**Factors which support "Generally Regarded as Safe (GRAS)" status for Mesozeaxanthin (MZ)**

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There are three xanthophyll carotenoids in the retinal pigment epithelium of the eye which together comprise more than 70% of the carotenoid content[1]. The carotenoids are lutein and two stereoisomers of zeaxanthin namely *all-trans* (3R,3'R)-zeaxanthin (Z) and *all-trans* (3R,3'S)-mesozeaxanthin (MZ). All three carotenoids are believed to play an important role in protecting the retinal pigment from oxidation but the two stereoisomers of zeaxanthin are predominantly found in the macula of the eye and may be particularly important role in protecting the visual process.

The source of the xanthophyll carotenoids for the retinol pigment is the diet. A binding protein has also been identified in the retinal layer of the eye that is selective for Z and MZ and may be responsible for the uptake of these carotenoids from the blood[2]. The average daily intake of lutein is ~1-3 mg of which 10-20% is Z[3]. MZ has also been reported in some potential foods namely fish skin, shrimp carapace and turtle fat[4] but there are no reports of MZ occurring naturally in blood[5] and most MZ is probably endogenously synthesised from lutein. Experiments in monkeys have identified lutein as a major source of MZ[6] and the binding protein may play a role in the metabolism of lutein to produce MZ.

However, MZ has been added to broiler diets for many years to colour both the chicken and their egg yolks. MZ concentrate has been supplied to 25-28% of the combined broiler and layer pigment market in Mexico for more than 10 years[3]. Colour density in Mexican eggs is relatively high and a recent analysis of an egg yolk lyophylisate from chickens fed Yemix® (Industrial Orgánica SA, Monterrey, N.L., Mexico) at 13-14 ppm found the mean total carotenoids per yolk to be 170 μg of which of 12 μg were MZ[3]. Several studies have looked at the lutein and zeaxanthin uptake from egg yolk and reported that plasma responses were 4-6 times (lutein) and two times higher respectively than from an equivalent amount of the xanthophyll in oily suspension[3]. Thus although the amount of MZ present in Mexican eggs was only 12 μg per egg yolk, the phospholipid environment of the egg yolk may well promote MZ absorption when consumed by man. In addition, egg consumption in Mexico is one of the highest in the world[7] at approximately one egg/person/day.

Evidence that MZ is absorbed by humans was obtained in a supplementation study done in Northern Ireland using LuteinPlus® (Holland & Barrett, Samuel Ryder House, Townsend Drive, Nuneaton, CV11 6XW, UK). Capules of LuteinPlus® contain 10.8 mg lutein, 1.2 mg Z and 8.0 mg MZ and one capsule of LuteinPlus®/day was fed to 19 volunteers for 21 days. MZ was present in the blood at days 10 and 22 when mean concentrations for all subjects were 0.121 and 0.209 μmol/L respectively[8]. Eighteen days has been shown previously to be sufficient to achieve plateau concentrations of lutein at doses as high as 20 mg/day[9] and 10 mg/d in the case of

zeaxanthin[10]. Thus although the measurements of MZ in Mexican blood has not been reported, MZ is absorbed in humans and therefore should be present in Mexican blood. Serum MZ concentrations were also shown to have increased in two subjects who were given capsules containing 20 mg MZ concentrate containing 68% MZ to 0.45 and 0.15 μmol/L after six weeks supplementation[11].

The MZ used in Yemix® and LuteinPlus® was prepared from lutein extracted from marigold flowers (*Targetes erecta*). The lutein extract was then processed using alkaline hydrolysis to convert some of the lutein to MZ and the non-esterified carotenoids were purified by a patented procedure[12]. The toxicological safety of the MZ concentrate was tested using oral gavage to give 2, 20 or 200 mg/kg/day or a corn oil control to rats for 13 consecutive weeks followed by a four week recovery to detect any delayed-onset toxicity or reversibility of any effects seen during the feeding. No compound-related mortality, clinical signs of toxicity, changes in body weights, ophthalmology, clinical pathology, gross pathology, or histopathology were noted[13]. The concentrate was also tested for potential mutagenic activity using the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A in both the presence and absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9). The doses of mesozeaxanthin concentrate in the metagenicity assay were 10.0, 33.3, 100, 333, 1000, and 5000 μg per plate along with concurrent vehicle and positive controls using three plates per dose. No dose caused a positive increase in the mean number of revertants per plate with any of the tester strains either in the presence or absence of microsomal enzymes[14].

The top concentrations tested in the toxicity and metagenicity trials were 200 mg/kg/day and 5 mg/plate respectively. The concentration of MZ in the concentrate used in the toxicity experiment was ~200 g/kg. Therefore in the toxicity study, the top dose was 200 x 20% fed per kg (40 mg) which for a 70 kg man would be 2.8 g. If the usual intake of zeaxanthin is 0.02 mg/day then the amount MZ fed was $1.4x10^5$ larger. For the metagenicity study, the top dose was 5 mg/plate and the concentration of MZ was approximately 38% = 1.9 mg/plate. The testing of higher concentrations was prevented by the opacity of the MZ solution. As 100 µl was added to a plate, the amount used was equivalent to a concentration of 34.5 µmol/L which was approximately 700 times greater than the amount of zeaxanthin found in plasma in a recent study (0.05 µmol/L)[8].

Various preparations of the MZ concentrate are available commercially to supplement the xanthophyll pigments in the diet for the prevention of age-related macular degeneration (AMD) : two products in Europe: LuteinPlus® ( Holland & Barrett Ltd, Nuneaton, Warwickshire CV11 6XW) and Macushield® (Macuvision Europe Ltd, Station Lane, Lapworth, Solihull B94 6JJ) and two products in the USA :LuteinPlus ® (QuantumNutritionals LLC 8585 P.G.A.Drive ,Walled Lake MI 48390) and LMZ3® (Ad-med LLC 4114 West Maple Rd Bloomfield Hills MI 48301 ). Lastly a MZ supplement was used in a recent study to determine its effect on macular pigmentation[11]. Ten subjects were given 20 mg/d of a xanthophyll supplement containing 68% MZ, 6% Z and 25% lutein for 120 days. Macular pigment optical density at 460 nm rose at an average rate of 0.59 milli-absorbance units/d which was significantly different from -0.17 milli-

absorbance units/d in 9 control subjects. Thus the macular pigment increased in response to the supplement but we cannot be certain of the contribution attributable to MZ. MZ was a major component in the supplement used but the subjects were also receiving 5 mg lutein/day. Further work on the efficacy of MZ must await work with the pure material.

In summary, MZ is potentially present in the diet in some fish products and has been available for many years in eggs in Mexico. Two studies have shown that it can be absorbed by humans. Its safety in toxicological and metagenicity studies has been shown at many times the concentration in diets to which man is likely to be exposed. It has been available commercially in both Europe and the USA for over 5 years with only good reports on possible benefits. Lastly, feeding studies over 120 days showed increases in macular pigment optical density.

Reference List

1. Landrum JT & Bone RA (2001) Lutein, zeaxanthin and the macular pigment. *Arch Biochem Biophys* **385,** 28-40.

2. Bhosale P, Larson AJ, Frederick JM, Southwick K, Thulin CD & Bernstein PS (2004) Identification and characterization of a Pi isoform of glutathione S-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye. *J Biol Chem* **279,** 49447-49454.

3. Thurnham DI (2007) Macular zeaxanthins and lutein - a review of dietary sources and bioavailability and some relationships with macular pigment optical density and age-related macular disease. *Nutrition Research Reviews* **20,** 163-179.

4. Maoka T, Arai A, Sinuzu M & Matsuno T (1986) The first isolation of entiomeric and meso-zeaxanthin in nature. *Comp Biochem Physiol B* **83,** 121-124.

5. Bone RA, Landrum JT, Hime GW, Cains A & Zamor J (1993) Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci* **34,** 2033-2040.

6. Johnson EJ, Neuringer M, Russell RM, Schalch W & Snodderly DM (2005) Nutritional manipulation of primate retinas, III: Effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci* **46,** 692-702.

7. Gallardo Nieto JL, Villamar L, Ruiz Sanchez N, Castillo Mangas MA & Guzman H (2003) Situation and outlook of table egg production in Mexico 2003. *http://www sagarpa gob mx/Dgg/estudio/sithuevo03i pdf* 1-27.

8. Thurnham DI, Tremel A & Howard AN (2008) A supplementation study in humans with a combination of *meso*-zeaxanthin, (3R,3'R)-zeaxanthin and (3R.3'R, 6')-lutein. *Brit J Nutr* **accepted,**

9. Thurmann PA, Schalch W, Aebischer C-P, Tenter U & Cohn W (2005) Plasma kinetics of lutein, zeaxanthin, and 3'-dehydro-lutein after multiple doses of a lutein supplement. *Am J Clin Nutr* **82,** 88-97.

10. Hartmann D, Thurmann PA, Spitzer V, Schalch W, Manner B & Cohn W (2004) Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am J Clin Nutr* **79,** 410-417.

11. Bone RA, Landrum JT, Cao Y, Howard AN & Alvarez-Calderon F (2007) Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutr Metabol* **4:12,**

12. Montoya-Olvera, R, Elizondo-Mireles, J-R, Torres-Gomez, C-J, & Torres-Quiroga, J-O (2003) Process to obtain xanthophyll concentrates of high purity -US patent no 6,504,067. [M Industrial Organica S.A.DE C.V.(Monterrey editor]. Application no. 449229. issued 2003. (filed 1999).

13. Chang, CJG (2006) Thirteen-week oral (gavage) toxicity of mesozeaxanthin in Han Wistar rats with a 4-week recovery. Gene Logic Study Number: 1567-04370, 1-344. 2006. Gaithersburg, MD, Gene Logic Laboratories Inc.

14. Mecchi, MS (2006) *Salmonella-Escherichia coli/*Mammalian-microsome reverse mutation assay with a confirmatory assay with mesozeaxanthin. Report no. 7609-100; Genetic Toxicology Assay No. 26471-0-409OECD, pp 1-27. 2008. Vienna, Virginia , Covance Laboratories Inc.