European Food Safety Authority

The EFSA Journal (2008) 728, 1-27

Opinion of the safety of 'synthetic Zeaxanthin as an ingredient in food supplements'[1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2007-078)

Adopted on 24 April 2008

PANEL MEMBERS

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SUMMARY

Following a request from European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of 'Zeaxanthin as an ingredient in food supplements' (max 20 mg per day) in the context of Regulation (EC) N° 258/97. In particular, EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by Member States.

After lutein ((3R,3'R,6'R)-β,ε-carotene-3,3´-diol), zeaxanthin is the most common xanthophyll in human diet. Important sources of zeaxanthin include yellow/orange/red fruits and green vegetables such as cabbage, corn, broccoli, Brussels sprouts, green beans, kale, mandarins, oranges, peas, pumpkins, spinach, and squash, as well as egg yolk. Estimations of the dietary intake of xanthophylls make usually no distinction between lutein and zeaxanthin due to analytical reasons.

Zeaxanthin is produced in a multi-step chemical synthesis. The predominant zeaxanthin isomer in the product is all-trans-zeaxanthin (not less than 96 %). Analytical data indicate that the amount of impurities, such as lead and other heavy metals are present in acceptable residue levels. Synthetic zeaxanthin is not intended to be marketed in its crystalline form, but in three different formulations: two spray-dried powders of either gelatine or starch base "beadlets" each containing 5 % zeaxanthin with α-tocopherol and ascorbyl palmitate added as antioxidants, and a corn oil suspension containing 20 % zeaxanthin with added α-tocopherol.

[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of 'Zeaxanthin as an ingredient in food supplements'. *The EFSA Journal* (2008) 728, 1-28.

The stability of the ingredient formulated into stabilized beadlets or corn oil suspensions has been demonstrated.

Synthetic zeaxanthin is not nutritionally essential and possesses practically no provitamin A-activity. Bioavailability of zeaxanthin in humans may vary and may depend on individual factors, the food matrix in which it is ingested, and on effects of co-ingested food. Based on the data provided by the applicant, it appears that the bioavailability of zeaxanthin from food is generally quite low. After intake of zeaxanthin formulated into tablets at doses of 1 and 10 mg/day by healthy adults, mean zeaxanthin plasma levels increased substantially (from approximately 50 nmol/L to 200 nmol/L and 920 nmol/L, respectively). The relative absorption at the high dose was 40 % lower than at the low dose. Up to 20 mg/day intake of zeaxanthin had no effect on the plasma levels of other common carotenoids in healthy human volunteers. At doses of 30 mg/day, zeaxanthin accumulated in the macula of the eye. In rats, zeaxanthin accumulated in adipose tissue, the liver and several other organs, whereas in monkeys zeaxanthin accumulated in the adipose tissue, liver and eye.

In European countries, the average intake level of zeaxanthin via food was estimated to be between 0.2 and 0.9 mg/day and for people with a high intake of zeaxanthin-rich vegetables and fruits this could result in a level of 1.8 mg/day (95^{th} percentile). The applicant proposes use levels of up to 20 mg/person/day of synthetic zeaxanthin as an ingredient in food supplements. Thus, the proposed level of use would lead to intake levels that would substantially increase the dietary intake of zeaxanthin resulting up to 100 times higher intakes for adults compared to the estimated average intake from natural sources.

Toxicity studies in rodents, dogs and monkeys showed no effects other than discolouration of the faeces and adipose tissue, as well as increases in plasma and liver zeaxanthin concentrations. These were not considered toxicologically relevant. In studies on embryotoxicity and teratogenicity using rats and rabbits there were no indications of maternal toxicity, embryotoxic or teratogenic effects. There was no indication for genotoxicity. No studies on chronic toxicity and carcinogenicity have been performed.

In studies provided by the applicant, no zeaxanthin-related adverse effects were reported for healthy human volunteers who took daily doses of 10-30 mg zeaxanthin up to 4-6 months.

Structural similarities between zeaxanthin and β-carotene were considered as it has been shown that supplementation with β-carotene increases risk of lung cancer in heavy smokers. While the available data indicate that zeaxanthin in food would not be expected to have similar effect there are no data concerning zeaxanthin in the form of supplements. There are differences between zeaxanthin and β-carotene regarding their chemical structure, stability, absorption, metabolism and functionality. However, there are no studies on the effects of zeaxanthin on the enzymes of xenobiotic metabolism, nor has zeaxanthin been tested in the tobacco smoke exposed ferret animal model which was used to study the enhancement of lung metaplasia by ß-carotene. On the basis of available data, it is not possible to assess whether additional synthetic zeaxanthin at the proposed level of use would increase the risk of lung cancer in heavy smokers as reported for ß -carotene.

The proposed levels of use of up to 20 mg/person/day of synthetic zeaxanthin as an ingredient in food supplements would lead to intake levels that would substantially increase the average dietary intake of synthetic zeaxanthin resulting in up to 100 times higher intakes for adults.

These intake levels are within the range of the group ADI 0-2 mg/kg body weight for lutein and synthetic zeaxanthin as established by JECFA. However, in the opinion of the Panel, the toxicological data on synthetic zeaxanthin are not sufficient to derive an acceptable daily intake.

Based on the existing data, the Panel concludes that the safety of zeaxanthin as an ingredient in food supplements at the proposed use level of up to 20 mg/person/day has not been established.

Key words:

Zeaxanthin, novel food ingredient, food supplement, CAS Registry Number 144-68-3

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 1 June 2004, Bioresco on behalf of DSM Nutritional Products submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the Competent Authorities of the Netherlands for placing on the market 'Zeaxanthin' as a novel food ingredient to be used in food supplements, foods for particular nutritional purposes and regular foods.

On 16 June 2005, the Competent Authorities of the Netherlands forwarded to the Commission their initial assessment report, which had reached the conclusion that the data provided were not sufficient to complete the safety assessment. The reason was that there was no guarantee that acceptable consumption levels of zeaxanthin would be exceeded under the proposed use conditions. Therefore the Competent Authorities were of the opinion that the range of products needs to be restricted and precisely defined.

On 1 August 2005, the Commission forwarded the initial assessment report to the other Member States. Several Member States agreed with the conclusions of the initial assessment. Some Member States submitted additional comments.

In consequence, a Community Decision is required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

On 2 February 2007 the applicant informed the Commission that the use of zeaxanthin should be limited to be used only as an ingredient in food supplements.

Community interest

Following the notification of the initial assessment report of the product concerned and the Member States' comments, an additional assessment is required. The issues of scientific nature can be summarized as follows (comments, no longer relevant due to limitations of the application to zeaxanthin as an ingredient only in food supplements, are not included):

- The lead levels should be minimized and the level of permissible residues of triphenylphosphine oxide (TPPO) should be reduced.
- While the stability data for zeaxanthin showed that it is stable in a range of food matrices, there was nevertheless measurable degradation during the shelf life of the products.
- Doubts were expressed toward plausibility of the applicant's "adequate intake" figure of 2-20 mg/person/day. "At risk" groups such as elderly people who could be particularly high consumers were suggested to be given special consideration. There was no information on potential effects in infants, children and young people.
- Intake estimates of zeaxanthin from natural sources were questioned due to assumed lack of analytical methods to separate zeaxanthin from lutein.
- It was questioned whether naturally occurring and synthetic zeaxanthin do not differ with regard to bioavailability. Information is needed about potential inhibitory effect of zeaxanthin on the absorption of β-carotene.
- Is potential accumulation of zeaxanthin in certain organs or tissues a conceivable hazard of the intake?

- Regarding the studies on genotoxicity it was questioned whether the endpoint chromosome mutations, including aneugenicity, was sufficiently examined. A study on chronic toxicity/carcinogenicity in laboratory animals was considered necessary since the substance might be consumed over a prolonged period of time. In the case that intake levels are considerably higher than the intake from natural sources, additional studies in humans including bioavailability determinations should be carried out. Further information, e.g. studies in the ferret model, should be provided to demonstrate that increased intake of zeaxanthin does not increase the risk of developing cancer in certain at risk groups, as it has been reported for the related carotenoid ß-carotene.
- Need to evaluate the implication of the formation of "polarising structures" in the eyes of tested monkeys (52-week study) given high doses of zeaxanthin in relation to high level consumers.

In view of these questions and the Community interest in this matter, the European Commission has decided to seek the opinion of the European Food Safety Authority (EFSA).

Existing authorizations and evaluations

According to the applicant the synthetic zeaxanthin under assessment has already been accepted for use in food supplements in many countries, including the US, Australia, Mexico and Taiwan.

Lutein, an isomer but not a stereoisomer of zeaxanthin, extracted from the natural strains of edible fruits and plants, grass, lucerne (alfalfa) and *Tagetes erecta* (marigold flowers), is authorised within the EU as food colour (E161b) (European Commission, 1995).

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) of EFSA has issued an Opinion on lutein extracted from *Tagetes erecta* and from some species of edible plants for use in foods for special medical purposes (FSMPs). The Panel concluded that the use of lutein is not of safety concern under the proposed use levels, which are in the range of the regular dietary intake of lutein, provided that it is in compliance with the existing EU specifications of the food additive (EFSA, 2006).

Tagetes extract containing xanthophylls at low concentrations was considered by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 31st meeting. Since no toxicological data were available at that time no evaluation was made. Tentative specifications were prepared, which were later superseded by full specifications (WHO, 1987; WHO, 2001).

In June 2004, at its 63rd meeting, JECFA evaluated the safety of synthetic zeaxanthin intended to be used as a food colour and nutritional supplement, as well as preparations from *Tagetes erecta* L. with a high lutein content (>80 %). In view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin, the Committee decided to include zeaxanthin in the ADI (acceptable daily intake) of 0-2 mg/kg bw/day derived for lutein from *Tagetes erecta*, which had a stronger toxicological database, and to make this a group ADI for these two substances. However, the Committee also concluded that they were unable to assess whether lutein and zeaxanthin in the form of supplements would

have adverse effects in heavy smokers as they have been reported for supplements containing ß-carotene (WHO, 2006a). Specifications were prepared for both substances (WHO, 2004; WHO, 2006b). The JECFA specification for synthetic zeaxanthin is in accordance with the specification proposed in this application.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Zeaxanthin as an ingredient in food supplements' (max 20 mg per day) in the context of Regulation (EC) N° 258/97.

In particular, EFSA is asked to consider the elements of a scientific nature in the comments/objections raised by the other Member States.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Jean-Louis Bresson, Karl-Heinz Engel, Marina Heinonen, Pagona Lagiou, Bevan Moseley, Andreu Palou, Annette Pöting, Seppo Salminen, Hendrik Van Loveren and Hans Verhagen.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC, the synthetic zeaxanthin belongs to class 6. "Foods produced using a novel process". The assessment of the safety of the ingredient will be based upon the data provided by the applicant to comply with the information required for novel foods of Class 6, i.e. the structured schemes I, II, III, IX, X, XI, XII, and XIII.

I. Specification of the novel food (NF)

Zeaxanthin, a naturally occurring xanthophyll pigment, is an oxygenated carotenoid with formula $C_{40}H_{56}O_2$. The molecular weight of zeaxanthin is 568.9 daltons and the CAS Registry Number is 144-68-3. Its structural formula is:

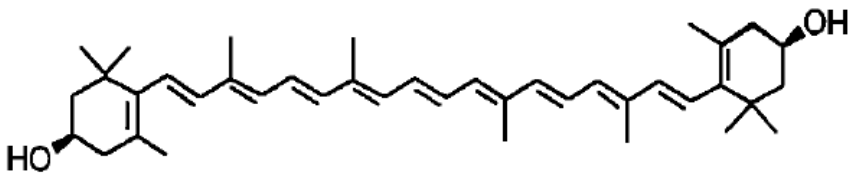

Fig 1. **Structural Formula**

Specification for the synthetic zeaxanthin has been provided by the applicant and is presented in Table 1. According to the specification synthetic zeaxanthin contains at least 96 % of the all-trans isomer (all-trans-(3R, 3'R)-β,β-carotene-3,3'-diol; (3R, 3'R)-dihydroxy-β,β-carotene) and not more than 2 % of cis isomers. By-products can be present in small quantities. The most common by-products are three other carotenoids, which are found in combination concentrations not exceeding 1.1 %. For triphenylphosphine oxide (TPPO) originally the maximum residue level of not more than 100 mg/kg was suggested, which is in accordance with the specifications prescribed by JECFA. However, the applicant accepts to lower the maximum level of TPPO to 50 mg/kg, since the batch analyses of zeaxanthin reveal TPPO concentrations that are substantially below the 100 mg/kg limit. The lead content does not exceed 2 mg/kg, which is in accordance with the JECFA specifications and below the maximum levels laid down in the specifications of other carotenoids authorised for use in food in the EU (European Commission, 1995). The applicant argues that this lead level should be acceptable for zeaxanthin which is consumed at levels of not more than 20 mg/day. Examples given by the applicant describe consumption of α-tocopherol as vitamin E at levels of 10 mg/day and consumption of ascorbic acid at levels of 60 mg/day, both with a residual lead content of not more than 5 mg/kg.

Synthetic zeaxanthin will not be marketed in its pure crystalline form, but in three different zeaxanthin formulations: two spray-dried powders of either gelatine or starch base "beadlets" each containing 5 % zeaxanthin with α-tocopherol and ascorbyl palmitate added as antioxidants, and a corn oil suspension containing 20 % zeaxanthin with added α-tocopherol.

Table 1. **Specification of synthetic zeaxanthin as provided by the applicant**

Analysis	**Method**	**Specification**
Description	Observation	Orange-red crystalline powder, with little or no odour
Identification	A. Solubility[a] B. Appearance in 1 % chloroform solution[b] C. UV-spectrometry	Soluble in chloroform, practically insoluble in water Clear, intense orange-red Absorption maximum at 450 nm to 454 nm in ethanol/ dichloromethane/cyclohexane (98/1/1, v/v/v)
All-trans zeaxanthin	HPLC[c]	Not less than 96.0 %
Cis-zeaxanthins	HPLC[c]	Not more than 2.0 %
Other carotenoids	HPLC[c]	12'-apo-Zeaxanthinal (CAS no 62742-02-3) not more than 0.1 % Diatoxanthin (CAS no. 31063-73-7), not more than 0.2 % Parasiloxanthin (CAS no. 62994-48-3), not more than 0.8 %
Triphenylphosphine oxide (CAS no. 791-28-6)	HPLC[d]	Not more than 50 mg/kg
Lead	AAS[a]	Not more than 2 mg/kg
Heavy metals (determined as Pb)	not specified	< 20 mg/kg
Loss on drying	Oven[a]	Not more than 0.2 %

[a] As specified in the JECFA Guide to Specifications (FAO Food and Nutrition Paper 5 Rev 2.)
[b] As specified in the European Pharmacopoeia 4th edition, 2002
[c] HPLC method by Barth and Lernhardt, 2002
[d] HPLC method by Demougeot, 1999

II. Effect of the production process applied to the NF

The dossier describes in detail the six steps in the novel chemical synthesis of zeaxanthin from smaller molecules. At the end of each step the intermediate product is purified by precipitation, filtration and in relevant cases by cleaning with an organic solvent or water, and drying. After the final reaction step zeaxanthin precipitates after filtration to be followed by recrystallization to form the pure crystalline end product. The starting material and all the intermediate products are identified and physicochemically characterized. Traces of organic solvents used in the process account for less than 0.2 % of the end product. Analytical data on heavy metals present (<20 mg/kg) indicate that the catalysts added are efficiently removed. During the reaction, other carotenoid compounds are also formed, up to 1.1 % of the end product. Triphenylphosphine oxide (TPPO) structurally unrelated to zeaxanthin, is formed during the process and is present in the final product in concentration less than 50 mg/kg.

The applicant states that crystalline synthetic zeaxanthin will not be used as such but will be formulated into stabilized beadlets or oily suspensions. Thus, data on the heat stability of pure zeaxanthin may not be extrapolated to the stability of zeaxanthin in the beadlets and other stabilized forms. According to applicant's standard operation procedures pure crystalline

zeaxanthin will be stored for no more than six months before it is used for the production of beadlets or oily suspension. Crystalline zeaxanthin (in amber glass ampoules, sealed under argon, protected from light) remained stabile at -3°C to +5°C for three years. Also formulated zeaxanthin products are stable at low temperatures and when protected from oxygen and light. The stability data provided by the applicant indicates that in a gelatine matrix zeaxanthin is stable at 15°C (when packaged in aluminium bags) for 48 months. The zeaxanthin content remained constant in a starch matrix up to three months and in corn oil suspension up to six months (15°C). In the gelatine matrix zeaxanthin remained stable for four to eight weeks after incorporation into orange juice, biscuits, yoghurt, and ice cream. A minor degradation of 5.1 % occurred when zeaxanthin in a tablet prepared with several formulation aids (in glass bottles) was stored at 25°C for 12 months. When similarly stored in combination with several vitamins, minerals and other carotenoids the zeaxanthin decrease was up to 7.7 %. For zeaxanthin in these tablets no degradation products were found within two months. According to the applicant possible oxidative degradation of zeaxanthin has not been investigated. However, studies with other carotenoids (lycopene, β-carotene) indicate that oxidation results in wide array of degradation products (Caris-Veyrat *et al*., 2001, 2003; Weedon, 1971), some of which also occur in nature (Britton *et al*., 2004; Khachik *et al*, 1997).

III. History of the organism used as the source of the NF

Chemically synthesised zeaxanthin does not have a biological source.

IX. Anticipated intake/extent of use of the NF

After lutein ((3R,3'R,6'R)-β,ε-carotene-3,3´-diol), zeaxanthin is the most common xanthophyll in human diet. Important sources of zeaxanthin include yellow/orange/red fruits and green vegetables such as cabbage, corn, broccoli, Brussels sprouts, green beans, kale, mandarins, oranges, peas, pumpkins, spinach, and squash, as well as egg yolk (Holden et al., 1999, Humphries and Khachik, 2003). The content of cis-isomers in naturally occurring zeaxanthin has been reported to vary between 30-80 % in green vegetables and between 0-15 % in yellow-orange fruits and vegetables (Humphries and Khachik, 2003).

Estimations of the dietary intake of xanthophylls make usually no distinction between lutein and zeaxanthin due to analytical reasons. The lutein to zeaxanthin ratio measured in the average US diet is about 5:1 (Mohamedshah *et al*., 1999). On the basis of limited data from national food consumption surveys (France and Germany) and several other studies, the intake of zeaxanthin from natural sources was estimated in European countries. The average intake level of zeaxanthin via food was estimated to be between 0.2 and 0.9 mg/day and for people with a high intake, i.e. those consuming diets which include high amounts of zeaxanthin-rich vegetables and fruits, could result in a level of 1.8 mg/day (95th percentile roughly estimated from the mean intake in a specific North Italian population group) (Franceschi *et al*., 2000). In two Dutch cohort studies the average combined lutein and zeaxanthin intake was 2.5 mg/person/day for the elderly and 2.9 mg/person/day for individuals aged between 25 and 45 (Goldbohm *et al*., 1998; O'Neill *et al*., 2001). German data from the 1998 Federal Nutrition Survey arrive at a mean zeaxanthin intake of 0.22 mg/person/day, and 0.49 mg/person/day in the 95th percentile. Data from the US (NHANES III survey) estimated mean and the 90th percentile consumption of lutein and zeaxanthin to 1.7 and 3.0 mg/person/day and to 3.8 and 7.3 mg/person/day for those who met the national recommended daily intake of vegetables.

Assuming that the 5 to 1 ratio of lutein to zeaxanthin is applicable also to this subgroup of population, the zeaxanthin intake may be estimated at about 0.6 (mean) and 1.2 mg/day (90th percentile). Higher intakes have been reported in other countries such as in different islands of the South Pacific with a mean lutein intake ranging from 3.9 - 23.6 mg/day among females and from 3.2 - 25.7 mg/day among males (Le Marchand et al., 1995). Applying the 5 to 1 ratio of lutein to zeaxanthin, the zeaxanthin intake may exceed 4 mg/day in the subgroup with the highest xanthophyll intake of native Fijians.

The applicant proposes levels of use of up to 20 mg/person/day of synthetic zeaxanthin as an ingredient in food supplements. Thus, the proposed level of use would lead to intake levels that would substantially increase the dietary intake of zeaxanthin resulting in 22-100 times higher intakes for adults compared to the estimated average intake from natural sources in European countries. Compared with individuals consuming diets with high amounts of zeaxanthin-rich vegetables and fruits, the intake would be 11 times higher. Compared with individuals consuming exceptionally high amounts, e.g. up to 4 mg zeaxanthin/day as reported for specific populations, the intake would be 5 times higher.

X. Information from previous human exposure to the NF or its source

Synthetic zeaxanthin has not previously been available on the European market. Outside EU, food supplements are available that contain natural lutein and zeaxanthin, and supplements that contain synthetic zeaxanthin. However, no reliable data are available concerning the consumption of such products.

According to the applicant zeaxanthin intake via food supplements generally appears to be very low, since the levels of consumption recommended by the manufacturers generally do not exceed 2 mg/day. However, supplements have been available in the US with recommended daily dose of 10 mg.

The applicant discusses results of various studies involving human subjects. One study involved five male and five female volunteers, all healthy, who took a daily dose of 10 mg of synthetic zeaxanthin in capsule form over a period of 42 days (Cohn *et al*., 2002). No zeaxanthin-related adverse effects were found. Other studies reported by the applicant concern primarily the effect of elevated zeaxanthin intake on the pigment density in the retina. In these studies healthy volunteer subjects took daily doses of 10-30 mg zeaxanthin up to 4-6 months with no adverse effects reported (Bone *et al*., 2003; Haartmann *et al*., 2004; Schalch *et al*., 2004; Rodriguez-Carmona *et al*., 2004).

XI. Nutritional information on the NF

Zeaxanthin is not nutritionally essential and possesses practically no provitamin A activity. The applicant states that neither zeaxanthin as such nor the materials used for the preparation of the beadlets (or oily suspension) are expected to have nutritional impact other than the intended zeaxanthin supplementation. Zeaxanthin and lutein together form the macular pigment which plays an important role in the protection of the eye retina from the deleterious effects of light (Hammond *et al*., 2001). The effect of increased zeaxanthin intake on the optical density of the macular pigment is currently being investigated in relation to the risk of AMD (age-related macular degeneration).

This opinion does not include an assessment of the possible benefits of zeaxanthin.

Bio-availability

The distribution of zeaxanthin after oral administration was examined in rats. Animals received doses of approximately 0.8 or 8 mg/kg bw/day for 5 weeks. The test material was a preparation containing 5 % zeaxanthin ("Carophyll Golden") which was not further specified. Examination of selected organs and tissues after 35 days showed dose-dependent increases of zeaxanthin levels in all these organs and tissues except the thyroid gland and the eye. The highest zeaxanthin concentrations were found in small intestine and spleen followed by liver, adipose tissues and adrenal glands. A decrease in zeaxanthin concentration during the following 5 week treatment free period was noted in all tissues with detectable zeaxanthin. Plasma levels after 5 weeks were increased to 0.3 and 1.9 µg/L in the low and high dose group respectively compared with the control. Increased plasma levels were reversed after 7 days in the low dose group and after 21 days in the high dose group.

The absorption of zeaxanthin in humans may vary in individuals and depends the food matrix in which zeaxanthin is ingested, and effects of co-ingested food. It appears that the bioavailability of zeaxanthin from food is generally quite low. When spinach (60 g/day, corresponding to 10.8 mg lutein/day and 0.29 mg zeaxanthin/day) and maize (150 g/day corresponding to 0.4 mg lutein/day and 0.29 mg zeaxanthin/day) were included in the normal diet of seven healthy subjects for 15 weeks, mean serum zeaxanthin levels were statistically significantly increased only at week 4 of supplementation, i.e. 42 µg/L compared with baseline levels of 33 µg/L (Johnson *et al*., 2000). The applicant thus claims that supplementation in higher amounts or more bioavailable forms of zeaxanthin are required to increase zeaxanthin levels in the blood.

A pharmacokinetic study was provided involving two groups of 5 male and 5 female healthy volunteers receiving 1 or 10 mg, respectively, of zeaxanthin a day for 42 days (Cohn *et al*., 2002; Hartman *et al*., 2004). The test material was a beadlet formulation of synthetic zeaxanthin embedded in a matrix of gelatine with alpha-tocopherol and ascorbyl palmitate added as antioxidants (Zeaxanthin 5 % TG (tablet grade)). Mean plasma concentrations of all-trans zeaxanthin increased from 27 µg/L at baseline to 113 µg/L and 523 µg/L at steady state after administration of 1 and 10 mg zeaxanthin, respectively. The relative absorption of the 10 mg dose was about 40 % lower than after the 1 mg dose. The effective half-life for accumulation was approximately 5 days, and after 17 days of dosing >90 % of the steady state concentrations were reached in both groups. The terminal elimination half-life was 12 days. Multiple doses of 1 or 10 mg zeaxanthin did not affect plasma concentrations of other carotenoids, retinol, α-tocopherol or lipids. As plasma lutein concentrations remained unaffected by zeaxanthin dosing, the observed increase in 3´-dehydrolutein was postulated to be derived from zeaxanthin.

In another study 30 mg zeaxanthin were administered daily to one male subject for 120 days (subject A), and to another one for 60 days (subject B). The test material was described as crystalline, unesterified zeaxanthin encapsulated in gelatine/starch beadlets, which were suspended in canola oil. In subject A the zeaxanthin serum concentration increased from 55 µg/l before supplementation within about 30 days to reach a plateau of approximately 320 µg/L. After 40 days of supplementation a gradual increase in the macular pigment (MP) optical density was observed in both eyes. For subject B the serum concentration increased

from 49 µg/L within about 10 days to approximately 273 µg/L. The macular pigment (MP) optical density increased from approximately 25 days of supplementation (Bone et al., 2003). According to the applicant, the relatively lower increases in plasma levels observed after administration of 30 mg zeaxanthin/day compared with the administration of 10 mg/day in the study described above, are most likely attributable to a different bioavailability of zeaxanthin from the formulations used.

There is evidence for a competition between simultaneously ingested carotenoids for intestinal absorption (Zaripheh and Erdman, 2002). For example, the addition of spinach or lutein to tomato purée reduced the absorption of lycopene. However, the medium-term plasma response to carotenoid supplementation was not diminished (Tyssandier *et al*., 2002). This result was confirmed in another study where ingestion of zeaxanthin, 10 mg/day in beadlets for 42 days, had no effect on plasma carotenoids, retinol or α-tocopherol concentrations (Hartmann *et al*., 2004). New information has become available (Schalch and Barker, 2005) showing that the intake of zeaxanthin at levels of 10 mg/day for six months (92 subjects) followed by an intake of 20 mg/day for another six months (14 subjects) had no effect on the plasma levels of other common carotenoids in healthy human volunteers.

Metabolism

A number of compounds derived from lutein and zeaxanthin have been identified in human tissues and in non-primate animal models (Fig 1). These metabolites result principally from three types of reactions involving the end groups of these xanthophylls – oxidation, reduction and double-bond migration (Khachik *et al*., 1995, 1997, 2002). Lutein and zeaxanthin can exist in equilibrium involving the intermediate carotenoid 3´-epilutein. Allylic oxidation of lutein at C3 results in the formation of oxolutein B, which can exist in equilibrium with lutein and 3´-epilutein through reduction reactions. 3´-Epilutein and zeaxanthin can also exist in equilibrium through reversible double-bond migration. Thus, presence of 3´-epilutein in human serum may be due to conversion of lutein and/or zeaxanthin. Acid-catalyzed dehydration is another reaction of carotenoids with 3-hydroxy end groups. Lutein is believed to undergo degradation in the stomach acid to form anhydroluteins that have been isolated from serum and detected in breast milk. The toxicological importance of these compounds is not known.

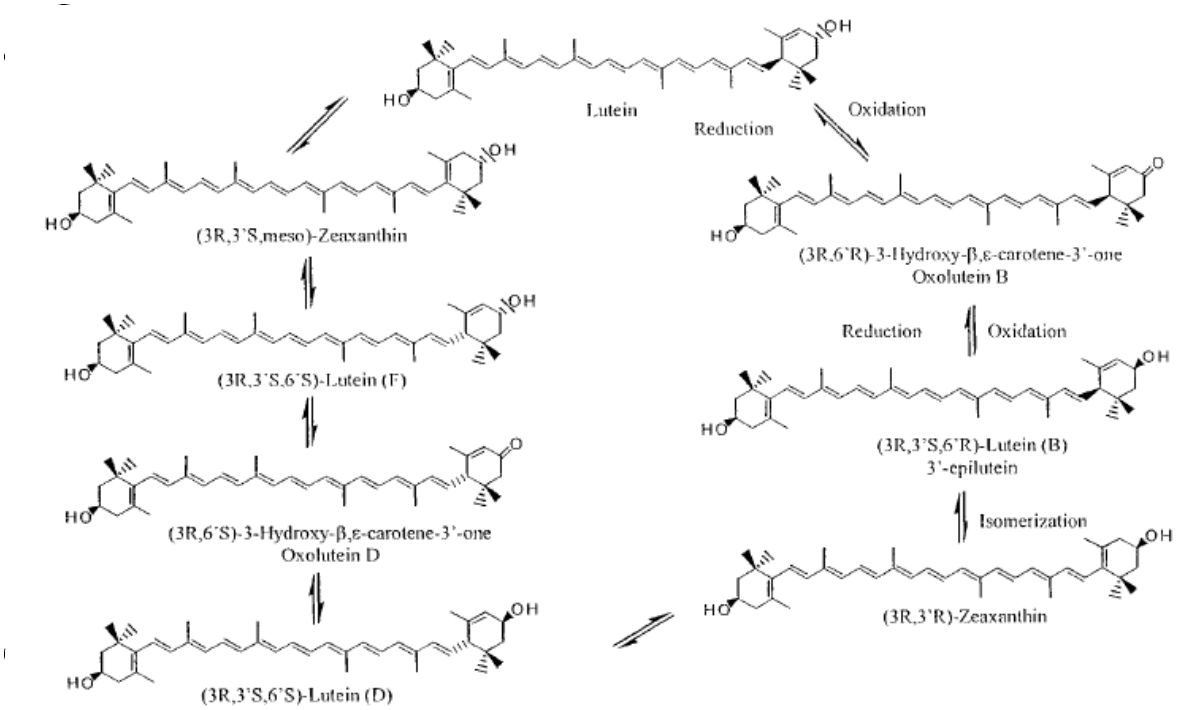

Fig 2. **Proposed in-vivo transformation processes of dietary zeaxanthin and lutein (Khachik *et al.*, 2002)**

At normal levels of intake, zeaxanthin accumulates at high local concentrations in the macula of the human eye (Hammond *et al*., 1997, 2001). The applicant presents animal studies (Froescheis *et al*. 2001; Neuringer *et al*., 2005; Johnson *et al*., 2005) showing a rate of absorption of ca. 4 % and 1 % at zeaxanthin intakes of 2 mg/kg bw and 20 mg/kg bw for 14 days, respectively, as well as rapid clearance, mainly in the faeces, and no evidence regarding accumulation of zeaxanthin in tissues of rats after administration of a single dose of radiolabelled zeaxanthin. Only about two times higher zeaxanthin concentrations were found in adipose tissue in a study with xanthophyll-free monkeys where the intake and plasma levels of zeaxanthin in the supplemented group were many times higher compared to the control group.

XII. Microbiological information on the NF

Micro-organisms are not used in the production of crystalline synthetic zeaxanthin. Adequate microbiological production standards are demonstrated by the applicant for beadlets containing substances other than zeaxanthin. The applicant maintains that growth of micro-organisms in the beadlets is not expected since their moisture content is too low to support microbial growth.

XIII. Toxicological information on the NF

Acute oral toxicity

A series of studies was conducted in mice and rats in order to determine the lethal dose of synthetic zeaxanthin after repeated oral or intraperitoneal (i.p.) administration. These relatively

old studies from 1977 were not carried out according to the current standard (OECD Guideline 401 from 1981) for acute toxicity testing. The LD50 of zeaxanthin (purity not specified) after repeated oral administration for 10 days was higher than 8000 mg/kg bw in mice and rats. After i.p. administration the LD50 was 840 and 1100 mg/kg bw in mice and rats, respectively. Using a 10 % zeaxanthin formulation, which was not further specified, the oral LD50 in rats and mice was higher than 8000 mg/kg bw corresponding to 800 mg zeaxanthin/kg bw.

Subchronic toxicity

In a 13-week feeding study not complying with GLP principles, groups of 10 male and 10 female mice received zeaxanthin at doses of approximately 250, 500 or 1000 mg/kg bw/day. Zeaxanthin was added to the diet in the form of a water-soluble beadlet formulation with a zeaxanthin content of 9.3 %. By addition of placebo beadlets all test diets contained the same amounts of beadlets. One control group received a diet containing placebo beadlets and a second one a standard rodent diet. No toxic effects were observed up to the highest dose administered zeaxanthin.

In a comparable 13-week feeding study in rats not complying with GLP principles groups of 16 male and 16 female animals received zeaxanthin in the diet (added in the form of a water-soluble beadlet formulation with a zeaxanthin content of 9.3 %). The beadlets were described as spherical particles (0.1-0.5 mm in diameter) containing zeaxanthin emulsified with ascorbylpalmitate and embedded in a mass of gelatine, sucrose and peroxide free peanut oil with dl-alpha-tocopherol as antioxidant. The surface was coated with corn-starch. Intended doses were approximately 0 (control group), 250, 500 or 1000 mg zeaxanthin/kg bw/day. All diets contained the same amount of beadlets. A second control group received a standard rodent diet. A precise determination of the exposure was not possible as the zeaxanthin intake was reduced towards the end of the treatment period due to avoidance of beadlets, in particular in the highest dose group (in females to approximately 40-50 %, in males to approximately 60-70 %). In addition, the zeaxanthin content of the feed admixture in weeks 12 and 13 was slightly lower as expected, which was attributed by the authors to degradation processes. The zeaxanthin content in the feed admixture of the high dose males was reduced by one third, which could not be explained by the authors.

In all groups receiving beadlets body weight gains were reduced compared with the group fed a standard rodent diet. According to the authors, haematology and clinical-chemical analyses, eye examinations as well as macroscopic and histological examinations of selected organs 'revealed only presumably spontaneous alterations without obvious or suspected relation to treatment'.

In a second 13-week rat feeding study complying with GLP principles groups of 12 male and 12 female animals received doses of approximately 250, 500 or 1000 mg zeaxanthin/kg bw/day in the diet (a water soluble beadlet formulation - 10 % WS with an actual zeaxanthin content of 9.4 %). One control group received a diet containing beadlets without zeaxanthin, and a second control group a standard rodent diet. The beadlets contained gelatine, sucrose, dextrin yellow, nipagin (p-hydroxybenzoic acid methylester), nipasol (p-hydroxy-benzoic acid propylic ester), NaOH, ascorbyl-palmitate, water and maize starch. Methylenchloride (approximately 150-250 ppm) was contained as solvent residual from the production process of the beadlets for the animal studies.

Feed analyses at the beginning and end of the treatment period showed relatively large variations in the zeaxanthin-content of the diets (79-109 % of the desired concentration), but the actual intake correlated well with the intended dosage. Unlike the previous study, the animals could not avoid beadlet intake from the feed. Animals in all groups receiving zeaxanthin, in particular those receiving the high dose, showed a yellow-orange discolouration of the faeces. Feed intake was reduced in females in all groups receiving beadlets compared with the controls fed a standard rodent diet. High dose females showed reduced mean body weight gain from week 6-10 compared with the placebo control group, and overall weight gain at study termination was also reduced. In the haematology and clinical-chemistry analyses at the end of the treatment period there were some statistically significant differences in relation to the placebo control group, i.e. lower white blood cell counts in males (high dose), lower bilirubin levels in males (high dose) and females (high and intermediate dose), lower total protein and higher albumin levels as well as a higher albumin/globulin ratio in females (high dose), higher Na levels in females (high and intermediate dose), but lower Na levels in males (high dose). According to the study report, all values were within the normal biological ranges. However, when considering the historical control data provided, the Panel cannot confirm this conclusion for all parameters. There were no relevant differences in the weights of selected organs and tissues between groups. Macroscopic examination revealed an orange discolouration of fat tissue in all groups receiving zeaxanthin, which is not considered adverse, and the histopathological examinations also gave no indications of adverse effects. The Panel noted that the study was not carried out according to the current standard (OECD Guideline 408) with regard to the statistical evaluation of data (in particular body weight data), data presentation (in particular urinalysis data) and the number of organ weight determinations. The Panel concludes that the study did not give indication of adverse effects up to the highest dose.

In a 13-week study in Beagle dogs complying with GLP principles groups of 3 male and 3 female animals received zeaxanthin at doses of 123, 204 or 422 mg/kg bw/day and 104, 238 or 442 mg/kg bw/day, respectively, in the diet (a water-soluble beadlet formulation with a zeaxanthin content of 9.4 %). The control group received a diet containing beadlets without zeaxanthin. Gelatine, sucrose, dextrin yellow, maize starch, ascorbyl-palmitate, nipasol and nipagin were specified as components of the beadlets. This study was not in accordance with OECD Guideline 409 with regard to the recommended number of animals/group (3 instead of 4 animals per sex were used).

According to the study report body weights were not influenced by the treatment and there were no remarkable findings in the eye examinations. Regular observation of the animals did not reveal obvious signs of toxicity. Faeces of animals receiving zeaxanthin showed an orange colour, in particular in the high dose group. There were no toxicologically relevant findings in haematology, clinical-chemistry and urine analyses. Macroscopic inspections at necropsy revealed a yellow to reddish discolouration of the adipose tissue of male dogs of the intermediate and particularly of the high dose group. Livers of high dose males showed a yellowish colour. Several changes in organ weights (higher absolute and relative thyroid weight in males and females of the low and high dose groups, lower absolute heart weight in females of the low dose group and lower relative kidney weight in males and females of the intermediate dose group) were not accompanied by histopathological changes and therefore not considered toxicologically relevant. It was concluded that the highest dose of 422 mg/kg bw/day was not toxic to dogs under the conditions of the study.

Upon request of the Panel the applicant has also provided the 13-week feeding study in rats with lutein, which formed the basis for the derivation of the ADI by JECFA. In the study, which complied with GLP, groups of 10 male and 10 female Wistar rats received lutein (according to JECFA from marigold petals containing about 79 % lutein and 5 % zeaxanthin), formulated as beadlets incorporated into the diet, at doses of 0 (control), 2, 20 or 200 mg/kg bw/day for 13 weeks. In addition, 5 male and 5 female animals were included in the control and the high-dose groups for an additional 4-week treatment-free period. One low-dose female died in week 13. There were no clinical signs and no relevant differences in body weight, body weight gain and food consumption between the groups. A battery of neurotoxicity tests and ophthalmoscopic examinations did not reveal treatment-related differences. Haematology examinations showed a statistically significant increase in the activated partial thromboplastin time (APPT) in females of all dose groups. Low-dose females in addition showed an increase in another blood clotting parameter (prothrombin time) compared with the controls. High-dose females showed an increase in white blood cell counts which was related to an increase in lymphocyte counts. Clinical-chemistry examinations revealed a dose-related reduction of plasma creatinin levels and total protein levels in males as well as an increase in the phosphate level in high-dose males. There were no treatment-related differences in organ weights. A statistically significant increase in testis/epididymis weight relative to body weight in high-dose males was not accompanied by histopathological alterations and therefore not considered toxicologically relevant. Microscopic examinations of selected organs and tissues at the end of the treatment period revealed inflammatory cell foci in all male and female animals examined including the controls. In females of all dose groups the incidence of liver vacuolation and kidney tubular degeneration/regeneration was higher than in the controls. These effects were not observed after the 4 week recovery period.

Plasma concentrations of lutein and zeaxanthin were determined in weeks 8 and 13 and found to be comparable at these time points. Lutein plasma concentrations showed a dose-related and under-proportional increase in both sexes resulting in 5-10 times higher values. Liver concentrations of lutein and zeaxanthin were also dose-related increased and revealed high inter-individual differences (up to 20 times), in particular in females. After the 4-week recovery period a decrease in the concentrations of both substances was observed in males but not in females. This was attributed to 2 females with very high values in the high-dose group.

According to the study report (and JECFA) the NOEL (no-observed effect level) in this study was 200 mg lutein/kg bw per day (208 mg of lutein + zeaxanthin/kg bw per day), the highest dose tested.

In a 52-week feeding study with cynomolgus monkeys complying with GLP principles, which was designed primarily to examine potential effects on the eyes, groups of 2 male and 2 female animals received doses of 0.2 or 20 mg zeaxanthin/kg bw/day by gavage (a beadlet formulation containing 10 % zeaxanthin). The control group received beadlets without zeaxanthin. An additional group of 1 male and 1 female animal received 20 mg zeaxanthin/kg bw/day for 26 weeks.

All animals in the high dose group showed an orange-yellow discolouration of the faeces. Food intake and body weight gain were comparable in all groups. In the haematology, clinical-chemistry and urine analyses, organ weight determinations and histopathological examinations no toxicologically relevant effects were observed. The animals showed a dose-related increase

in zeaxanthin levels in plasma and liver. Most animals showed a yellow discolouration of the mesenterial adipose tissue.

Determination of the zeaxanthin content in the eyes of animals of the high dose group after 52 weeks using HPLC analysis revealed a higher content in the macula lutea (38 versus 8 ng/macula), in the peripheral area of the retina (70 versus 2 ng/peripheral retina) and in the lens (0.8 versus 0.1 ng/lens) when compared with the placebo group.

Extensive eye examinations were carried out including ophthalmoscopy and biomicroscopy examinations, fundus photography and electroretinography (ERG). *Post mortem* examinations of the retina included macroscopic inspection, microscopic pathology under polarised and bright light for peripheral retina and macula, confocal microscopy of the macula and histopathology examination of the peripheral retina. According to two expert opinions provided by the applicant there was no evidence for treatment-related adverse changes in the eyes of the animals, in particular, there were no indications of crystal formation in the eyes.

Chronic toxicity and carcinogenicity

No studies on chronic toxicity and carcinogenicity have been performed.

Reproductive and developmental toxicity

A study on embryotoxicity and teratogenicity in rats complying with GLP was provided. Groups of 36 mated Füllinsdorf albino rats received doses of 0 (control), 250, 500 and 1000 mg zeaxanthin/kg bw/day from day 7 through day 16 of gestation. Zeaxanthin was administered in the form of beadlets (containing 9.4 % zeaxanthin) in the diet. By addition of placebo beadlets all diets contained the same amount of beadlets. On day 21 of gestation 2 subgroups were formed. The dams of one subgroup were killed on day 21 and the uteri were examined for implantations and resorptions. Foetuses from 15 litters per group were weighed and examined for skeletal or soft tissue malformations. The dams of the second subgroup reared their young up to weaning. Litter size was registered and all animals were weighed. At day 23 of lactation the young were killed and examined for abnormalities. According to the study report, there were no signs of maternal toxicity. The weight development of the dams was similar in all groups. No relevant differences between groups were found in the resorption rate, average litter size, mean body weight of live foetuses and the survival rate of the foetuses. The skeletal and soft tissue examinations as well as the examinations of body functions in the rearing subgroup did not reveal relevant abnormalities. One foetus in the high-dose group showed severe malformations, which can be regarded as an isolated finding not related to treatment. There were no indications of embryotoxic or teratogenic effects up to the highest dose of 1000 mg zeaxanthin/kg bw.

In a similar study complying with GLP groups of 20 mated Füllinsdorf albino rabbits received by gavage daily doses of 0, 100, 200 and 400 mg/kg bw of zeaxanthin in seed oil from day 7 through day 19 of gestation. The test material was not further specified. All rabbits were killed on day 30 of gestation and the uteri were examined for the number and location of implantations and resorptions. The foetuses were weighed and examined for viability, soft tissue and skeletal malformations. According to the study report, there were no signs of maternal toxicity. Although a statistical analysis of the relevant body weight data was not provided, it was shown that the weight development of the dams in the high-dose group was similar to that of the control group. There were no relevant differences in the resorption rate,

the average litter size, the mean body weight of live foetuses and the survival rate of the foetuses. Some foetuses with malformations were identified. Since the effects did not show a consistent pattern and were distributed among all groups including the controls they are not considered toxicologically relevant. There were no indications of embryotoxic or teratogenic effects up to the highest dose of 400 mg zeaxanthin/kg bw.

Genotoxicity

The potential of zeaxanthin to induce gene mutations was examined in the Ames test using *Salmonella enterica* Typhimurium strains TA1535, TA1537, TA1538, TA97, TA98, TA100 and TA102. Due to solubility problems (the substance was dissolved in DMSO but precipitated upon addition to the aqueous medium at concentrations of approximately 50 µg/plate) zeaxanthin (> 96 % purity) was tested at a concentration range of 2.4-1500 µg/plate using the plate incorporation method and 5-500 µg/plate using the pre-incubation method. Zeaxanthin did not induce an increase in the number of revertant colonies with and without metabolic activation (S9-mix) while the responsiveness of the tester strains and the activity of the S9-mix were verified by including appropriate controls.

In tests on gene mutations using mammalian cells (V79/HGPRT-test), no mutagenic activity up to the highest tested concentration of 16 µg/ml with and without metabolic activation (S9-mix) was found.

In tests on unscheduled DNA synthesis (UDS-test) using freshly isolated primary rat hepatocytes, zeaxanthin did not induce DNA repair synthesis up to the highest tested concentration of 16 µg/ml.

In tests on chromosomal aberrations using human peripheral blood lymphocytes an increase in the number of cells with chromosome aberrations was found at the highest tested concentration of 70 µg/ml without metabolic activation (S9-mix). This effect was not confirmed when the test without S9-mix was repeated using concentrations up to 80 µg/ml. In the presence of S9-mix there was no increase in chromosomally aberrant cells up to the highest concentration of 120 µg/ml. A second study carried out by a different laboratory did not show an increased number of cells with chromosome aberrations in the presence of S9-mix up to the highest dose of 120 µg/ml. However, in this study test was incomplete in that the respective test without S9-mix was not conducted. These studies were not carried out according to OECD Guideline 473 with regard to the duration of exposure, duplication of cultures and determination of cytotoxicity of the test material.

In an *in vivo* micronucleus assay not complying with GLP regulations, mice received by gavage aqueous suspensions of a 10 % water-soluble beadlet formulation with an actual zeaxanthin-content of 8.9 %. Doses of 44.5, 89.0 and 178.0 mg zeaxanthin/kg bw were administered twice, i.e. 30 and 6 hours prior to sacrifice of the animals. The analysis of polychromatic erythrocytes of the bone marrow did not reveal an increased number of micronuclei up to the highest tested dose of 178 mg/kg bw administered twice. However, the study was not conducted according to the current standard (OECD Guideline 474) with regard to the timing of dosing and bone marrow sampling as well as data presentation and evaluation. In particular the numbers of immature erythrocytes among total erythrocytes was not considered in the study results.

Structural similarity to β-carotene

In view of the structural similarities between zeaxanthin and β-carotene, JECFA (WHO, 2006a) considered the outcome of two trials that showed that supplementation with β-carotene increases risk of lung cancer in heavy smokers (Omenn *et al*., 1996; The α-Tocopherol and β-Carotene Cancer Prevention Study Group, 1994). JECFA concluded that the available data indicated that zeaxanthin in food would not be expected to have similar effect. However, JECFA was unable to assess whether zeaxanthin in the form of supplements would have the reported effect in heavy smokers.

Several hypotheses have been proposed to explain the effects of β-carotene in association with cigarette smoke (Palozza et al., 2006). The carotenoid may (i) activate and/or induce phase I carcinogen-bioactivating enzymes, including activators of cigarette smoke carcinogens, such as polycyclic aromatic hydrocarbons (Paolini et al., 1999, 2001, Fuster et al., 2008); (ii) decrease the absorption of other carotenoids with better antioxidant profile (Grievink et al., 1999); and (iii) induce at high doses the formation of metabolites, which may cause diminished retinoic acid signalling by down-regulating RARβ expression and up-regulating AP-1 with consequent possible acceleration of lung tumorigenesis (Wang et al., 1999; Crowe et al., 2003). A further hypothesis is linked to pro-oxidant characteristics of β-carotene (Handelman et al., 1996; Palozza et al., 1998, 2004) with the pro-oxidant character further enhanced by a high concentration. It has been shown using rat lung microsomal membranes that β-carotene auto-oxidation measured as 5,6-epoxy-β,β-carotene was faster at high (100-150 mmHg, in lung) than at low partialO_2 (15 mmHg). Thus β-carotene may enhance cigarette smoke-induced oxidative stress and exert potential deleterious effects at the pO_2 normally present in lung tissue (Palozza 2006). The loss of the antioxidant effects of β-carotene in lung membranes under relatively high pO_2 could be due to an increased formation of β-carotene peroxyl radicals (Burton and Ingold, 1984). Several studies show that tobacco smoke oxidizes β-carotene resulting in different oxidation products such as 4-nitro-β-carotene, β-apocarotenals, and β-carotene epoxides (Arora et al. 2001, Baker et al. 1999, Wang et al., 1999,).

There are differences between zeaxanthin and β-carotene regarding their chemical structure, stability, absorption, metabolism and functionality. Zeaxanthin is an oxygenated carotenoid with a more hydrophilic nature than β-carotene. Zeaxanthin is reported to be more stable than β-carotene or lycopene to pro-oxidant exposure *in vitro* (Siems et al., 1999), differing from β-carotene also with a much lower or absent pro-oxidant potential (Beutner et al., 2001, Martin et al., 1999). In contrast to β-carotene, zeaxanthin is not a provitamin A active carotenoid and thus retinoic acid is not a metabolite of zeaxanthin. Zeaxanthin is metabolized similarly to lutein by different oxidation-reduction reactions leading to formations of 3´-epilutein, mesozeaxanthin, and various mono-and diketo-carotenoids. Regarding accumulation in humans and other species (e.g. rats, monkey) a distinct feature is the accumulation of zeaxanthin in the eye tissue. However, there are no studies on the effects of zeaxanthin on the enzymes of xenobiotic metabolism, nor has zeaxanthin been tested in the tobacco smoke exposed ferret animal model which was used to study the enhancement of lung metaplasia by ß-carotene.

Allergenicity

There are no reports on allergic reactions due to zeaxanthin.

DISCUSSION

Data on the intake of zeaxanthin from foods based on current and representative dietary surveys in EU Member States have not been provided. According to an assessment by the applicant, which was largely based on food intake data from specific populations, the average dietary intake of zeaxanthin in European countries is between 0.2 and 0.9 mg/day and for people with a high intake, i.e. those consuming diets which include high amounts of zeaxanthin-rich vegetables and fruits, up to 1.8 mg/day (95th percentile). On this basis the proposed level of use would lead to intake levels that would substantially increase the dietary intake of zeaxanthin resulting in 22-100 times higher intakes for adults compared with the estimated average intake from natural sources in European countries. Compared with individuals consuming diets with high amounts of zeaxanthin-rich vegetables and fruits, the intake would be 11 times higher. Compared with individuals consuming exceptionally high amounts, e.g. up to 4 mg zeaxanthin/day as reported for specific populations, the intake would be 5 times higher.

Synthetic zeaxanthin has not previously been available on the European market. No reliable data are available concerning consumption of zeaxanthin-containing food supplements. According to the applicant, the resulting levels generally do not exceed 2 mg/day. However, supplements have been available in the US with recommended daily dose of 10 mg. According to the applicant, synthetic zeaxanthin is intended to be consumed at levels of up to 20 mg/person/day as an ingredient in food supplements.

No mutagenicity was observed in tests on gene mutations in bacteria (Ames test) and mammalian cells (HGPRT test). *In vitro* tests on chromosomal aberrations and an *in vivo* micronucleus test had some shortcomings but did not provide evidence for genotoxicity. The Panel notes that the concentrations and doses of synthetic zeaxanthin used in the genotoxicity studies were generally low due to solubility reasons.

Toxicity studies in rodents, dogs and monkeys showed no relevant effects other than discolouration of the faeces and adipose tissue as well as increases in plasma and liver zeaxanthin concentrations. These were not considered toxicologically significant. In studies on embryotoxicity and teratogenicity using rats and rabbits there were no indications of maternal toxicity, embryotoxic or teratogenic effects. No studies on chronic toxicity and carcinogenicity have been performed using synthetic zeaxanthin, the toxicological data are therefore not sufficient to derive an acceptable daily intake (ADI).

No zeaxanthin-related adverse effects were found in studies provided by the applicant with healthy human volunteers who took daily doses of 10-30 mg zeaxanthin up to 4-6 months.

Similarities between zeaxanthin and β-carotene were considered as it has been shown that supplementation with β-carotene increases risk of lung cancer in heavy smokers. While the available data indicate that zeaxanthin in food would not be expected to have similar effect there are no data concerning synthetic zeaxanthin in the form of supplements. There are differences between zeaxanthin and β-carotene regarding their chemical structure, stability, absorption, metabolism and functionality. However, no studies on the effects of zeaxanthin on the enzymes of xenobiotic metabolism have been provided, nor has zeaxanthin been tested in appropriate animals with exposure to tobacco smoke which was used to study the enhancement of lung metaplasia by ß-carotene.

CONCLUSIONS AND RECOMMENDATIONS

The proposed levels of use of up to 20 mg/person/day of synthetic zeaxanthin as an ingredient in food supplements would lead to intake levels that would substantially increase the average dietary intake of synthetic zeaxanthin resulting in up to 100 times higher intakes for adults. These intake levels are within the range of the group ADI 0-2 mg/kg bw for lutein and synthetic zeaxanthin as established by JECFA. However, in the opinion of the Panel the toxicological data on zeaxanthin are not sufficient to derive an acceptable daily intake.

On the basis of available data, it is not possible to assess whether additional synthetic zeaxanthin at the proposed level of use would increase the risk of lung cancer in heavy smokers as reported for b-carotene.

Based on the existing data, the Panel concludes that the safety of zeaxanthin as an ingredient in food supplements at the proposed use level of up to 20 mg/person/day has not been established.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission to the Chairman of the European Food Safety Authority with the request for an opinion on the safety of 'Zeaxanthin as an ingredient in food supplements'. SANCO E4/AK/bs (2007) D/540182.
2. Initial assessment report by the Bureau Nieuwe Voedingsmiddelen (NL) concerning the assessment of 'Zeaxanthin as an ingredient in food supplements'.
3. Letters from Member States with comments on the initial assessment report on 'Zeaxanthin as an ingredient in food supplements' from Bureau Nieuwe Voedingsmiddelen (NL).
4. Response to Member States comments on the Netherland Opinion for 'Zeaxanthin as an ingredient in food supplements' as a novel food ingredient.
5. Application under regulation No 258-97 for the use of 'Zeaxanthin as an ingredient in food supplements' as a novel food ingredient.

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