To Whom It May Concern:

This letter contains 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

[Signature]

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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 Retinas

<table>
<thead>
<tr>
<th>Retinal Section</th>
<th>Lutein 3R,3’R-Zeaxanthin (µmol/g)</th>
<th>3R,3’S-Zeaxanthin (µmol/g)</th>
<th>Lutein:3R,3’R Zeaxanthin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>17.04</td>
<td>12.07</td>
<td>9.94</td>
</tr>
<tr>
<td>Medial</td>
<td>20.46</td>
<td>8.74</td>
<td>3.44</td>
</tr>
<tr>
<td>Outer</td>
<td>22.30</td>
<td>6.86</td>
<td>2.09</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Lutein  µmol/L</th>
<th>Zeaxanthin  µmol/L</th>
<th>Lutein:Zeaxanthin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>All</td>
</tr>
<tr>
<td>Ascherio et al, 1992 (N=307)</td>
<td>0.28</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Olmedilla et al, 1997 (N=450)</td>
<td>0.19</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>Rock et al, 2001 (N=2786)</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>Curran-Celantano et al, 2001 (N=280)</td>
<td>-</td>
<td>-</td>
<td>0.28</td>
</tr>
<tr>
<td>Broekmans et al, 2002 (N=376)</td>
<td>0.16</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean</td>
<td>0.23</td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>

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 \( \alpha \)-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>
<thead>
<tr>
<th>Carotenoid</th>
<th>Tocopheryl radical cation(^a)</th>
<th>Singlet oxygen(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R,3’R-zeaxanthin</td>
<td>26.4</td>
<td>12.6</td>
</tr>
<tr>
<td>Lycopene</td>
<td>13.5</td>
<td>16.8</td>
</tr>
<tr>
<td>( \beta )-carotene</td>
<td>10.2</td>
<td>13.5</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>8.8</td>
<td>13.2</td>
</tr>
<tr>
<td>Lutein</td>
<td>5.3</td>
<td>6.6</td>
</tr>
</tbody>
</table>

*a* Bohm et al, 1997

b. Conn et al, 1992

2\(^{nd}\) order rate constants M\(^-1\)s\(^{-1}\); \(^a\): in hexane, \(^b\): in benzene
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**


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


