hydroxide and sonicate for 20 min until the sodium hydroxide is dissolved. Adjust to pH 8.7 ± 0.3 with 4 M NaOH and fill to a volume of 100 mL with water. Add 0.025 g sodium dodecylsulphate and sonicate for 20 min at room temperature (may not be completely dissolved). Prepare daily.

the organic phase into 10 mL of 5:95 isopropanol:hexane and mix thoroughly. Filter this final solution through 0.45 micron PTFE syringe filter and inject into the HPLC.

(3) Oil suspensions or oleoresins (typically 5-20% zeaxanthin)

(Note: See preparation 5 for products that may contain zeaxanthin esters.)
Use ultrasonic agitation to dissolve 100-150 mg of liquid sample in 50 mL HEAT[1] or dichloromethane. Add 0.10 mL of this solution to an empty glass vial with at least 15 mL capacity and evaporate residual solvent under nitrogen flow.[2] Add 10 mL of 5:95 isopropanol:hexane to the vial, cap and mix thoroughly to dissolve. Filter this final solution through 0.45 micron PTFE syringe filter and inject into the HPLC.

(4) Soft gels (typically 2-4 mg zeaxanthin per capsule)

(Note: See preparation 5 for products that may contain zeaxanthin esters.)
Carefully slice two soft gel capsules lengthwise and place both together into in an open glass vessel (these will provide about 500-700 mg of liquid fill). Add 50 mL of HEAT[1] or dichloromethane and apply ultrasonic agitation for 5 min to dissolve the xanthophylls. Add 0.10 mL of this solution to an empty glass vial with at least 15 mL capacity and evaporate residual solvent under nitrogen flow.[2] Add 10 mL of 5:95 isopropanol:hexane to the vial, cap and mix thoroughly to dissolve. Filter this final solution through 0.45 micron PTFE syringe filter and inject into the HPLC.

(5) Saponification procedure.

Some liquid extracts may contain naturally-occurring zeaxanthin esters. Because meso-forms are generally not prepared in ester-form, analysis of these samples without saponification could underestimate the relative amount of natural (3R,3R') isomer or overestimate the relative amount of meso (3R,3S') isomer. If accurate determination of minimum meso isomer level is necessary in these extracts, the following method should be used to hydrolyze the natural esters prior to HPLC analysis.

a) In a 10 mL culture tube with sealable cap, combine 600 µL of ethanol and 250 µL of 30% methanolic KOH.
b) Add 100 µL of the first dilution made in preparation 4 or 5 above.
c) Vortex this mixture for 15 s and let rest at 22-28ºC for 30 min or 2-10ºC for 18 h.
d) Allow the sample to return to room temperature. Uncap sample and add 2.0 mL of 35% saturated sodium chloride solution to the culture tube.
e) Add 4.5 mL of 5:95 isopropanol/hexane and vortex sample for 30 s.
f) Let sit for 3-5 minutes for two layers to appear.
g) Transfer the top layer from the culture tube into a 10.0 mL volumetric flask. Make sure that no aqueous (bottom) layer is transferred.
h) Repeat steps e – g and combine both top layer fractions.
i) Fill the 10.0 mL volumetric flask containing supernatant to the mark with 5:95 isopropanol:hexane and mix well.
j) Filter the solution through a 0.45 µm Teflon™ (PTFE) into an amber 2 mL vial and analyze by HPLC.

## Peak Identification

The peaks of the (3R,3'R) –, *meso*-(3R,3'S) – and (3S,3'S) – all-E-zeaxanthin isomers and lutein are identified by injection of reference substance of at least one of these stereoisomers and use of the relative retention times given in the table below. The retention time for (3R,3'R)-E-zeaxanthin is typically between 30-40 min. In complex mixtures it is recommended to confirm the identity of the peaks by comparison of their UV/VIS-spectra with that of the reference substance using a diode array detector (DAD). Reference standards for (3R,3'R) – E-zeaxanthin and lutein are available from several research chemical suppliers. The ZTA is presently working to provide a commercially-available *meso*-(3R,3'S) – all -E-zeaxanthin standard.

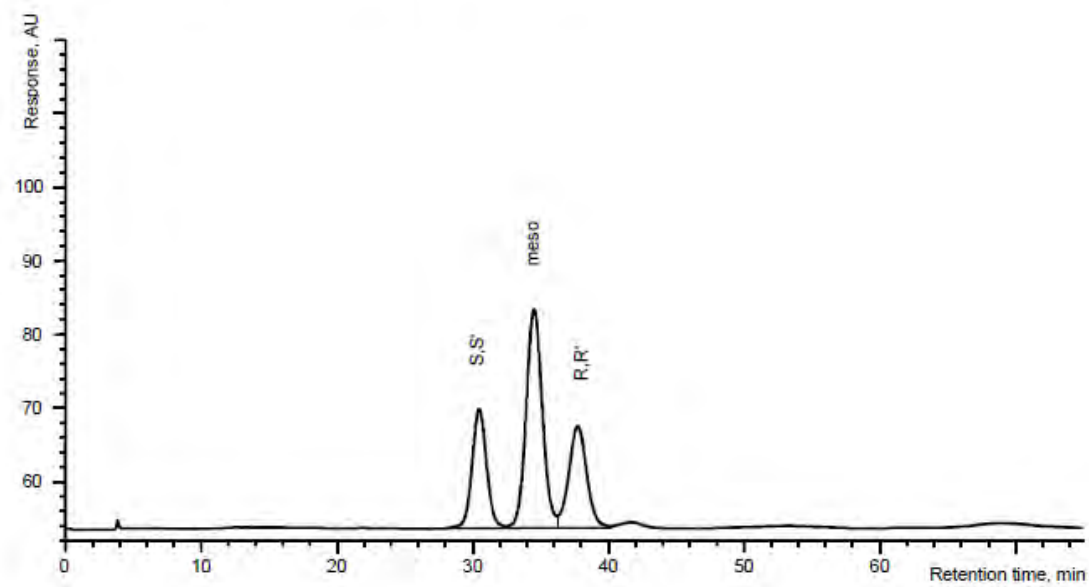
| Component | Retention time / retention time of 3R,3'R |
|---|---|
| (3S,3'S)-E-Zeaxanthin | 0.82 |
| *meso*-(3R, 3'S)-E-Zeaxanthin | 0.92 |
| (3R,3'R)-E-Zeaxanthin | 1.00 |
| (3R,3'R,6'R)-E-Lutein | 1.12 |

## Quantitation

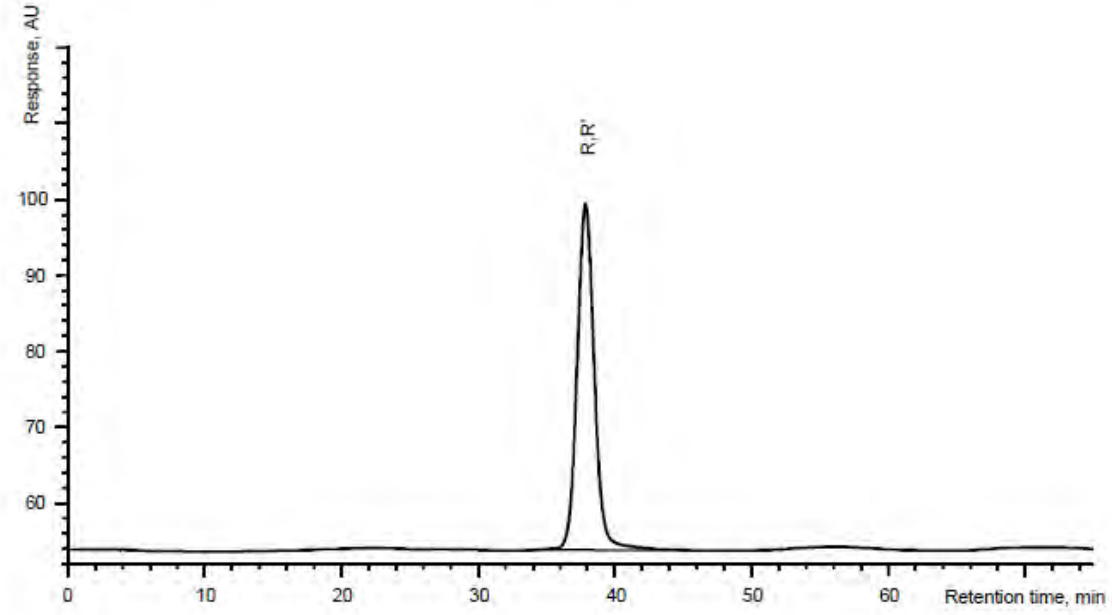
No calibration is necessary. Results are reported as the relative area percent of the three stereoisomers of E-zeaxanthin (normalized to a sum total of 100%).

## Representative Results

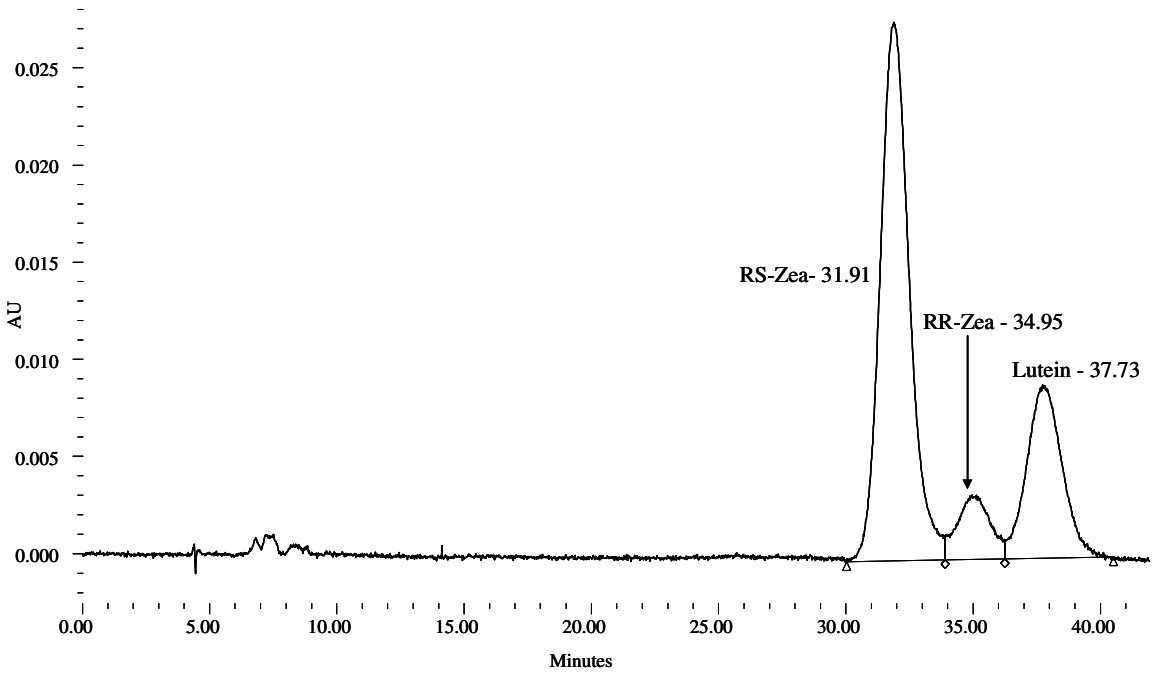
**Typical Chromatogram of Racemic all-E-Zeaxanthin:**



**Typical Chromatogram of (3R,3'R) all-E-Zeaxanthin:**




**Chiral chromatography of 20 ppm RS-Zea, RR-Zea and Lutein before isomerization:**



Pages 000093-000474 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 000053-000059.

SUBMISSION END