

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

June 25, 2004

Reference: **Docket: 2004Q-0180 - Qualified Health Claim (QHC): Lutein and Eye Disease**

To Whom It May Concern:

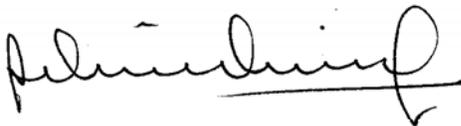
This letter contain comments to the qualified health claim petition - Xangold® Lutein Esters and development of certain eye diseases, docket 2004Q-0180.

DSM Nutritional Products, Inc. believes that there is sufficient data to justify the use of a qualified health claim on conventional foods and dietary supplements regarding the relationship between lutein and zeaxanthin and certain eye diseases.

We have all the references cited in these comments, and they are available at your request.

Please feel free to call me, at your convenience if we can be of any assistance in the evaluation of these comments.

DSM Nutritional Products, Inc



Associate Director Regulatory Affairs
45 Waterview Boulevard
Parsippany, NJ 07054
Telephone 973 257 8325
Fax 973 257 8414

Attachment

We agree with the petitioner that the scientific literature provides evidence to support a protective role for lutein against age-related macular degeneration and cataract. However, we propose to widen the scope of a qualified health claim in two ways.

- 1) A qualified health claim should include lutein derived from a variety of dietary and supplemental sources. This would eliminate the need for additional health claims to support the potential benefit of consuming sources of lutein and zeaxanthin other than lutein esters.
- 2) Include zeaxanthin in the qualified health claim. The scientific data described by the petitioner supports a role for both xanthophylls in age-related macular degeneration and cataract. Additional studies, described below, provide evidence to include zeaxanthin in a qualified health claim.

Additional Scientific Rationale for Lutein and Zeaxanthin

The studies below provide additional support to include zeaxanthin along with lutein in a health claim for age-related macular degeneration and cataract.

- 1) Lutein and zeaxanthin are the only carotenoids that accumulate in the human retina (Handelman et al, 1988; Sommerburg et al, 2000).
 - a) The concentration of lutein and zeaxanthin in the central macula of the retina is estimated to be around 1 mM, which is three orders of magnitude higher than typical carotenoid concentrations in other human tissues (Landrum et al, 1999). This degree of selective accumulation suggests that both xanthophylls may have biological significance in the retina.
 - b) The distribution of lutein and zeaxanthin in the retina varies in a systematic manner (Landrum et al, 1999; Bone et al, 1997). Zeaxanthin concentrations are highest in the central macula whereas lutein concentrations dominate in the peripheral retina (Table 1). Only the dietary stereoisomer of lutein (3R,3'R,6'R-Lutein) was detected, whereas zeaxanthin was present as two stereoisomers, 3R,3'R-zeaxanthin and 3R,3'S-zeaxanthin. More than 50% of total zeaxanthin was present as 3R,3'R-zeaxanthin, which is found in the diet and in the blood. The other stereoisomer, 3R,3'S-zeaxanthin or meso-zeaxanthin, is not present in the diet or in the blood. It is hypothesized that that meso-zeaxanthin is derived from lutein. Bhosale and coworkers (2004) identified and characterized a specific xanthophyll-binding protein that only interacts with 3R,3'R-zeaxanthin and 3R,3'S-zeaxanthin, and to a less extent with 3R,3'R,6'R-lutein.
 - c) The ratio of 3R,3'R,6'R-lutein to 3R,3'R-zeaxanthin in the central retina (1.4) is lower than the ratio of these two carotenoids in the blood (3.3) (Table 2) suggesting a preferential accumulation of 3R,3'R-zeaxanthin in the location of highest light exposure. The ratio of 3R,3'R,6'R-lutein to 3R,3'R-zeaxanthin in the outer retina (3.3) is equivalent to that in the blood.

Table 1. Levels of Dietary Lutein & Total Zeaxanthin in Human Retinas¹

Retinal Section	Lutein µmoles	3R,3'R- Zeaxanthin µmoles	3R,3'S- Zeaxanthin µmoles	Lutein:3R,3'R Zeaxanthin Ratio
Central	17.04	12.07	9.94	1.4
Medial	20.46	8.74	3.44	2.3
Outer	22.30	6.86	2.09	3.3

¹ mean µmoles from 16 normal human retinas (Landrum et al, 1999)

Table 2. Blood Levels of Lutein and Zeaxanthin in Men and Women

Reference	Lutein µmol/L			Zeaxanthin µmol/L			Lutein:Zeaxanthin Ratio		
	Men	Women	All	Men	Women	All	Men	Women	All
Ascherio et al, 1992 (N=307)	0.28	0.27	0.28	0.07	0.06	0.07	4.0	4.5	4.3
Olmedilla et al, 1997 (N=450)	0.19	0.18	0.19	0.06	0.06	0.06	3.2	4.53	3.1
Rock et al, 2001 (N=2786)	-	-	0.20	-	-	0.07	-	-	2.9
Curran-Celantano et al, 2001 (N=280)	-	-	0.28	-	-	0.09	-	-	3.1
Broekmans et al, 2002 (N=376)	0.16	0.19	0.18	0.05	0.05	0.05	3.2	3.8	3.6
Mean			0.23			0.07			3.3

2) Lutein and zeaxanthin are the only carotenoids that accumulate in the human lens (Yeum et al, 1995, 1999).

a) The ratio of lutein to zeaxanthin in normal human lenses (1.6 to 2.2) is within the range of that observed for the retina (Yeum et al, 1995). In the cataractous lens, a higher concentration of lutein and zeaxanthin is present in the epithelial/cortical layer than in the nuclear layer (Yeum et al, 1999) suggesting a depletion of these xanthophylls.

b) Although lutein and zeaxanthin are found in lower concentrations in the lens than in the retina, a protective role for these xanthophylls in the lens is supported by studies reporting an inverse relationship between macular pigment and lens density (Hammond et al 1997; Berendschot et al (2002). This observation suggests that macular lutein and zeaxanthin may be markers for lutein and zeaxanthin in the lens.

3) There is evidence to suggest that lutein and zeaxanthin have different biological actions in ocular tissues.

a) Structural differences between lutein and zeaxanthin molecules may contribute to different protective effects in the retina. The differences in the stereochemical

structure of lutein and zeaxanthin lead to different organizations in the lipid phase of model membrane systems. Zeaxanthin adopts a perpendicular orientation relative to the membrane whereas lutein exhibits two orientation pools, one following the perpendicular orientation of zeaxanthin and the second orienting parallel to the membrane. The appearance of two orientation pools may explain the higher efficiency of lutein to filter blue light compared to zeaxanthin in model membrane systems (Junghans et al, 2001; Sujak et al, 2002). It may also explain the enhanced photoprotection of zeaxanthin compared to lutein in egg yolk lecithin membranes exposed to prolonged UV radiation (Sujak et al, 1999). Enhanced photoprotection by zeaxanthin supports the proposed protective role of zeaxanthin in the central retina, the site where this xanthophyll preferentially accumulates. Recent work by Milanowska et al (2003) showed that the organization of geometric isomers of zeaxanthin in membranes is also different. The all-trans and 13-cis isomers of zeaxanthin adopt vertical and horizontal orientations, respectively, in the lipid membrane, which may provide favorable conditions for screening damaging light.

- b) Lutein and zeaxanthin have intrinsic antioxidant properties that directly quench potentially damaging reactive species (Conn et al., 1992). This quenching capability may depend, in part, on the number of conjugated double bonds present in the molecule. Lutein has 10 conjugated double bonds and zeaxanthin has eleven such bonds. In two *in vitro* systems, 3R,3'R-zeaxanthin has a higher quenching ability for reactive species than lutein (Table 3 from Schalch et al, 1999). Alpha-tocopherol is present in the retina (Friedrichson et al, 1995) and the lens (Yeum et al, 1995,1999), so it is possible that lutein and zeaxanthin may act to quench the tocopheryl radical in these tissues. In fact, two recent studies by Wrona and coworkers showed that zeaxanthin acted synergistically with α -tocopherol and/or ascorbic acid to protect liposomes against lipid peroxidation induced by photodynamic damage (Wrona et al, 2003) and retinal pigment epithelial cells against photo-induced oxidative stress (Wrona et al, 2004). Although *in vitro* results are not readily transferable to *in vivo* situations, these experiments support the theory that lutein and zeaxanthin may act as antioxidants in the retina and lens and that zeaxanthin may be a stronger antioxidant than lutein.

Table 3. Relative Free Radical Quenching Ability of Carotenoids

Carotenoid	Tocopheryl radical cation ^a	Singlet oxygen ^b
3R,3'R-zeaxanthin	26.4	12.6
Lycopene	13.5	16.8
β -carotene	10.2	13.5
Canthaxanthin	8.8	13.2
Lutein	5.3	6.6
2 nd order rate constants M ⁻¹ s ⁻¹ ; ^a : in hexane, ^b : in benzene		

a. Bohm et al, 1997

b. Conn et al, 1992

- 4) Animal studies provide evidence that zeaxanthin may play a specific protective role in age-related macular degeneration.
 - a) Thomson and colleagues (2002a, 2002b) provided the first experimental evidence that lutein and zeaxanthin protect photoreceptors from light damage in quails consuming diets supplemented with zeaxanthin (from *Flavobacterium*). Quails lack a macula, but their cone-rich retinas, similar to human retinas, accumulate xanthophylls (Bowmaker et al, 1993) and can form drusen, a marker for increased risk of age-related macular degeneration (Fite et al, 1994). In a subsequent report of this work Dorey and coworkers (2003) showed that zeaxanthin and lutein concentrations in the lens were enhanced in these birds, which provided the first experimental evidence that lens xanthophylls can be enhanced by dietary manipulation (Dorey et al, 2003).
 - b) Primates have maculas very similar to those of humans, and thus are considered the most appropriate animal model to study age-related macular degeneration. Studies in primates demonstrated increased blood levels of zeaxanthin and macular pigment optical densities following supplementation of zeaxanthin beadlets (Snodderly et al, 1997) and *fructus lycii* berries containing a high concentration of zeaxanthin (Leung et al 2001). In carotenoid-depleted monkeys, supplementation of lutein or zeaxanthin increased foveal concentrations of xanthophylls and restored macular photoprotection against blue-light damage (Barker et al, 2002).
- 5) Additional human studies, not included in the current petition, demonstrated increased blood levels of zeaxanthin and increased macular pigment following consumption of various supplemental sources of zeaxanthin, one from the *Lycium barabarum* berry (Khachik et al 1995) and another from *Flavobacter* (Bone et al, 1999). A more recent plasma kinetic study using 1-mg and 10-mg doses of synthetic zeaxanthin incorporated into gelatin beadlets increased plasma zeaxanthin concentrations approximately 4- and 20-fold, respectively (Hartmann et al, 2004). Serum responses and changes in macular pigment density resulting from zeaxanthin supplementation studies were recently summarized by Bone et al (2003).

Conclusions

Based on the current scientific evidence supporting a protective role for lutein and zeaxanthin in age-related macular degeneration and cataract, we propose that a health claim include lutein and zeaxanthin from a variety of dietary and supplemental sources.

References

Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L and Willett WC. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.* 1992. 122:1792-1992.

Barker FM, Neuringer M, Johnson EJ, Snodderly DM, Sandstrom MM, Schalch W, Koepcke W. Supplementation of carotenoid-depleted monkeys with lutein (L) or

zeaxanthin (Z) restores protection against photochemical blue-light damage. 13th International Carotenoid Symposium. Honolulu, Hawaii January 6-11, 2002.

Berendschot TT, Broekmans WM, Klopping-Ketelaars IA, Kardinaal AF, Van Poppel G, Van Norren D. Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. *Arch Ophthalmol*. 2002 Dec;120(12):1732-7.

Bhosale P, Larson AJ, Southwick K, Thulin CD, Bernstein PS. Identification and characterization of a zeaxanthin binding protein purified from human macula. Annual Meeting of Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL April 25-29, 2004.

Bone RA, Landrum JT, Friedes LM, Gomez CM, Kilburn MD, Menendez E, Vidal I, Wang W. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res*. 1997 Feb;64(2):211-8.

Bone RA, Landrum JT, Guerra LH, Moore LL, Sprague KE, Chen Y. Oral supplements of zeaxanthin enhance macular pigment, *Invest. Ophthalmol. Vis. Sci.*, 39 (Suppl.), S385, 1998. (Abstract)

Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J Nutr*. 2003 Apr;133(4):992-8.

Bowmaker JK, Kovach JK, Whitmore AV, Loew ER. Visual pigments and oil droplets in genetically manipulated and carotenoid deprived quail: a microspectrophotometric study, *Vision Res.*, 33, 571-578, 1993.

Broekmans WM, Berendschot TT, Klopping-Ketelaars IA, de Vries AJ, Goldbohm RA, Tijburg LB, Kardinaal AF, van Poppel G. 2002. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr*. 2002 Sep;76(3):595-603.

Conn PF, Lambert C, Land EJ, Schalch W, Truscott GT. Carotene-oxygen radical interactions. *Free Radical Res Commun*. 1992 16:401-408.

Curran-Celentano J, Hammond BR Jr, Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am J Clin Nutr*. 2001 74(6):796-802.

Dorey CK, Cheng KM, Gierhart DL, Craft NE. Dietary manipulation of lens zeaxanthin in quail. Annual Meeting of Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL May 4-9, 2003.

- Fite KV, Bengston CL, Cousins F. Drusen-like deposits in the outer retina of Japanese quail, *Exp. Eye Res.*, 59, 417-424, 1994.
- Friedrichson T, Kalbach HL, Buck P, van Kuijk FJ. Vitamin E in macular and peripheral tissues of the human eye. *Curr Eye Res.* 1995 14(8):693-701.
- Hammond BR Jr, Wooten BR, Snodderly DM. Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. *Optom Vis Sci.* 1997 74(7):499-504.
- Handelman GJ, Dratz EA, Reay CC, van Kuijk JG. Carotenoids in the human macula and whole retina. *Invest Ophthalmol Vis Sci.* 1988 29(6):850-5.
- Hartmann D, Thurmann PA, Spitzer V, Schalch W, Manner B, Cohn W. Plasma kinetics of zeaxanthin and 3'-dehydro-lutein after multiple oral doses of synthetic zeaxanthin. *Am J Clin Nutr.* 2004 Mar;79(3):410-7.
- Junghans A, Sies H, Stahl W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch Biochem Biophys.* 2001 15;391(2):160-4.
- Khachik F, Beecher GR, Smith JC. Lutein, Lycopene, and their oxidative metabolites in chemoprevention of cancer, *J. Cell. Biochem.*, 22:236-246, 1995.
- Landrum JT, Bone RA, Moore LL, Gomez CM. Analysis of zeaxanthin distribution within individual human retinas, *Methods Enzymol.*, 299, 457-467, 1999.
- Leung IYF, Tso MOM, Li WWY, and Lam TT. Absorption and tissue distribution of zeaxanthin and lutein in rhesus monkeys after taking *Fructus lycii* (Gou Qi Zi) extract, *Invest. Ophthalmol. Vis. Sci.* 42,466-471, 2001.
- Milanowska J, Polit A, Wasylewski Z, Gruszecki WI. Interaction of isomeric forms of xanthophyll pigment zeaxanthin with dipalmitoylphosphatidylcholine studied in monomolecular layers. *J Photochem Photobiol B.* 2003 Dec 5;72(1-3):1-9.
- Olmedilla B, Granado F, Gil-Martinez E, Blanco I and Rojas-Hidalgo R. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic Spanish subjects. *Clin. Chem.* 1997. 43(6):1066-1071.
- Rock CL, Thornquist MD, Neuhouser ML, Kristal AR, Neumark-Sztainer D, Cooper DA, Patterson RE and Cheskin LJ. 2002. Diet and lifestyle correlates of lutein in the blood and diet. *J. Nutr.* 132:525S-530S.
- Schalch W, Dayhaw-Barker P, Barker FM, The carotenoids of the human retina. In: Taylor A, ed., *Nutritional and environmental influences on the eye*, CRC Press, Boca Raton, 1999, pp. 215-250.

Snodderly DM, Shen B, Land RI, Krinsky NI. Dietary manipulation of plasma carotenoid concentrations of squirrel monkeys (*Saimiri sciureus*), *J. Nutr.* 127, 122-129, 1997.

Sommerburg O, Siems WG, van Kuijk FJ. Localization of carotenoids in different eye tissues. *Biofactors.* 2000 11(1-2):3-6.

Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys.* 1999 Nov 15;371(2):301-7.

Sujak A, Mazurek P, Gruszecki WI. Xanthophyll pigments lutein and zeaxanthin in lipid multibilayers formed with dimyristoylphosphatidylcholine. *J Photochem Photobiol B.* 2002 Aug;68(1):39-44.

Thomson LR, Toyoda Y, Langner A, Delori FC, Garnett KM, Craft N, Nichols CR, Cheng KM, Dorey CK. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Invest Ophthalmol Vis Sci.* 2002a 43(11):3538-49.

Thomson LR, Toyoda Y, Delori FC, Garnett KM, Wong ZY, Nichols CR, Cheng KM, Craft NE, Kathleen Dorey C. Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. *Exp Eye Res.* 2002b 75(5):529-42.

Wrona M, Korytowski W, Rozanowska M, Sarna T, Truscott TG. Cooperation of antioxidants in protection against photosensitized oxidation. *Free Radic Biol Med.* 2003 Nov 15;35(10):1319-29.

Wrona M, Rozanowska M, Sarna T. Zeaxanthin in combination with ascorbic acid or alpha-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radic Biol Med.* 2004 May 1;36(9):1094-101.

Yeum KJ, Shang FM, Schalch WM, Russell RM, Taylor A. Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr Eye Res.* 1999 19(6):502-5.

Yeum KJ, Taylor A, Tang G, Russell RM. Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Invest Ophthalmol Vis Sci.* 1995 36(13):2756-61.