European Food Safety Authority

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SCIENTIFIC OPINION

Safety of use of colouring agents in animal nutrition[1]

Part III: β-apo-8'-carotenal, ethyl ester of β-apo-8'-carotenoic acid, lutein, zeaxanthin and concluding remarks

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2003-060)

Adopted on 12 May 2009

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of use of colouring agents in animal nutrition.

β-apo-8'-carotenal (trans-β-apo-8'-carotenaldehyde, known also as CI Food Orange 6) occurs abundantly in nature, although in very low concentrations.

The effective feed concentration to colour egg yolk is 40 mg β-apo-8'-carotenal kg^{-1} complete feed for laying hens. However, when a red-pigmenting carotenoid like canthaxanthin is additionally used, the same effect will be reached with 10 mg β-apo-8'-carotenal kg^{-1} complete feed. Data for skin pigmentation are not available.

Very limited information is available concerning the metabolic fate of β-apo-8'-carotenal in poultry. Dietary β-apo-8'-carotenal is deposited in the egg yolk as its oxidation product β-apo-8'-carotenoic acid. No further β-oxidation of the unsaturated side chain of β-apo-8'-carotenoic acid occurs.

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* One member of the Panel did not participate in the discussion on the subject referred to above because of possible conflicts of interest.

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Data on the safety of β-apo-8'-carotenal for the target animals is not available. However, given the natural occurrence of the compound and considering the molecular structure of the carotenoid, the Panel on Additives and Products or Substances use in Animal Feed (FEEDAP) does not see reasons for concern.

Several *in vitro* studies, performed with β-apo-8'-carotenal in both prokaryotic and eukaryotic test systems, do not give rise to safety concerns with respect to the genotoxicity of the compound.

Based on the limited and not recent dataset, no toxic effects have been reported in humans or in animals following oral exposure. Available data do not raise concern at doses of 100 mg kg^{-1} bw of β-apo-8'-carotenal given for 34 weeks in rats and at approximately 50–60 mg kg^{-1} bw day^{-1} for 14 weeks in dogs, the highest doses studied. In a multi-generation rat study, 5000 mg β-apo-8'-carotenal kg^{-1} diet did not induce reproductive toxicity.

If 10 mg β-apo-8 kg^{-1} feed (together with a red-pigmenting carotenoid) is taken as a realistic feed concentration, a worst case intake of eggs of 100 g (containing 27 g of egg yolk) would lead to a daily intake of 0.010 mg kg^{-1} bw per day for a 60 kg person, most likely as β-apo-8-carotenoic acid, which can be compared to 0.053 mg β-apo-8 kg^{-1} bw per day from the consumption of 0.5 L orange soft drinks. Residues present in eggs would not be essentially different from those formed in laboratory animals and humans in the metabolism of β-apo-8'-carotenal. The FEEDAP Panel therefore concludes that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8'-carotenal supplemented diets.

Data to estimate human exposure from poultry tissues is not available. However, safety concerns are not likely.

Data for the safety for the user is not available. A skin sensitisation study indicated that a sensitisation potential was unlikely.

Ethyl ester of β-apo-8'-carotenoic acid (known also as CI Food Orange 7) is available from chemical synthesis. It is an effective colourant for both egg yolk and broiler skin pigmentation. To obtain intensively coloured egg yolk, 4–10 mg β-apo-8'-carotenoid acid ethyl ester together with a red pigment are required. For skin colouration, at least 30 mg β-apo-8'-carotenoid acid ethyl ester alone or 15 mg β-apo-8'-carotenoid acid ethyl ester together with a red pigment are required.

Very limited information is available concerning the metabolic fate of β-apo-8'-carotenoid acid ethyl ester in poultry. It is deposited in the egg of laying hens. The hydrolysis of the ester occurs at a limited extent.

Data on the safety of β-apo-8'-carotenoid acid ethyl ester for the target animals is not available. However, given the molecular structure of the carotenoid and considering the similarity in the metabolism of β-apo-8'-carotenoid acid ethyl ester and β-apo-8'-carotenal, the FEEDAP Panel does not see reasons for concern.

The FEEDAP Panel confirms the conclusion of the SCF, according to which there are too few data to conclude on the safety of β-apo-8'-carotenoid acid ethyl ester for the consumer. However, it must be noted that none of the available data indicate reasons for concern.

Eggs and edible poultry tissues are likely to be the primary contributors to the human intake of β-apo-8'-carotenoid acid ethyl ester. A daily exposure of 0.27 mg per person can be calculated, equivalent to 0.0045 mg β-apo-8'-carotenoid acid ethyl ester kg^{-1} bw.

A worst case intake would be based on a supplementation level of 40 mg β-apo-8'-carotenoid acid ethyl ester kg^{-1} feed for laying hens and for chickens for fattening. The total intake from

egg and skin/fat consumption would be 0.08 mg kg^{-1} bw. Refining egg and skin/fat consumption results in 0.034 mg kg^{-1} bw.

Lutein (3R,3'R,6'R)-β,ε-carotene-3,3'-diol) and **Zeaxanthin** (all-trans-(3R,3'R)-β-carotene-3,3'-diol) occur closely associated at varying ratios (about 1:0.1–0.8) in feedingstuffs and additives. A separate assessment of the pigmenting efficiencies of lutein and zeaxanthin under practical feeding conditions is therefore not possible. Both xanthophylls alone are effective in colouring yolk and skin of chickens for fattening.

Lutein or zeaxanthin supplementations are only seldom calculated as such but rather as total xanthophylls. Poultry diets rich in corn and corn products may contain about 10 mg lutein and about 5 mg zeaxanthin, and supplemented diets about 20–30 mg lutein kg^{-1} and 8–12 mg zeaxanthin kg^{-1}. Those dietary levels may lead to egg yolk concentrations of 13–25 mg lutein kg^{-1} and 8–10 mg zeaxanthin kg^{-1}.

Lutein is absorbed in the small intestine, zeaxanthin predominantly in the ileum. Both xanthophylls enter the blood stream in its free form and are re-esterified when entering the target cells. No cleavage of the isoprenoic chain occurs. Metabolisation is characterised by the oxidation of the hydroxyl groups. In humans, the interconversion of both xanthophylls is observed.

Data on the safety of lutein and zeaxanthin for the target animals are not available. However, given the widespread natural occurrence of the compounds and considering the molecular structure of the xanthophylls, the FEEDAP Panel does not see any reason for concern.

Lutein is not mutagenic, based on the limited studies available. In repeated dose studies on rats, lutein did not indicate adverse effects at the highest doses tested (600 mg kg^{-1} bw in a four-week study, 200 kg^{-1} bw in a 13-week study). **Zeaxanthin** is not mutagenic and there is no evidence for genotoxity, embryotoxicity or teratogenicity. In 13-week toxicity studies, zeaxanthin did not indicate either adverse effects at the highest doses tested (100 mg kg^{-1} bw in rats, 422 mg kg^{-1} bw in beagle dogs). From a one-year monkey study, it could be concluded that 20 mg zeaxanthin kg^{-1} bw day^{-1} does not cause adverse effects in the eyes. A group ADI of 0–2 mg kg^{-1} bw day^{-1} for lutein (from *Tagetes erecta*) and synthetic zeaxanthin has been established by JECFA, but not confirmed by the NDA Panel.

The human intake of **lutein** (including the consumption of eggs and edible poultry tissues) has been estimated by EFSA's former scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) at 0.8–2.5 mg day^{-1} and is considered not to present any safety concern. The highest reported human exposure to **zeaxanthin** is 1.8 mg day^{-1}, corresponding to about 0.03 mg kg^{-1} bw. This is less than 2 % of the JECFA group ADI for lutein and zeaxanthin.

Lutein intake from organic eggs would correspond to 0.17 mg day^{-1} and from cage eggs to 0.06 mg day^{-1}. The contribution of eggs to the maximum total human intake (2.5 mg) is then calculated to be between 2 % (cage husbandry) and 7 % (organic production).

Zeaxanthin intake from organic eggs would correspond to 0.1 mg day^{-1} and from cage eggs to 0.03 mg day^{-1}. The contribution of eggs to the maximum total human intake (1.8 mg) is then calculated to be between 2 % (cage husbandry) and 6 % (organic production).

No data is available to asses the lutein and zeaxanthin intakes from edible poultry tissues.

Taking into account the human lutein and zeaxanthin intake from all sources, the contribution from food of animal origin (eggs and poultry tissues produced with lutein- and zeaxanthin-containing diets) would be a very small proportion of the total intake which varies with the consumption pattern in different countries. It does not require a particular safety assessment.

β-apo-8’-carotenal, β-apo-8’-carotenoid acid ethyl ester, lutein and zeaxanthin are natural substances or with close structural relation (e.g. esters). Taking also into account the susceptibility to degradation of carotenoids, the FEEDAP Panel considers it unlikely that the use of those substances as feed additives would pose a risk for the environment.

General remarks and recommendations

The FEEDAP Panel recommends to introduce specifications for each carotenoid/xanthophyll and to adjust/supplement the existing specifications.

The FEEDAP Panel recommends to exclude β-carotene from the list of colouring carotenoids/xanthophylls and to introduce maximum contents for the individual carotenoids/xanthophylls.

The consumer safety of canthaxanthin is based on an ADI. A safety evaluation of astaxanthin could be completed after setting an ADI.

At present, an ADI cannot be allocated to the carotenoids/xanthophylls because of the withdrawal of the previous group ADI for β-carotene, including carotenoids/xanthophylls. As long as no other (structurally related to β-carotene) carotenoids/xanthopylls have been tested, the conclusion on consumer safety based on total exposure will retain a degree of uncertainty. The FEEDAP Panel recommends therefore that the maximum content authorised for the individual carotenoids/xanthophylls should be adjusted to the maximum levels required to obtain the desired effect in animal products, considering also the combined use of red and yellow carotenoids.

Key words: sensory additives, colourants, carotenoids, xanthophylls, β-apo-8’-carotenal, ethyl ester of β-apo-8’-carotenoic acid, lutein, zeaxanthin, pigments, egg yolk colour, skin pigmentation

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BACKGROUND AS PROVIDED BY EC

In its opinion of April 2002 on the use of canthaxanthin in feedingstuffs, the SCAN (EC, 2002) suggested that the required levels of canthaxanthin should be reviewed in order that human exposure to canthaxanthin remains within the Acceptable Daily Intake (ADI) established for that compound. The lowering of the levels of this pigment would lead to an increasing use of alternative colorants.

Other substances are indeed authorised for use in feedingstuffs as colouring agents, as described in the table hereafter. In its opinion on canthaxanthin, as well as in the report on an astaxanthin-rich product, the SCAN drew the attention of the European Commission to the fact that no risk assessment has ever been carried out and that no ADI has ever been established for carotenoids other than canthaxanthin.

TERMS OF REFERENCE AS PROVIDED BY EC

In the light of these opinions on some of the colouring agents, EFSA is asked to assess the safety of use of capsanthin (E160c), β-apo-8'-carotenal (E160e), ethyl ester of β-apo-8'carotenoic acid (E160f), lutein (E161b), cryptoxanthin (E161c), zeaxanthin (E161h), citranaxanthin (E161i), astaxanthin (E161j) in feedingstuffs for laying hens, other poultry, salmon, trout, on the basis of currently available scientific literature.

In making its assessment, EFSA is requested to prioritise the substances that may be used as alternatives to canthaxanthin.

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Table 1. **Annex Entry**

EC No.	Additive	Chemical formula, descrip-tion	Species or category of animal	Maxi-mum age	Mini-mum content	Maximum content	Other provisions	End of period of authorisation
					mg kg^{-1} of complete feedingstuff			
Colourants including pigments								
1.Carotenoids and xanthophylls								
E 160c	Capsanthin	$C_{40}H_{56}O_3$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 160e	Beta-apo-8'-carotenal	$C_{30}H_{40}O$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 160f	Ethyl ester of beta-apo-8'-carotenoic acid	$C_{32}H_{44}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161b	Lutein	$C_{40}H_{56}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161c	Cryptoxanthin	$C_{40}H_{56}O$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161h	Zeaxanthin	$C_{40}H_{56}O_2$	Poultry	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161i	Citranaxanthin	$C_{33}H_{44}O$	Laying hens	-	-	80 (alone or with the other carotenoids and xanthophylls)	-	Without a time limit
E 161j	Astaxanthin	$C_{40}H_{52}O_4$	Salmon, trout	-	-	100	Use only permitted from the age of 6 months onwards. The mixture of astaxanthin with canthaxanthin is allowed provided that the total concentration of the mixture does not exceed 100 mg kg-1 in the complete feedingstuff.	Without a time limit
			Ornamental fish	-	-	-	-	Without a time limit

ASSESSMENT

On request from the European Commission, the FEEDAP Panel first assessed and adopted an opinion on astaxanthin (Part I), then on the other red-colouring carotenoids (capsanthin, citranaxanthin, and cryptoxanthin) (Part II). In the present opinion, the FEEDAP Panel focuses on the yellow-colouring carotenoids: β-apo-8'-carotenal, ethyl ester of β-apo-8'-carotenoic acid, lutein and zeaxanthin (Part III).

In egg yolk colouring, yellow and red pigments are usually supplemented together if a DSM-YCF > 11 is required (see Part I, Section 1.6). For skin colouration, yellow pigments may also be used alone.

Generally, carotenoids are now analysed and efficiently separated by HPLC. This technique, however, has only been available since the late seventies, thus in many earlier studies it has not been possible to separate certain carotenoids such as lutein and zeaxanthin or apo-carotenals. This has to be considered when interpreting older results.

1. β-apo-8'-carotenal (E 160e)

1.1. Specifications

The specification of the European Union on the colouring compound β-apo-8'-carotenal (known also as CI Food Orange 6) applies predominantly to all trans isomers of β-apo-8'-carotenal together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from β-apo-8'-carotenal meeting those specifications and include solutions or suspensions of β-apo-8'-carotenal in edible fats or oils, emulsions and water dispersible powders. Those preparations may have different cis/trans isomer ratios.

Preparations should contain not less than 96 % of the total colouring matters as β-apo-8'-carotenal. However, the percentage of the total colouring matters is not specified.

The EU specification also reports information on the following contaminants: sulfated ash (not more than 0.1 % of total colouring matters), carotenoids other than β-apo-8'-carotenal (not more than 3.0 %), arsenic (not more than 3 mg kg^{-1}), lead (not more than 10 mg kg^{-1}), mercury and cadmium (not more than 1 mg kg^{-1}) and other heavy metals (not more than 10 mg kg^{-1}).

1.2. General characteristic

β-apo-8'-carotenal (β-apo-8) is found in abundance in nature in different vegetables, citrus fruits and fodder plants (lucerne, alfa alfa meal, grass), but generally at low concentrations. Its most common source is oranges and tangerines. The predominant source in animal nutrition is the synthetically produced compound. The carotenoid is mainly in the all trans form.

β-Apo-8'-carotenal (trans-β-apo-8'-carotenaldehyde) (Figure 1), CAS number 1107-26-2 (the corresponding number in EC database is 214-171-6), has a molecular formula of $C_{30}H_{40}O$ and a molar mass of 416.65 g mol^{-1}.

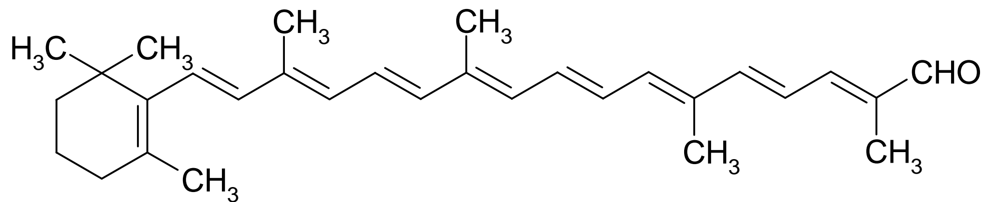

Figure 1. **Molecular structure of β-apo-8'-carotenal**

The melting point of β-apo-8 is 136 ºC–140 ºC and a logP value of 7.685 is reported. β-apo-8 forms dark violet crystals with metallic lustre or crystalline powder. The maximum absorption occurs at a wavelength of 460–461 nm in cyclohexane. β-apo-8 is insoluble in cold water, slightly soluble in hot water and soluble in diethyl ether, acetone and cyclohexane.

In poultry, β-apo-8 is mainly deposited to yolks and tissues. β-apo-8 can at least partly be metabolised to vitamin A (Euler et al., 1938). The vitamin A activity of β-apo-8 is about 66 % of β-carotene (Hencken, 1992).

1.3. Analytical methods

The CRL reports that no ISO and CEN methods could be found as the official analytical method for the determination of β-apo-8 in feedingstuffs or other relevant matrices.

HPLC is currently the method of choice for carotenoid analysis since it gives the most accurate, sensitive and reproducible quantitative analyses of carotenoid content and composition. β-apo-8 was analysed by using TLC and HPLC with UV detection from red pepper (Minguez-Mosquera et al., 1995). Recently, β-apo-8 was analysed from retail foods and beverages by using HPLC and photodiode array (Scotter et al., 2003). The limit of detection (LOD) and the limit of quantification (LOQ) of β-apo-8 in their method were 0.01 and 0.1 mg kg^{-1}, respectively. The response was linear over the range of 0–50 mg L^{-1}. β-apo-8 was well separated from the trans and cis isomers of β-carotene.

1.4. β-apo-8'-carotenal in poultry feeding

In general, β-apo-8 is an effective carotenoid for egg yolk and broiler skin pigmentation (Marusich and Bauernfeind, 1970a; El Boushy and Raterink, 1992; Hencken, 1992; Steinberg et al., 2000).

1.4.1. Sources

The natural sources of β-apo-8 show too low concentrations for effective pigmentation of eggs and tissues. Therefore, synthetic products of β-apo-8 are used in poultry feeding.

The European Feed Manufacturers Federation (FEFAC) made available some non-quantitative market data on the use of β-apo-8, covering responses from nine EU Member States (MS) (FEFAC, 2004). According to those data, β-apo-8 is not used very frequently in the EU for egg yolk and poultry skin pigmentation. β-apo-8 is not used at all in four MS, by a small proportion of feed manufacturers in three MS and by half of the feed manufacturers in two other MS.

1.4.2. Pigmenting efficacy

The pigmenting effectiveness of β-apo-8 in relation to β-apo-8'-carotenoid acid ethyl ester (β-apo-8-ester) is given by El Boushy and Raterink (1992) as 1:1.9 by weight. Marusich and Bauernfeind (1970a,b) used β-apo-8 as beadlets (107 g kg^{-1} product) with and without combination with canthaxanthin for yolk pigmentation at supplementation levels of 2.5 to 60 mg kg^{-1} (Table 2). It is clearly demonstrated that yolk visual score is increasing with increasing supplementation level of β-apo-8, but the increase is more distinct in combination with canthaxanthin.

Data on the use of β-apo-8 in broiler pigmentation are not available. Based on the results from the use of β-apo-8'-carotenoic acid ethyl ester for broiler skin pigmentation (Marusich and Bauernfeind, 1970b), it can be estimated that supplementation levels of β-apo-8 of 20 to 40 mg kg^{-1} feed will result in a skin pigmentation sufficient for market requirements.

Table 2. **Yolk pigmentation of β-apo-8'-carotenal (Marusich and Bauernfeind, 1970a)**

Dose (mg kg^{-1})	β-apo-8'-carotenal*		β-apo-8'-carotenal + canthaxanthin (4:1)	
	µg $yolk^{-1}$	DSM-YCF	µg $yolk^{-1}$	DSM-YCF
2.5	125	7.0	117	10.0
5	236	9.0	211	11.9
10	365	10.2	569	14.0
20	765	11.6	1093	>15
40	1246	13.6	1528	>15
60	1735	14.8	2303	>15

* by colourimetric determination (A.O.A.C., 1960)

1.4.3. Deposition of β-apo-8'-carotenal in the egg and tissues

Deposition rates of β-apo-8 are available for egg yolk (Table 3), whereas no information was found for tissues.

Table 3. **Deposition rates of β-apo-8'-carotenal to egg yolks**

β-apo-8-carotenal	β-apo-8 in egg yolk (µg g^{-1})	Mean deposition rate (%)	Reference
1-4 mg hen^{-1} d^{-1}	18-101*	29.0	Marusich and Bauernfeind (1970a)
2.5-60 mg kg^{-1} feed	8-108*	30.9	Marusich and Bauernfeind (1970a)
-	-	37.0	Marusich and Bauernfeind (1981)

* calculated from original data by using a yolk weight of 16 g

The mean deposition rate of β-apo-8 into egg yolk can be assumed with 32 %. However, the deposition rate is dose-dependent and decreased from 40 % (2.5 mg β-apo-8 kg^{-1} diet) to 23 % (60 mg β-apo-8 kg^{-1} diet) (Marusich and Bauernfeind, 1970a). More recent findings (Schiedt et al., 1991; see Section 1.5.1), which show the deposition of dietary β-apo-8 as β-apo-8-carotenoic acid in the yolk, support the assumption that the colourimetric determination applied by Marusich and Bauernfeind in 1970 was not specific to β-apo-8.

1.5. Metabolism of β-apo-8'-carotenal

1.5.1. Poultry

Very limited information is available concerning the metabolic fate of β-apo-8 in animals, and especially poultry. Poultry is naturally exposed to β-apo-8 as it occurs in plants. But β-apo-8 is also formed endogenously as an intermediate of the conversion of β-carotene to vitamin A (Glover and Redfearn, 1954).

The transfer of β-apo-8 to the egg has been studied in groups of laying hens that received a dose of 10 mg β-apo-8 kg^{-1} diet (Schiedt et al., 1991). It was shown that (i) β-apo-8 was oxidised to β-apo-8'-carotenoic acid, (ii) only β-apo-8'-carotenoic acid was deposited in the egg which amounted to 10 % of the administered β-apo-8, (iii) no further β-oxidation of the unsaturated side chain of β-apo-8'-carotenoic acid occurred due to the presence of a methyl group in the α position of the carboxyl.

1.5.2. Human

β-apo-8 given to humans as a single oral dose was extensively metabolised mainly to the corresponding alcohol, acid and palmitate ester (JECFA, 1975).

1.6. Human intake estimates

Limited data from HPLC analysis of β-apo-8 in a variety of vegetables and fruits indicates that β-apo-8 is, if at all, present only in traces (Schweigert, 2007, personal communication). Natural sources are vegetables (citrus fruits). Major contributions to human intake come from the use of β-apo-8 as the food additive CI Food Orange 6. It is found (Scotter et al., 2003) for example in beverages (orange juice, ~7 mg kg^{-1}), confectionary (~4 mg kg^{-1}) and dried tomato soup (~3 mg kg^{-1}).

At a supplementation level of 10 mg kg^{-1} feed, 22.5 µg g^{-1} egg yolk could be expected (Table 2), but whether and to what extent β-apo-8 occurs as such in eggs is questionable. Based on the results of Schiedt et al. (1991), β-apo-8-carotenoic acid would occur in eggs and eggs products as a result of feeding of β-apo-8. Schlatterer and Breithaupt (2006) did not find β-apo-8 in eggs, in a field study with unknown supplementation levels of commercial eggs of different origin. No data are available for the content of β-apo-8 in edible tissues, particularly skin.

The very limited contribution of β-apo-8 to human consumption is reflected by the fact that this carotenoid has not been described so far in human plasma.

If 10 mg β-apo-8 kg^{-1} feed (together with a red-pigmenting carotenoid) is taken as a realistic feed concentration, a worst case intake of 100 g eggs (containing 27 g of egg yolk) would lead to a daily intake of 0.010 mg kg^{-1} bw per day for a 60 kg person, most likely as β-apo-8-carotenoic acid, which can be compared to 0.053 mg β-apo-8 kg^{-1} bw per day from consumption of 0.5 L orange soft drinks.

1.7. Safety studies

The JECFA (1974) evaluated β-apo-8 and β-apo-8-ester in 1974 and expressed an ADI of 0–5 mg kg^{-1} bw day^{-1} as the sum of β-apo-8 and β-apo-8-ester together with β-carotene. In 1975, the SCF endorsed the JECFA group ADI of 5 mg kg^{-1} bw day^{-1}.

Later on, the SCF (2000) decided to withdraw the previous ADI for β-carotene. The decision was based on the adverse effects of β-carotene observed in human studies conducted in male smokers and on the fact that the ADI relied on animal studies where the model had been shown to lack relevance with regard to human risk assessment.

As a consequence, an ADI for β-apo-8 and β-apo-8-ester does not exist any more because β-apo-8 and β-apo-8-ester were included in the former ADI.

1.7.1. Acute toxicity

JECFA (1974) reported an LD_{50} higher than 10 g kg^{-1} bw day^{-1} for β-apo-8 in mice.

1.7.2. Genotoxicity

Two studies were reported and assessed by BIBRA (1996). β-apo-8 was shown not to be mutagenic using the Ames test, with *Salmonella typhimurium*, and no genotoxicity was observed for β-apo-8 in cultured hamster lymphocytes exposed for 48 hours.

A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell lines was reported by Ishidate et al. (1988). On the basis of the evaluation of the results abstracted from the literature, they concluded that 'apocarotenal', assumed to be β-apo-8 (250 and 1000 µg mL^{-1}), is not clastogenic in mammalian cell cultures.

In a study on the antimutagenic and anticarcinogenic effects of carotenoids, Azuine et al. (1992) also tested the mutagenicity of β-apo-8 with and without metabolic activation in *Salmonella typhimurium* strains TA 98 and TA 100. β-apo-8 was not mutagenic in this test system.

Rauscher et al. (1998) examined β-apo-8 at concentrations up to 100 µg β-apo-8 per plate for mutagenicity in the histidine-deficient strains of *Salmonella typhimurium* TA98, TA 98NR and/or TA100. They found negative results.

The treatment of calf thymus DNA with β-apo-8 significantly increased the levels of mutagenic adducts (Marques et al., 2004). β-apo-8 induced DNA damage in a comet assay already at a concentration of 2 µM (Yeh and Wu, 2006). However, the relevance of those results to genotoxicity is not established.

Other mutagenicity studies in which β-apo-8 was tested focused on the antimutagenic and *in vivo* anticlastogenic effects of series of carotenoids (Shah et al., 1992; Gradelet et al., 1996, Gradelet et al., 1997, Gradelet et al., 1998; Rauscher et al., 1998; Durnev et al., 1999). β-apo-8 was reported to be a strong inducer of liver cytochromes P450 1A1 and 1A2 in rats (Gradelet et al., 1996). β-apo-8 (and other carotenoids) was shown to inhibit aflatoxin B_1-induced liver preneoplastic foci, DNA damage, carcinogenicity and genotoxicity in rats (Gradelet et al., 1997; Gradelet et al., 1998). The authors suggested that β-apo-8 (like other carotenoids) may modulate aflatoxin metabolism towards increased detoxification to less genotoxic products, like aflatoxin M_1.

In conclusion, several *in vitro* studies, performed with β-apo-8 in both prokaryotic and eukaryotic test systems, do not give rise to safety concerns with respect to the genotoxicity of the compound.

1.7.3. (Sub)chronic toxicity studies

The JECFA monograph (1975) summarises the results from anonymous studies conducted in 1962 and 1966 provided by a petitioner. After oral administration of β-apo-8 to monkeys (dose and duration not specified), an orange discolouration of liver and fat was observed (JECFA, 1975).

Groups of 16 male rats received 0 (control), 100 or 500 mg β-apo-8 kg^{-1} bw intragastrically five days per week for 34 weeks. No adverse effects were seen on body weight gain, general health survival, liver and kidney function and organ weights. The testicular weight of the high-level test group was significantly lower than that of the controls. Microscopic findings were normal except for granular pigment deposition in the liver and kidneys of the test animals. JECFA (1975) indicated that fertility was not affected. Because pigmentation is not considered as an adverse effect, a NOAEL of 100 mg β-apo-8 kg^{-1} bw can be deduced from this study.

Groups of two or three female dogs and three to four male dogs received daily 0, 100 mg or 1000 mg β-apo-8, administered by gelatine capsules, for 14 weeks (because bw was not given, an estimation based on liver weights resulted in approximate doses of 0, 5–6 and 50–60 mg kg^{-1} bw day^{-1}). All animals remained healthy and no significant effects were noted. No pathological lesions related to the test substance were seen post-mortem. Peripheral blood picture, liver function tests, serum enzymes, blood urea and organ weight were normal. The only microscopic finding was pigmentation of the adipose tissue, kidney and adrenal cortex (Bagdon et al., 1962).

1.7.4. Reproductive toxicity studies, including developmental toxicity

A three-generation study from 1966, conducted with rats at doses of 0, 1000, 2000 and 5000 mg β-apo-8 kg^{-1} diet for two years, showed no adverse effects in any generation (JECFA, 1975).

1.7.5. Human data

A study with 135 persons with urticaria or atopic dermatitis and 123 persons with contact dermatitis given oral doses of 100 mg β-apo-8 with 100 mg β-carotene showed no specific sensitazion response to β-apo-8 (study quoted by BIBRA, 1996).

1.8. Risk assessment

Several *in vitro* studies, performed with β-apo-8 in both prokaryotic and eukaryotic test systems, do not give rise to safety concerns with respect to the genotoxicity of the compound.

Based on the limited and not recent dataset, no toxic effects have been reported in humans or in animals following oral exposure. Available data do not raise concern at doses of 100 mg kg^{-1} bw of β-apo-8 given for 34 weeks in rats and of approximately 50–60 mg kg^{-1} bw day^{-1} given for 14 weeks in dogs, the highest doses studied. In a multi-generation rat study, 5000 mg β-apo-8 kg^{-1} diet did not induce reproductive toxicity.

Based on the assumption from older studies that β-apo-8 is deposited in the egg, the following scenario can be described. For DSM-YCF values of about 14, a concentration of β-apo-8 of 10 mg kg^{-1} feed (when given simultaneously with canthaxanthin in a ratio 4:1) or 40 mg kg^{-1} feed (when given alone) is required. Those dietary concentrations would result in 22.5 and 76.9 mg β-apo-8 kg^{-1} yolk, respectively (Table 2). A daily consumption of 100 g egg (Directive

2001/79/EEC[2]) would result in an intake of 0.608 and 2.066 mg β-apo-8, corresponding to 0.010 mg and 0.034 mg kg^{-1} bw, respectively. This intake is about 5000 and 1500, respectively, lower than the lowest NOAEL observed in toxicity studies with laboratory animals (50 mg kg^{-1} bw). The consumption of 0.5 L orange soft drink, which would result in an intake of 0.053 mg of β-apo-8 kg^{-1} bw, would amount to the 1.6 to 5.0 fold of that from 100 g egg.

Egg consumption refined on the basis of SCOOP data (EC, 2004) reduces human exposure to β-apo-8 from eggs to 0.004 and 0.012 mg kg^{-1} bw at feed concentrations of 10 and 40 mg β-apo-8, respectively. The safety factors would increase to 12500 and 4200, respectively.

Considering the direct intake of β-apo-8 from the use of this carotenoid as food additive and the rare use of the substance for egg yolk pigmentation in the EU Member States, the contribution from eggs of hens fed 10–40 mg β-apo-8 kg^{-1} diet to the total intake by the consumer appears rather small. The FEEDAP Panel therefore concludes that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8 supplemented diets.

Data to estimate human exposure from poultry tissues are not available. However, safety concerns are not likely.

More recent findings indicate that the consumer is not exposed to β-apo-8 in eggs from the use of this feed additive in laying hens. The use of β-apo-8 as CI Food Orange 6 appears to be the only source for consumer exposure apart from citrus fruits. Residues present in eggs would not be essentially different from those formed in laboratory animals and humans in the metabolism of β-apo-8. The FEEDAP Panel therefore concludes that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8 supplemented diets.

1.9. Environment

No data is available for a qualified assessment of the environmental impact of β-apo-8 used in poultry feed.

According to Directive 2001/79/EEC,[3] an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, the use of which will not alter the concentration or distribution in the environment. β-apo-8 occurs abundantly in plants and the terrestrial environment.

Given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers unlikely that the use of β-apo-8 in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

1.10. Conclusions and recommendations

1.10.1. Conclusions

β-apo-8 occurs abundantly in nature, although in very low concentrations.

The effective feed concentration to colour egg yolk up to a DSM-YCF of 14 is 40 mg β-apo-8 kg^{-1} complete feed for laying hens. However, when a red-pigmenting carotenoid like canthaxanthin is additionally used, the same effect will be reached with 10 mg β-apo-8 kg^{-1} complete feed. Data for skin pigmentation are not available.

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[2] OJ L 267, 06.10.2001, p. 1

[3] OJ L 267, 06.10.2001, p. 1

Very limited information is available concerning the metabolic fate of β-apo-8 in animals, and especially poultry. In poultry, dietary β-apo-8 is deposited in the egg yolk as such or, according to more recent studies, as its oxidation product β-apo-8'-carotenoic acid. No further β-oxidation of the unsaturated side chain of β-apo-8'-carotenoic acid occurs.

Data on the safety of β-apo-8 for the target animals is not available. However, given the natural occurrence of the compound and considering the molecular structure of the carotenoid, the FEEDAP Panel does not see reasons for concern.

Several *in vitro* studies, performed with β-apo-8 in both prokaryotic and eukaryotic test systems, do not give rise to safety concerns with respect to the genotoxicity of the compound.

Based on the limited and not recent dataset, no toxic effects have been reported in humans or in animals following oral exposure. Available data do not raise concern at doses of 100 mg kg^{-1} bw of β-apo-8 given for 34 weeks in rats and of approximately 50–60 mg kg^{-1} bw day^{-1} for 14 weeks in dogs, the highest doses studied. In a multi-generation rat study, 5000 mg β-apo-8 kg^{-1} diet did not induce reproductive toxicity.

Following the assumption that β-apo-8 is deposited in the egg as such, feeding the maximum proposed content of 40 mg β-apo-8 kg^{-1} diet would result in 76.9 mg kg^{-1} yolk. This would correspond to a daily intake of 2.07 mg from eggs of 100 g. A refinement of egg consumption according to SCOOP data results in a daily intake of 0.74 mg β-apo-8, corresponding to a daily intake of 0.012 mg kg^{-1} bw. This value is 4200 times lower than the lowest NOAEL.

Considering the direct intake of β-apo-8 from the use of this carotenoid as food additive and the rare use of the substance for egg yolk pigmentation in the EU Member States, the contribution from eggs of hens fed 10–40 mg β-apo-8 kg^{-1} diet to the total intake by the consumer appears rather small. The FEEDAP Panel therefore concludes that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8 supplemented diets.

More recent findings indicate that the consumer is not exposed to β-apo-8 in eggs from the use of this feed additive in laying hens. The use of β-apo-8 as CI Food Orange 6 appears to be the only source for consumer exposure apart from citrus fruits. Residues present in eggs would not be essentially different from those formed in laboratory animals and humans in the metabolism of β-apo-8. The FEEDAP Panel therefore concludes that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8 supplemented diets.

Data to estimate human exposure from poultry tissues are not available. However, safety concerns are not likely.

Data for the safety for the user are not available. A skin sensitisation study indicated that a sensitisation potential was unlikely.

No data is available for a qualified assessment of the environmental impact of β-apo-8 used in poultry feed. Considering the abundant occurrence of β-apo-8 in plants and the terrestrial environment, and the oxidative susceptibility of carotenoids, the FEEDAP Panel considers unlikely that the use of β-apo-8 in poultry feed at concentrations adequate for egg would affect the environment.

1.10.2. Recommendations

The FEEDAP Panel recommends to limit the maximum content of β-apo-8 to 40 mg kg^{-1} complete feed. Higher dosages will not result in any additional colouring effect.

Data on efficacy and deposition (of the substance and its metabolite) in edible poultry tissues (skin/fat) should be provided when the additive is intended to be applied for skin pigmentation during the re-evaluation process.

2. Ethyl ester of β-apo-8'carotenoic acid (E 160f)

2.1. Specifications

The specification of the European Union[4] on the colouring compound ethyl ester of β-apo-8'carotenoic acid (known also as CI Food Orange 7) applies predominantly to all trans isomers of ethyl ester of β-apo-8'-carotenoid acid, together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from ethyl ester β-apo-8'-carotenoic acid meeting those specifications and include solutions or suspensions of ethyl ester of β-apo-8'- acid in edible fats or oils, emulsions and water dispersible powders. Those preparations may have different cis/trans isomer ratios.

Preparations should contain not less than 96 % of the total colouring matters as ethyl ester of β-apo-8'-carotenoic acid. However, the percentage of the total colouring matters is not specified.

The EU specification also reports information on the following contaminants: sulfated ash (not more than 0.1 %), carotenoids other than ethyl ester of β-apo-8'carotenoic acid (not more than 3.0 % of total colouring matters), arsenic (not more than 3 mg kg^{-1}), lead (not more than 10 mg kg^{-1}), mercury and cadmium (not more than 1 mg kg^{-1}) and other heavy metals (not more than 10 mg kg^{-1}).

2.2. General characteristics

Significant natural sources of ethyl ester of β-apo-8'-carotenoic acid (β-apo-8-ester) have not been reported. Two products containing synthetic β-apo-8-ester are available on the market in powder and beadlet formulation for egg yolk and poultry tissue pigmentation.

β-apo-8-ester (β-apo-8'-carotenoid acid ethyl ester, ethyl 8'-apo-β-caroten-8'oate) (Figure 2), CAS number 1109-11-1 (the corresponding number in EC database is 214-173-7), has a molecular formula of $C_{32}H_{44}O_2$ and a molar mass of 460.70 g mol^{-1}.

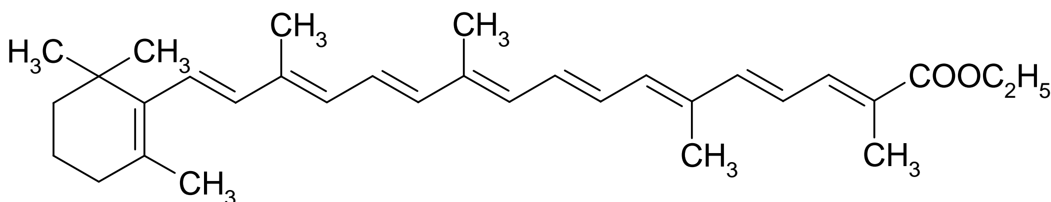

Figure 2. **Molecular structure of ethyl ester of β-apo-8'carotenoic acid**

A logP value of 8.317 is reported. Ethyl ester of β-apo-8'-carotenoid acid exists as red to violet crystals or crystalline powder. The maximum absorption occurs at 449 nm in cyclohexane. This synthetic carotenoid may have different cis/trans isomer ratios in various preparations.

The vitamin A activity of β-apo-8-ester is estimated as 25 % of β-carotene (Hencken, 1992). β-apo-8-ester shows antioxidant properties, has a positive impact on the immune system and is highly bioavailable (Adams, 2000).

[4] O.J. L 226, 22.9.1995, p. 1 (Directive 95/45/EC laying down specific purity criteria concerning colours for use in foodstuffs)

2.3. Analytical methods

The CRL reports that no ISO and CEN methods could be found as the official analytical method for the determination of β-apo-8-ester in feedingstuffs or other relevant matrices. However, a reference method for the determination of the β-apo-8-ester in concentrated butter and butter was established in 1996 (Commission Regulation (EC) No 1082/96).

β-apo-8-ester was analysed in human serum by using HPLC (Zeng et al., 1992). In egg yolks, β-apo-8-ester was determined by using HPLC and the positive identification of this particular carotenoid was accomplished using HPLC/API/MS (Schlatterer and Breithaupt, 2006).

2.4. Ethyl ester of β-apo-8'carotenoic acid in poultry feeding

2.4.1. Sources

Only synthetic β-apo-8-ester is used for egg yolk and poultry tissue pigmentation.

The European Feed Manufacturers Federation (FEFAC) made available some non-quantitative market data on the use of β-apo-8-ester covering responses from nine EU Member States (FEFAC, 2004). According to those data, β-apo-8-ester is used for laying hens by the majority of feed manufacturers in three MS, by about half of the feed manufacturers in two MS and in a small proportion in three MS. In one MS this carotenoid is not used at all.

For other poultry, β-apo-8-ester is used by a small proportion of the feed manufacturers in four MS. It is used by the majority of the feed manufacturers in one MS and by half of the feed manufacturers in two MS. In two other MS, the carotenoid is not used for skin pigmentation.

2.4.2. Pigmentation efficacy

In general, the β-apo-8-ester is an effective carotenoid for both egg yolk and broiler skin pigmentation (Marusich and Bauernfeind, 1970a; El Boushy and Raterink, 1992; Hencken, 1992; Steinberg et al., 2000; Sidibé, 2001; Fru et al., 2006a,b). The pigmenting efficacy of β-apo-8-ester is significantly higher than that of β-apo-8 (1.9:1; El Boushy and Raterink, 1992) and of *Tagetes* oleoresins, as a source of lutein and zeaxanthin (Sirri et al., 2006). This ratio can also approximately be confirmed (for dietary level > 10 mg kg^{-1}) when comparing the DSM-YCF values of Marusich and Bauernfeind (1970a) given in Table 2 and 4.

Table 4. **Yolk pigmentation of ethyl ester of β-apo-8'-carotenoic acid**

Dose (mg kg^{-1})	Ethyl ester of β-apo-8'-carotenoic acid		
	µg $yolk^{-1}$*	DSM-YCF*	µg $yolk^{-1}$**
2.5	211	7.8	-
5	381	9.2	259
10	675	10.8	496
20	1616	13.6	973
40	3161	>15	2301
60	4430	>15	-

* Marusich and Bauernfeind (1970a); ** Steinberg et al., 2000

From the data in Table 4, it is obvious that supplementation of the β-apo-8-ester alone is capable to produce intensively coloured egg yolks. It can be expected that 10 mg β-apo-8-ester kg^{-1} in combination with a red xanthophyll will result in DSM-YCF values of approximately

14. Balnave and Bird (1996) used 2.8 mg β-apo-8-ester kg^{-1} diet and found 10.8 µg β-apo-8-ester g^{-1} yolk, which is comparable to the results of Marusich and Bauernfeind (1970a; 211 µg $yolk^{-1}$ corresponds to 13.2 µg g^{-1} yolk). Steinberg et al. (2000) concluded as well that in combination with a red pigment, up to 10 mg β-apo-8-ester kg^{-1} feed will be sufficient for effective yolk pigmentation (DSM-YCF 13-15), but that for the production of liquid eggs higher supplementation levels will be necessary. On the contrary, Grashorn and Seehawer (1999) stated that 3 mg β-apo-8-ester kg^{-1} in combination with 4 mg canthaxanthin kg^{-1} will be sufficient for yolk pigmentation in fresh eggs (DSM-YCF 12-13), whereas for hard-boiled eggs at least 7 mg kg^{-1} should be used. The first finding is also confirmed by Sidibé (2001). However, even in combination with a red-pigmenting carotenoid, the level of β-apo-8-ester required for satisfactory yolk pigmentation depends to a high extent on the composition of the diet, particularly on the level of corn and corn gluten meal as potent sources of xanthophylls (Sidibé, 2001).

Shank pigmentation (Table 5) increased with the supplementation level of β-apo-8-ester. The additional supplementation of canthaxanthin resulted in a more intensive colour (ratio 3:1 or 4:1). For a skin colouration leading to a DSM-YCF > 10, at least 30 mg β-apo-8-ester have to be supplemented kg^{-1} feed, if used as single source of pigmenting carotenoids.

Table 5. **Colouring efficiency of ethyl ester of β-apo-8'-carotenoic acid in broilers. Shank colour (Marusich and Bauernfeind, 1970b)**

Dose (mg kg^{-1})	Ethyl ester of β-apo-8'-carotenoic acid (DSM-YCF)	Ethyl ester of β-apo-8'-carotenoic acid + canthaxanthin (4:1) (DSM-YCF)
7.5	7.0	-
15.0	9.4	12.0
30.0	10.8	12.4

2.4.3. Deposition of ethyl ester of β-apo-8'carotenoic acid in the egg and tissues

Deposition rates of β-apo-8-ester to egg yolk normally exceed 50 % (Table 6). According to Marusich and Bauernfeind (1970a) and Steinberg et al. (2000), the deposition rates did not decrease with increasing supplementation level (dietary ranges: 2.5–60 and 5–40 mg kg^{-1}, respectively).

Table 6. **Deposition rates of ethyl ester of β-apo-8'-carotenoid acid to egg yolks**

Supplementation level of ethyl ester of β-apo-8'-carotenoic acid	Ethyl ester of β-apo-8'-carotenoic acid in egg yolk (µg g^{-1})	Mean deposition rate (%)	Reference
2.5-60 mg kg^{-1}	13.2-277*	62.1	Marusich and Bauernfeind (1970a)
1and4 mg hen^{-1} d^{-1}	34.3 and 137*	54.7	Marusich and Bauernfeind (1970a)
2.8 mg kg^{-1}	10.8	50.5	Balnave and Bird (1996)
5.0-40 mg kg^{-1}	16.2-144	53.5	Steinberg et al. (2000)
0-40.5 mg kg^{-1}	-	51.0	Huyghebaert (2004)
2.5-5.0 mg kg^{-1}	9.2-18.0	62.3	Fru et al. (2006b)

* calculated from original data by using a yolk weight of 16 g; determination by colourimetric method

Only few studies using β-apo-8-ester for broiler skin pigmentation are available.

Fru et al. (2006a) compared a gelatine-based product of β-apo-8-ester with a gelatine-free preparation and observed comparable pigmentation efficiencies for broiler skin and shanks. The products were supplemented to a low xanthophyll diet in combination with 4 mg canthaxanthin kg^{-1}. Contents of β-apo-8-ester increased in skin and abdominal fat with increasing supplementation levels of this carotenoid (Table 7), reflecting a linear increase over the entire dose range (0–80 mg kg^{-1}). The authors did not calculate deposition rates.

Table 7. **Concentrations of carotenoids (mg kg^{-1}) in feed and skin (Fru et al., 2006a)**

Ethyl ester of β-apo-8'-carotenoic acid	Gelatine-based		Gelatine-free	
mg kg^{-1}	Skin	Abdominal fat	Skin	Abdominal fat
0	-	-	-	-
10	1.43	1.48	1.31	1.49
20	2.60	2.79	2.80	3.27
40	5.93	6.50	6.04	7.33
80	11.80	12.6	11.65	12.15

2.5. Metabolism of ethyl ester of β-apo-8'carotenoic acid

Very limited information is available concerning the metabolic fate of β-apo-8-ester in animals, especially poultry. Fru et al. (2006a) reported a high transfer rate of β-apo-8-ester from the intestine to the blood of broilers. Dietary supplementation levels of 10, 20, 40 and 80 mg kg^{-1} β-apo-8-ester corresponded to plasma levels of 5.34, 10.45, 21.70 and 42.25 mg L^{-1}.

Early findings suggest that β-apo-8-esters (ethyl and methyl esters) occur naturally in animal tissues as intermediates of the metabolism of β-apo-8 (Wiss & Thommen, 1963), which is confirmed by the more recent study of Schiedt et al. (1991). Laying hens were administered β-apo-8-ester at a dose of 10 mg kg^{-1} feed. The hydrolysis of the ester occurred at a limited extent. The deposition rate of β-apo-8-ester to the egg yolk amounted to 49 % of the administered dose, whereas only minor quantities (3–6 %) of the deposited carotenoid had been hydrolysed to β-apo-8'-carotenoic acid.

Without giving further information, JECFA mentioned that β-apo-8-ester is eliminated very rapidly from the blood of human infants (JECFA, 1975).

2.6. Human intake estimates

Significant natural sources of β-apo-8-ester have not been reported. The major contributions to the human intake of apo-esters come from animal products, especially eggs and egg products, and from foodstuffs, such as beverages and margarine, where it is used as pigment (CI Food Orange 7). In eggs depending on the feeding strategy, levels of up to 143 mg β-apo-8-ester kg^{-1} egg yolk and 43 mg β-apo-8-ester kg^{-1} liquid egg can be found (Steinberg et al., 2000). Schlatterer and Breithaupt (2006) determined, depending on the husbandry classes (ecological husbandry, cages), 6.7 to 8.1 mg β-apo-8-ester kg^{-1} yolk in a field study with unknown supplementation levels of ethyl ester of β-apo-8'-carotenoic acid. Based on the results of that field survey, a maximum level of 10 mg kg^{-1} egg yolk appears realistic, which would correspond to a supplementation level of 2.5 mg β-apo-8-ester kg^{-1} feed.

Assuming an intake of 100 g of egg containing 27 g of egg yolk, a daily intake of 0.27 mg per person per day can be calculated, which is equivalent to 0.0045 mg β-apo-8-ester kg^{-1} bw per day for a 60 kg person. Vegetables and fruits do apparently not contribute to the human intake of β-apo-8-ester as indicated by a limit screening (Schweigert 2007, personal communication;

unpublished data). Intake data for the consumption of foodstuffs such as beverages and margarine supplemented with CI Food Orange 7 are not available.

A worst case intake would be based on a supplementation level of 40 mg β-apo-8-ester kg^{-1} feed for laying hens and for chickens for fattening. Eggs of 100 g (containing 27 g of egg yolk) would lead to a daily intake of 0.066 mg kg^{-1} bw for a 60 kg person, 90 g skin/fat would lead to a daily intake of 0.01 mg kg^{-1} bw, resulting in a total intake from food of poultry origin of 0.08 mg kg^{-1} bw.

2.7. Safety studies

The allocated group ADI was withdrawn in 2000 (see Section 1.7).

No data on acute toxicity is available.

2.7.1. Genotoxicity

Rauscher et al. (1998) studied the mutagenicity and the anti-mutagenic properties of β-apo-8-ester using the Ames test (histidine-deficient strains of *Salmonella typhimurium* TA98, TA 98NR and/or TA100 with metabolic activation). No mutagenic activities could be detected at concentrations up to 100 µg β-apo-8-ester per plate.

2.7.2. (Sub)chronic toxicity studies

In a two-year study, 15 male rats were fed a diet containing 10 g β-apo-8-ester kg^{-1} diet. No adverse effects on mortality, weight, fertility and general health were reported (JECFA 1974, 1975).

2.7.3. Reproductive toxicity studies, including developmental toxicity

No data is available.

2.8. Risk assessment

Although none of the available data indicate any reason for concern, the SCF (2000) concluded that there are too few data to make a full conclusion about β-apo-8-ester.

The contribution to the human intake of β-apo-8-ester from foodstuffs such as beverages and margarine where the carotenoid is used as pigment (CI Food Orange 7) is unknown. From a limited screening of vegetables and fruits, it is concluded that natural sources do not contribute to human intake of β-apo-8-ester.

Eggs and edible poultry tissues are likely the primary contributors to the human intake of β-apo-8-ester. As derived from a field study with unknown supplementation levels of β-apo-8-ester, an upper content of 10 mg kg^{-1} egg yolk appears realistic. The β-apo-8-ester intake from eggs is estimated to 0.0045 mg kg^{-1} bw per day for a 60 kg person (see Section 2.6). A refinement of the egg consumption by SCOOP data reduces the daily intake to 0.002 mg kg^{-1} bw.

A worst case calculation (see Section 2.6) for β-apo-8-ester intake from eggs resulted in an intake of about 0.07 mg kg^{-1} bw. A more realistic value is obtained after refinement with 0.03 mg kg^{-1} bw. Data for the β-apo-8-ester content of edible tissues after treatment of chickens for fattening are not available, except for the skin. Skin/fat consumption would add 0.01 mg kg^{-1} bw, which is lowered to 0.0035 mg kg^{-1} bw after refinement by SCOOP data (assumption: 18

% of total meat is skin/fat). The total intake from both sources would then be about 0.08 mg kg^{-1} bw (worst case) or 0.034 mg kg^{-1} bw (refined calculation).

Although the exposure data presented above appear to be a reasonable basis for the risk assessment, the potential hazard from β-apo-8-ester required for the risk assessment is unknown due to a lack of toxicity data. Therefore, the FEEDAP Panel can only confirm the conclusion of the SCF (2000), according to which there are too few data to conclude on the safety of this compound for the consumer. However, it must be noted that none of the available data indicate any reason for concern. Although the β-apo-8-ester is considered as a naturally occurring metabolite (probably in very small amounts) in animal tissues, a full evaluation of the additive is recommended by the FEEDAP Panel.

2.9. Environment

No data is available for a qualified assessment of the environmental impact of β-apo-8-ester used in poultry feed.

β-apo-8-ester, when excreted, will enter the pool of β-apo-8 carotenoids. Given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers unlikely that the use of β-apo-8-ester in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

2.10. Conclusions and recommendations

2.10.1. Conclusions

The β-apo-8-ester is an effective carotenoid for both egg yolk and broiler skin pigmentation. Deposition rates of β-apo-8-ester to egg yolk normally exceed 50 % and are not dose-dependent. To obtain intensively coloured egg yolk, 4–7 (highest 10) mg β-apo-8-ester together with a red pigment are required. For a skin colouration leading to a DSM-YCF > 10, at least 30 mg β-apo-8-ester alone or 15 mg β-apo-8-ester together with a red pigment has to be supplemented kg^{-1} feed.

Very limited information is available concerning the metabolic fate of β-apo-8-ester in poultry. It is deposited in the eggs of laying hens. The hydrolysis of the ester occurs at a limited extent.

Data on the safety of β-apo-8-ester for the target animals are not available. However, given the molecular structure of the carotenoid and considering the similarity in the metabolism of β-apo-8-ester and β-apo-8, the FEEDAP Panel does not see reasons for concern.

The FEEDAP Panel confirms the conclusion of the SCF (2000), according to which there are too few data to conclude on the safety of β-apo-8-ester for the consumer. However, it must be noted that none of the available data indicate any reason for concern.

Vegetables and fruits do apparently not contribute to the human intake of β-apo-8-ester. Intake data for the consumption of foodstuffs such as beverages and margarine supplemented with CI Food Orange 7 are not available. Eggs and edible poultry tissues are likely to be the primary contributors to the human intake of β-apo-8-ester.

Based on the results of a field study (not more than 10 mg β-apo-8-ester kg^{-1} egg yolk) and on an intake of 100 g of egg, a daily exposure of 0.27 mg per person can be calculated, equivalent to 0.0045 mg β-apo-8-ester kg^{-1} bw.

A worst case intake would be based on a supplementation level of 40 mg β-apo-8-ester kg^{-1} feed for laying hens and for chickens for fattening. The total intake from egg and skin/fat

consumption would be 0.08 mg kg^{-1} bw. Refining egg and skin/fat consumption results in 0.034 mg kg^{-1} bw.

No data is available for a qualified assessment of the environmental impact of β-apo-8-ester used in poultry feed. However, given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers unlikely that the use of β-apo-8-ester in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

2.10.2. Recommendations

Although the β-apo-8-ester is considered as a naturally occurring metabolite in animal tissues, a full evaluation (particularly addressing metabolism and deposition, safety for the consumer, the user and the environment, and efficacy) of the additive is recommended by the FEEDAP Panel.

The FEEDAP Panel recommends to limit the maximum content of β-apo-8-ester to 30 mg kg^{-1} complete feed. Higher dosages would not result in any additional colouring effect.

3. Lutein (E161b)

3.1. Specifications

According to the specification of the European Union[5] on the colouring compound lutein (E161b), the product is obtained by solvent extraction of the natural strains of edible fruits and plants, grass, lucerne (alfalfa) and *Tagetes erecta* (marigold flowers). The main colouring principle consists of carotenoids for which lutein and its fatty acid esters account for the major part. Variable amounts of carotenes will also be present. Lutein may contain fats, oils and waxes naturally occurring in the plant material. Only the following solvents may be used for the extraction: methanol, ethanol, propan-2-ol, hexane, acetone, methyl ethyl ketone, dichloromethane and carbon dioxide.

Extracts should contain not less than 4 % of the total colouring matters calculated as lutein (absorption measured at 445 nm in chloroform/ethanol or in exane/ethanol/acetone). However, the percentage of the total colouring matters is not specified.

Concerning the purity criteria, the maximum levels of contaminants include: solvent residues (not more than 50 mg kg^{-1} of methanol, ethanol, propan-2-ol, hexane, acetone, methyl ethyl ketone singly or in combination, and not more than 10 mg kg^{-1} of dichloromethane), arsenic (not more than 3 mg kg^{-1}), lead (not more than 10 mg kg^{-1}), mercury and cadmium (not more than 1 mg kg^{-1}) and other heavy metals as Pb (not more than 40 mg kg^{-1}).

Lutein (INS 161b) from *Tagetes erecta* and other sources is a permitted food colour within the European Union (Council Directive 94/36/EC[6]). The authorisation of lutein as food colour is restricted to a range of 50–500 mg kg^{-1} foodstuff, depending on the food category.[7]

3.2. General characteristics

Lutein occurs in corn, alfalfa, green vegetables and fruits, such as broccoli, green beans, green peas, brussel sprouts, cabbage, kale, collard greens, spinach, lettuce, kiwi and honeydew. Lutein is also found in nettles, algae and the petals of many yellow flowers and *Tagetes erecta*. In green vegetables and fruits, lutein exists in the non-esterified form, but in plants also as

[5] OJ L 226, 22.9.1995, p. 1 (Directive 95/45/EC laying down specific purity criteria concerning colours for use in foodstuffs)
[6] OJ L 237, 10.09.94, p. 13
[7] http://ec.europa.eu/food/fs/ifsi/eupositions/ccfac/ccfac_2005-50_ec-comments_en.pdf

mono- or diesters of fatty acids (i.e. dipalmitates, dimyristates and mono myristates). Lutein is industrially produced by extraction of lutein rich plants or flowers such as the petals of the marigolds flowers. Synthetic lutein is not available.

Lutein (3R,3'R,6'R)-β,ε-carotene-3,3'-diol) (Figure 3), CAS number 127-40-2 (the corresponding number in EC database is 204-840-0), has the molecular formula of $C_{40}H_{56}O_2$ and molar mass 568.88 g mol^{-1}.

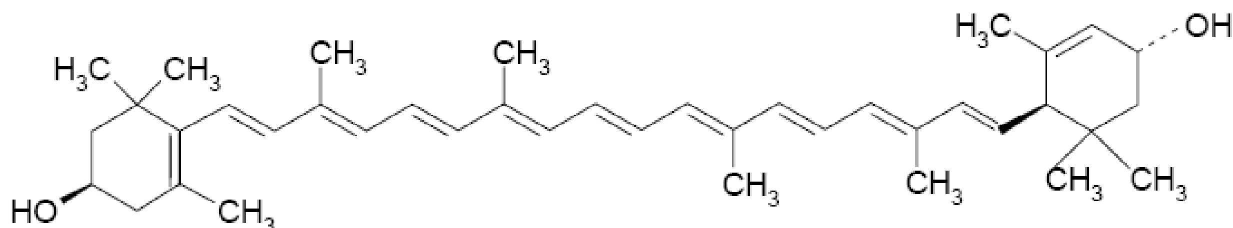

Figure 3. **Molecular structure of lutein (3R,3'R,6'R)-β,ε-carotene-3,3'-diol configuration)**

The melting point of lutein is 182 °C and a logP value of 8.546 is reported. Lutein exists as yellow to orange crystals or crystalline powder. The maximum absorption occurs at 446 nm in cyclohexane. It is soluble in most organic solvents but insoluble in water. Lutein has three chiral centers and there are eight stereoisomers. The principal natural stereoisomer of lutein is (3R,3’R,6’R)lutein.

According to the literature, the major carotenoids in lutein extract are all-trans and cis-trans isomers of lutein and its dipalmitate esters (Hadden et al., 1999; Kruger et al., 2002). Specific data on the occurrence of lutein and zeaxanthin and their proportions in raw materials and a marigold feed additive are given in Table 8.

3.3. Analytical methods

The CRL reports that no ISO and CEN methods could be found as the official analytical method for the determination of lutein in feedingstuffs or other relevant matrices.

Lutein can be analysed from various matrices by using capillary electrophoresis and HPLC (Calvo, 2005; Herrero-Martinez et al., 2006; Inbaraj et al., 2006). Recently developed HPLC methods allow to separate various lutein stereoisomers, providing LOD and LOQ values of 0.06 and 0.18 µg mL^{-1}, respectively (Inbaraj et al., 2006).

3.4. Lutein in poultry feeding

Lutein, one of the most abundant carotenoids in nature (Surai and Sparks, 2000), is ingested as part of natural diets by wild birds. It is routinely present in commercial diets for poultry flocks based on raw materials like corn and alfalfa meal, where it serves to pigment skin and eggs.

3.4.1. Sources

Most feedingstuffs used in poultry feeding contain both lutein and zeaxanthin, with lutein in a considerably higher amount. Lutein-rich raw materials comprise alfalfa meal, yellow corn, red corn and corn gluten. Pigmentation of egg yolks and broiler skin is normally achieved by using lutein- and zeaxanthin-containing feed materials supplemented with other natural or synthetic

carotenoid sources (β-apo-8, β-apo-8-ester and red-pigmenting carotenoids). Other natural sources rich in lutein are marigold flower (*Tagetes erecta*) as petal meal or extract (oleoresin). The term 'marigold' is used in many studies synonymously for *Tagetes*. A commercial product derived from a typical *Tagetes* petal meal contains 70–90 % lutein, 10–25 % zeaxanthin in the colouring matter and some other carotenoids which do not contribute to yolk or tissue pigmentation (Hoppe and Wiesche, 1988). The total content of xanthophylls in this product amounts to 1 to 4 %, with carotenoids mainly esterified. Some Mexican *Tagetes* varieties contain 0.3 to 2.3 % esterified xanthophylls.

The highest amount of zeaxanthin is found in corn, whereas *Tagetes* products show very low proportion of zeaxanthin. Some examples are summarised in Table 8.

Table 8. **Relation of lutein to zeaxanthin in some xanthophyll rich feedingstuffs and *Tagetes* products expressed as mg kg^{-1} (Huyghebaert, 2004)**

Source	Lutein (mg kg^{-1})	Zeaxanthin (mg kg^{-1})	Ratio lutein/zeaxanthin
Yellow corn	10	8	1.25
Corn gluten 60	140	110	1.27
Alfalfa concentrate	690	80	8.63
Tagetes product 1	8,510	1,190	7.15
Tagetes product 2	18,000	1,600	11.25
Tagetes product 3	13,700	1,900	7.21

The European Feed Manufacturers Federation (FEFAC) made available some non-quantitative market data on the use of lutein, covering responses from nine EU Member States (FEFAC, 2004). As no lutein product is available on the market, the presented figures are based mainly on the use of *Tagetes* products. According to those data, lutein is used for laying hens by the majority of feed manufacturers in six MS, by about half of the feed manufacturers in two MS and in a small proportion in one MS.

For other poultry, lutein is used by the majority of the feed manufacturers in four MS, by half of the feed manufacturers in one MS and by a small proportion of feed manufacturers in two MS. This carotenoid is reported not to be used in two MS.

3.4.2. Pigmenting efficacy

Lutein is effective for pigmentation of yolk and skin. As lutein leads to a yellow to green colour, incorporation of feed components rich in lutein in relation to zeaxanthin, as alfalfa meal and *Tagetes*, results only in an intensification of the yellow tone of eggs and skin. Thus, the use of lutein-rich materials for yolk pigmentation will produce yolk colours only up to a value of 9–10 DSM-YCF. However, lutein-containing feedingstuff with a relatively high content of zeaxanthin will allow more intensive egg yolk pigmentation (up to a value of 11 DSM-YCF).

In terms of yolk pigmentation, Fritsche and Schippel (1990) observed bioequivalence (8 DSM-YCF value) between 40 % corn, 20 % corn + 0.5 % *Tagetes* and 0 % corn + 1 % *Tagetes* meal in the diet. The authors stated that the use of alfalfa meal as a lutein source is limited due to its crude fiber content and the fast oxidation of xanthophylls.

Steinberg et al. (2000) supplemented a diet containing 55 % corn and 3 % corn gluten with *Tagetes* (xanthopyhll content 1.56 %, ratio of lutein to zeaxanthin 7.33) in doses of 15, 30, 60 and 120 mg total xanthophylls kg^{-1} diet (amounting to intended 13.2, 26.4, 52.8 and 105.6 mg

lutein, respectively). The authors observed (Table 9) both an increase in the lutein content of the yolk and in the yolk colour, measured by colour photometry (L*, a*, b*).

Table 9. **Content of lutein and zeaxanthin in yolk and yolk colour after supplementing a diet containing 55 % corn and 3 % corn gluten with different levels of *Tagetes* (Steinberg et al., 2000)**

Supplemented xanthophylls[1] (mg kg^{-1} diet)	Total Xanthophylls[2] (mg kg^{-1} diet)	Total Xanthophylls (mg kg^{-1} yolk)	Lutein (mg kg^{-1} yolk)	Zeaxanthin (mg kg^{-1} yolk)	Ratio Lutein/ Zeaxanthin in yolk	L*	a*	b*
Control	14.4	21.7	13.1	8.4	1.56	62.5	4.4	60.6
15	30.7	27.6	19.7	8.1	2.43	60.5	6.8	59.6
30	48.5	38.0	28.0	9.7	2.89	60.5	8.6	60.1
60	75.9	43.5	34.5	8.8	3.92	59.7	9.3	59.2
120	141.0	51.8	42.0	9.8	4.29	59.0	10.4	57.1

[1] Lutein content 88 %
[2] Analysed values
L* lightness, a* redness, b* yellowness, according to CIE-Lab System

The increase in total xanthophylls in egg yolk was not linear with increasing dietary inclusion levels of *Tagetes*. This was mainly due to the lower deposition rate of zeaxanthin compared to lutein (Table 10). Consequently, the amount of lutein in total xanthophylls of egg yolk increased with 61 % in the control diet to 81 % in the diet with 120 mg supplemented *Tagetes* xanthophylls kg^{-1}. The pigmenting efficacy of total xanthophylls (88 % lutein) seems to be less than the commonly assumed ratio of 1:3. This may be due to the high xanthopylls concentration studied in this experiment.

Leeson and Caston (2004) supplemented 1 kg of a corn, wheat, soybean meal layer diet with 0 to 1000 mg lutein from a commercial *Tagetes* product. At a supplementation level of around 500 mg *Tagetes* product kg^{-1}, yolk lutein reached a plateau.

Prabakaran et al. (2001) supplemented a diet with 200 and 400 mg kg^{-1} *Tagetes* product with a carotenoid concentration of 2 % (ratio of lutein to zeaxanthin not specified). The authors observed DSM-YCF values of 7.1 (400 mg kg^{-1}) and 10.6 (400 mg kg^{-1} + 1.2 g of a red pigment source kg^{-1}). The authors did not differ between lutein and zeaxanthin. Grashorn and Seehawer (1999) reported that 9 mg colouring matter kg^{-1} diet from 580 mg a *Tagetes* product (1.56 % xanthophylls; 88 % lutein), together with 4 mg canthaxanthin kg^{-1}, would lead to a yolk colour of 12 DSM-YCF in fresh eggs. However, 21 mg colouring matter + 4 mg canthaxanthin kg^{-1} would be required for the same pigmentation effect in hard-boiled eggs.

Hoppe and Wiesche (1988) conducted several experiments on the comparison of pigmenting efficiency of marigold (72 to 88 % lutein) with β-apo-8-ester. The supplementation levels were 5 to 120 mg total xanthophylls kg^{-1} and 0.75 to 16 mg β-apo-8-ester kg^{-1} diet, respectively. Pigmenting efficiency of xanthophylls from marigold was only one seventh of β-apo-8-ester.

Balnave and Bird (1996) supplemented diets with a saponified *Tagetes* oleoresin (at doses of 2.8, 5.5 and 8.3 mg total xanthophylls kg^{-1}) and determined yolk contents of 4.4, 6.9 and 8.6 mg xanthophylls kg^{-1} yolk. Pigmenting efficiency was 1:3 to 1:4 in relation to β-apo-8-ester. Hencken (1992) compared the colouring efficiency of esterified lutein from *Tagetes* extracts with saponified free lutein from *Tagetes* extracts. He observed significantly better yolk pigmentation for the saponified product. The xanthophyll content in yolk increased from 12.8 to 31.0 mg kg^{-1} (dietary supplementation levels 8 to 20 mg kg^{-1}).

Surai and Speake (1998) fed two diets (diet 1 with added lutein and citranaxanthin; diet 2 with added lutein, citranaxanthin, canthaxanthin and β-apo-8-ester) to layers. Carotenoids were deposited linearly to yolks until day 11 and reached a maximum on days 19–23. The content of the carotenoids in plasma, liver and heart of the newly hatched embryos was linearly correlated to feed level.

Supplementation of a marigold meal (5, 10 and 20 mg total xanthophylls kg^{-1}) to broiler diets resulted in a significant increase in shank pigmentation (6.9-9.4 DSM-YCF), whereas pigmentation levels from alfalfa meal and corn gluten were lower (Marusich and Bauernfeind, 1970b). Damron et al. (1990) used different *Tagetes* products (meal, extracts, oleoresins) with free or saponified xanthophylls in broiler diets and observed a better pigmenting efficiency for free lutein than for saponified lutein or lutein/zeaxanthin mixtures. In contrast, Fletcher et al. (1986) observed significantly better skin and shank pigmentation in broilers with a saponified marigold product. Calafat et al. (2005) used canthaxanthin and paprika oleoresins in combination with *Tagetes* for broiler skin pigmentation and observed a higher colouring efficiency for the combination of canthaxanthin with *Tagetes*.

3.4.3. Deposition of lutein in the egg and tissues

Three studies with graded supplementation levels of lutein from *Tagetes* products are available (Marusich and Bauernfeind, 1970a; Balnave and Bird, 1996; Steinberg et al., 2000), which allows the calculation of deposition rates for the egg yolk (data of Steinberg et al. 2000, Table 10). Whereas increasing the lutein supplementation resulted in higher lutein deposition in the yolk, the deposition rate (expressed in % of the amount ingested) decreased.

Table 10. **Deposition of lutein and total xanthophylls to egg yolks when feeding different levels of total xanthophylls from a *Tagetes* product expressed % of the amount ingested (Steinberg et al., 2000)**

Supplemented xanthophylls in diet (mg kg^{-1})[1]	Lutein (%)	Total xanthophylls (%)
15	8.7	6.6
30	8.9	8.1
60	6.8	6.0
120	4.4	4.1

[1] Supplemetation from *Tagetes* to a basal diet containing 14.4 mg total xantophylls kg^{-1}

Deposition of lutein was six to eight times lower than that of β-apo-8-ester and pigmenting efficacy was three to six times lower (Steinberg et al., 2000; Blanch et al., 2002; Beardsworth and Hernandez, 2004). Lutein is deposited more effectively than the natural carotenoid capsanthin (Lai et al., 1996).

No study was found with deposition rates of lutein to edible poultry tissues.

3.5. Metabolism of lutein

3.5.1. Poultry

The absorption and distribution of lutein in chickens has been established following administration of free lutein or its diacylester(s) from *Tagetes* petals administered in a dose range of 5–80 mg kg^{-1} diet (Osianu and Nicoara, 1984; Tyczkowski and Hamilton, 1986a and 1986b; Schiedt, 1998 for review).

No differences in absorption of free and esterified lutein have been observed in layers (Hoppe and Wiesche, 1988; Breithaupt et al., 2003). Lutein esters are hydrolysed in the intestine and free lutein is absorbed at the duodenum and jejunum levels. A linear increase of total lutein (free plus esters) with dietary lutein is observed in blood serum, liver and toe-web. Lutein occurs in blood mainly in its free form (96 %) and to a minor extent (4 %) as monoester(s). In liver, the distribution is similar (80 % and 20 %, respectively, plus traces of diester). Lutein is re-esterified by local enzymes when it enters other depository sites (e.g. 50 % lutein diester(s), 25 % lutein monoester(s) and 25 % free lutein in the toe-web).

The metabolic fate of lutein has been investigated following lutein diester administration to chickens (Schiedt, 1987). Two major metabolites were identified that correspond to the oxidation (dehydrogenation) of one hydroxy group (3 or 3'-oxolutein) followed by a second reaction on a second hydroxy group (lutein-3,3'-dione). No cleavage of the lutein molecule occurred, which is consistent with the fact that lutein does not exhibit vitamin A activity (Van Vliet, 1996). Lutein/metabolites deposition in the egg was studied following free lutein administration (20 mg kg^{-1} feed) to chickens (Schaeffer et al., 1988). The ratio 3 or 3'-oxolutein vs. lutein reached a plateau at 14 days that amounted to 0.12. A similar ratio was measured in the serum indicating that the oxidation most likely takes place in the liver and not in the ovaries.

3.5.2. Humans

The intestinal absorption of lutein, measured experimentally *in vivo* in the human, amounted to 2.5 % (O'Neill and Thurnham, 1998). However, it must be considered that the extent of the absorption of carotenoids, including lutein, depends on many nutritional (i.e. bioavailability is markedly reduced by including fiber, Riedl et al., 1999) and physiological factors (Yeum and Russel, 2002 for review).

Several studies have investigated the bioavailability of lutein in humans (Riedl et al., 1999; Johnson et al., 2000; Berendschot et al., 2000; Hininger et al., 2001; Granado et al., 2002; Bowen et al., 2002; Olmedilla et al., 2002; Lienau et al., 2003). The relative bioavailability of lutein was reflected by the serum (or plasma) concentration following oral exposure (different sources and amounts, different exposure periods). The results indicate that (i) lutein diester(s) are significantly more bioavailable (by 62 %) than unesterified lutein, (ii) diet supplementation with marigold extracts (15 mg lutein for 20 weeks or 10 mg for four weeks) led to a lutein concentration plateau in plasma after four weeks which corresponded to a fivefold or fourfold increase, respectively, compared to the initial values, and (iii) diet supplemented with frozen spinach (10.8 mg lutein per day during 15 weeks) significantly increased (twofold) the lutein concentration in serum within the first four weeks.

In humans, about 45 % and 10 % of a single dose of [14C]-lutein administered orally were excreted the first 48 hours in the faeces and urine, respectively (de Moura et al., 2005). The metabolic pathways in human include: (i) the oxidation at 3 and 3' giving rise to mono-oxoluteins and the di-oxolutein, (ii) the oxidation at 3 followed by reduction and epimerization (3'-epilutein), and (iii) the non-enzymatic dehydration at 3 giving rise to two didehydro compounds (Khachik et al., 1995).

It is noteworthy that the conversions of lutein to zeaxanthin and the reverse have been shown to occur *in vivo* in humans, through an equilibrium involving oxidation/reduction and double-bond isomerisation reactions, and the intermediate 3'-epilutein. This intermediate and zeaxanthin can also exist in equilibrium through reversible double-bond migration. Thus, the presence of 3'-epilutein in human serum may be due to the conversion of lutein and/or zeaxanthin (Khachik et al., 1995, Khachik et al., 2002).

3.6. Human intake estimates

A field study with 41446 volunteers from five Spanish regions on the intake of lutein and zeaxanthin (García-Closas et al., 2004) led to the conclusion that eggs and poultry meat do not significantly add to human intake of lutein as the estimated intake amounted to < 1 % of the total intake.

In 2006, EFSA's former scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) delivered an opinion on lutein for use in foods for particular nutritional uses (EFSA, 2006). In that opinion, the evaluation of the daily intake of lutein as such was based on published literature and it was estimated to vary from 0.8–2.5 mg.

Bräunlich published in 1974 (El Boushy and Raterink, 1992) that egg yolk in Germany would contain 44.2 mg total carotenoids kg^{-1}, in Sweden only 11.1 mg. In a recent field survey on commercial eggs, Schlatterer and Breithaupt (2006) determined lutein contents of egg yolk at 5.7 mg kg^{-1} in cage eggs and 17.6 mg kg^{-1} in organic eggs (free range). Based on those data and the above AFC estimate on total lutein intake, eggs (SCOOP data) would contribute in a range of 2–20 % to the total intake.

3.7. Safety studies

JECFA has established a group ADI of 0–2 mg kg^{-1} bw day for lutein from *Tagetes erecta* and synthetic zeaxanthin (FAO/WHO, 2004). However, this group ADI has been questioned later by the EFSA's scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) (EFSA, 2008) (see Section 4.7) and should not be applied to lutein alone when used in food. Although the FEEDAP Panel would agree with this position, given the use of the product in feedingstuffs and the very limited contribution to human exposure (see Section 3.8), it retains the group ADI as an indicative value for assessment purposes.

No data on acute toxicity is available.

3.7.1. Genotoxicity

A dose of 10 g dried spinach powder containing 11.3 mg lutein did not induce DNA damage in human lymphocytes, as detected by the Comet assay (Pool-Zobel et al., 1997).

The genotoxic potential of lutein has been reviewed by the AFC Panel (EFSA, 2006) and it was concluded that a lutein product, containing 79 % lutein and 5 % zeaxanthin, did not have mutagenic potential in the Ames test.

3.7.2. (Sub)chronic toxicity studies

Two repeated dose studies with a lutein product have been reviewed by the AFC Panel (EFSA, 2006). In a four-week study in rats, the lutein product given at doses up to 773.2 mg kg^{-1} bw day^{-1} (highest dose level used, equivalent to about 600 mg lutein kg^{-1} bw day^{-1}) for four weeks did not result in test article-related toxicity and was well tolerated by the rats.

A similar lutein product was used in a 13-week toxicity study in rats with a marigold extract. The AFC Panel (EFSA, 2006) concluded that no adverse effects were observed at the highest dose tested (260 mg lutein product kg^{-1} bw day^{-1}, equivalent to about 200 mg kg^{-1} bw day^{-1}).

3.7.3. Reproductive toxicity studies, including developmental toxicity

No data is available.

3.7.4. Human data

Epidemiological studies on lutein intake and human health effects generally report beneficial effects of increased lutein intake or plasma levels, such as an inverse association between lutein intake levels and cancer incidences (Goodman et al., 2003). Lutein slows the degeneration of macula and exhibits antioxidative properties (Rapp et al., 2000; Landrum and Bone, 2001) and is suggested to influence the development of cataract (Moeller et al., 2000).

The AFC Panel (EFSA, 2006) concluded that human studies have not revealed adverse effects, although the majority of those studies have not been designed to assess the safety of lutein. In one study, 40 % of a cohort supplemented for 20 weeks with 15 mg lutein per day showed carotenodermia, but no changes in biochemical and haematological indices were observed.

3.8. Risk assessment

Two lutein-containing products did not show mutagenicity in different test systems. It is therefore concluded that lutein is not mutagenic in the studies conducted. No evidence for adverse effects was observed in repeated dose studies. No data is available on reproduction toxicity.

The AFC Panel (EFSA, 2006) considered that the regular dietary lutein intake was not of safety concern, estimating it to be between 0.8 and 2.5 mg day^{-1}. As an indication, this amounts to 2 % of the JECFA group ADI for lutein and zeaxanthin. The contribution of animal products (eggs) to the total intake is estimated by literature and the FEEDAP Panel to be in the range of 1–20 %, the higher figures being from eggs produced by organic farming.

A worst case calculation is described by a daily intake of 100 g egg produced from hens fed diets containing the maximum content authorised for lutein, 80 mg kg^{-1} diet. This hypothetical assumption would result in a potential human daily intake (derived from the data in Table 9, assuming a maximum value of 40 mg lutein kg^{-1} egg yolk) of 1.08 mg lutein (0.018 mg kg^{-1} bw day^{-1}, corresponding to < 1 % of the JECFA group ADI). As a more realistic dietary lutein concentration, 20 mg lutein is considered (providing optimum pigmentation in the egg together with red carotenoids). This would result (derived from Table 9, 20 mg lutein kg^{-1} egg yolk) in a daily intake of 0.54 mg lutein (0.009 mg kg^{-1} bw day^{-1}).

The most realistic estimate of lutein intake from eggs can probably be derived from SCOOP consumption data (highest egg intake: 36 g day^{-1}) and the lutein contents of eggs collected in a field survey (17.6 mg kg^{-1} yolk (organic production), 5.7 mg kg^{-1} yolk (cage husbandry), Schlatterer and Breithaupt, 2006). Lutein intake from organic eggs would then correspond to 0.17 mg day^{-1}, from cage eggs to 0.06 mg day^{-1}. The contribution of eggs to the maximum total human intake (2.5 mg) is then calculated to be between 2 (cage husbandry) and 7 % (organic production).

No data is available to asses the lutein intake from edible poultry tissues.

Taking into account the human lutein intake from all sources (as an indication, about 2 % of JECFA group ADI), the contribution from food of animal origin (eggs and poultry tissues produced with lutein-containing diets) would represent a very small proportion of the total intake and varies with the consumption pattern in different countries. It does not require a particular safety assessment.

3.9. Environment

According to Directive 2001/79/EEC,[8] an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, the use of which will not alter the concentration or distribution in the environment. Lutein occurs abundantly in plants and the terrestrial environment.

Taking also into account the oxidative susceptibility of carotenoids, the FEEDAP Panel considers it unlikely that the use of lutein in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

3.10. Conclusions and recommendations

Lutein occurs in feedingstuff and additives in association with zeaxanthin at varying ratios (about 1:0.1–0.8). A separate assessment of the pigmenting efficiency of lutein under practical feeding conditions is therefore not possible, particularly because zeaxanthin leads to more intense visible colour than lutein. Lutein alone is effective in colouring yolk (up to DSM-YCF of 9-10) and skin of chickens for fattening.

A lutein supplementation is only seldom calculated as such but rather as total xanthophylls. Poultry diets rich in corn and corn products may contain about 10 mg lutein and supplemented diets about 20–30 mg lutein kg^{-1}. Those dietary levels may lead to egg yolk concentrations of 13–25 mg lutein kg^{-1}.

Lutein is absorbed in the small intestine and enters the blood stream in its free form; it is re-esterified when entering the target cells. No cleavage of the isoprenoic chain occurs. Metabolisation is characterised by the oxidation of the hydroxyl groups. In humans, the interconversion of both xanthophylls is observed.

Data on the safety of lutein for the target animals are not available. However, given the widespread natural occurrence of the compound, and considering the molecular structure of the carotenoid, the FEEDAP Panel does not see reasons for concern.

Lutein is not mutagenic, based on the limited studies available. A group ADI of 0–2 mg kg^{-1} bw day^{-1} for lutein from *Tagetes erecta* and synthetic zeaxanthin has been established by JECFA. The human intake of lutein (including the consumption of eggs and edible poultry tissues) has been estimated by the AFC Panel to 0.8–2.5 mg day^{-1} and is considered not to present any safety concern (as an indication, maximum 2 % of the group ADI).

A worst case calculation (daily intake of 100 g egg, 80 mg lutein kg^{-1} diet) would result in a potential human daily intake, of 1.08 mg lutein (0.018 mg kg^{-1} bw day^{-1}). A realistic estimate of lutein intake from eggs is derived from SCOOP consumption data (high egg intake 36 g day^{-1}) and the lutein contents of eggs collected in a field survey. Lutein intake from organic eggs would then correspond to 0.17 mg day^{-1}, from cage eggs to 0.06 mg day^{-1}. The contribution of eggs to the maximum total human intake (2.5 mg) is then calculated to be between 2 % (cage husbandry) and 7 % (organic production).

No data is available to asses the lutein intake from edible poultry tissues.

Taking into account the human lutein intake from all sources, the contribution from food of animal origin (eggs and poultry tissues produced with lutein containing diets) would be a very small proportion of the total intake and varies with the consumption pattern in different countries. It does not require a particular safety assessment.

[8] OJ L 267, 06.10.2001, p. 1

Taking into account the abundant occurrence of lutein in plants and the terrestrial environment, the FEEDAP Panel considers it unlikely that the use of lutein in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

4. Zeaxanthin (E161h)

4.1. Specifications

Although zeaxanthin has an E-number, it is not authorised in the EU as a food additive. Therefore, it has no specification on the food additive legislation. However, the sources for lutein listed in the specification for lutein (E161b) refer also to zeaxanthin.

4.2. General characteristics

Zeaxanthin is present in corn, green vegetables and fruits, such as broccoli, green beans, green peas, brussel sprouts, cabbage, kale, collard greens, spinach, lettuce, kiwi and honeydew. Zeaxanthin is also found in nettles, algae and the petals of many yellow flowers. Zeaxanthin is also chemically produced (Ernst, 2002).

Zeaxanthin (all-trans-(3R,3'R)-β-carotene-3,3'-diol) (Figure 4), CAS number 144-68-3, has the molecular formula of $C_{40}H_{56}O_2$ and a molar mass of 568.88 g mol^{-1}.

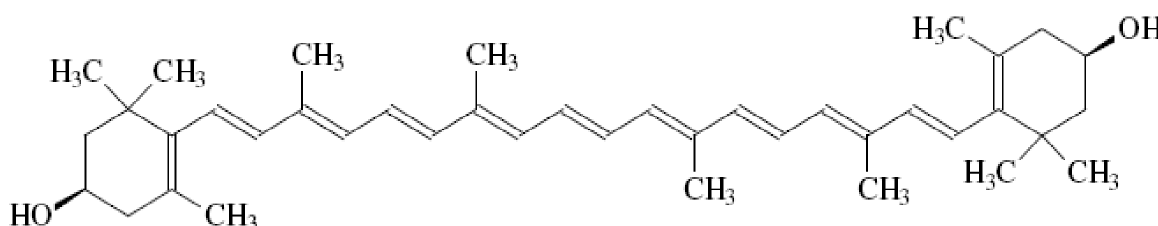

Figure 4. **Molecular structure of (3R3'R)-zeaxanthin**

The melting point of zeaxanthin is 215.5 ºC and a logP value of 9.156 is reported. Zeaxanthin exists as orange red crystals or crystalline powder. The maximum absorption occurs at 446 nm in cyclohexane. Zeaxanthin is insoluble in water but soluble in most organic solvents. Zeaxanthin has two chiral centers and therefore theoretically four stereoisomeric forms ((3R,3'R), (3S,3'S), (3R,3'S) and (3S,3'R)). However, since zeaxanthin is a symmetrical molecule, (3R,3'S) and (3S,3'R) are identical; therefore, only three stereoisomeric forms may occur. The predominant natural form of zeaxanthin is (3R,3'R)-zeaxanthin.

Zeaxanthin occurs in plants as non-esterified or in the form of mono- or diesters of fatty acids (e.g. dipalmitate).

The antioxidative capacity of zeaxanthin is estimated at 36 % of α-tocopherol (Matsufuji et al., 1998).

4.3. Analytical methods

The CRL reports that no ISO and CEN methods could be found as the official analytical method for the determination of zeaxanthin in feedingstuffs or other relevant matrices.

Zeaxanthin can be analysed from botanical materials by using HPLC and mass spectrometric techniques (Inbaraj et al., 2006; Hao et al., 2005). By using HPLC technique, LOD (0.02 µg ml^{-1}) and LOQ (0.06 µg ml^{-1}) were achieved (Inbaraj et al., 2006).

4.4. Zeaxanthin in poultry feeding

4.4.1. Sources

Most feed components used in poultry feeding contain both lutein and zeaxanthin, although the zeaxanthin content is distinctly less than that of lutein (see Section 3.4.1, Table 8). In contrast to lutein, a synthetic product is available, containing 60 g zeaxanthin kg^{-1}. The pigmentation of egg yolks and broiler skin is normally achieved by using lutein- and zeaxanthin-containing feed materials supplemented with other natural or synthetic carotenoid sources (β-apo-8, β-apo-8-ester and red-pigmenting carotenoids).

The European Feed Manufacuturers Federation (FEFAC) made available some non-quantitative market data on the use of zeaxanthin, covering responses from nine EU Member States (FEFAC, 2004). Obviously, this information is based on the supplementation of different marigold products to diets, as the market share of the zeaxanthin product is quite small. According to those data, zeaxanthin is used for laying hens by half of the feed manufacturers in four MS, by the majority of feed manufacturers in two MS and by a small proportion of the feed manufacturers in one MS. It is not used in one MS.

For other poultry, zeaxanthin is used by the majority of the feed manufacturers in two MS, by half of the feed manufacturers in two MS, by a small proportion of feed manufacturers in two MS and it is not used in two MS.

4.4.2. Pigmenting efficacy

The main effects of zeaxanthin have already been described in Section 3.4.2. As already mentioned, it is rather impossible to describe the effects of zeaxanthin separately but as zeaxanthin has an orange tone, it can be expected that the use of zeaxanthin or zeaxanthin-containing feed material will result in a more intense visual colour of yolks and broiler skin. The increase in a* value with increasing supplementation level of *Tagetes* to diets may partly be attributed to the content of zeaxanthin (Table 9). Colouring efficiency of zeaxanthin in relation to lutein is reported to be 5 to 1 (Marusich and Bauernfeind, 1970a).

Marusich and Bauernfeind (1970a) fed 9 and 18 mg zeaxanthin (from ether stabilised beadlets) kg^{-1} diet to layers and determined 17 and 36 mg zeaxanthin kg^{-1} yolk, respectively.

Steinberg et al. (2000) reported 8.1–9.8 µg zeaxanthin g^{-1} yolk when supplementing diets containing 5.3 mg zeaxanthin kg^{-1} with 2.4–15.7 mg zeaxanthin from *Tagetes* kg^{-1} diet (Table 9). It appears that in the range of dietary concentrations between 5 and 21 mg zeaxanthin from *Tagetes* kg^{-1}, the egg yolk concentration is only weakly affected. Leeson and Caston (2004) observed a significant decrease ($p < 0.01$) in yolk zeaxanthin content with increasing the supplementation levels of lutein (0–1000 mg supplemental lutein from a commercial *Tagetes* product kg^{-1} diet).

Meanwhile, a new *Tagetes* product is available with a lutein to zeaxanthin ratio of 30:70 % (Blanch et al., 2002). Standard *Tagetes* products include 100 % RR-Isomer of zeaxanthin, whereas the modified product consists of 6.5 % RR and 93.5 % RS-Isomers. As RS-Isomers are deposited less effectively, pigmentation efficacy of zeaxanthin in this *Tagetes* product is lower.

4.4.3. Deposition of zeaxanthin in egg and tissues

Deposition rates of zeaxanthin vary between 20 and 29 % (Table 11). Obviously, a higher deposition is achieved with concentrated products (beadlets). Lai et al. (1996) and Pérez-Gálvez et al. (2003) reported a better deposition of zeaxanthin than capsanthin. The deposition rate of zeaxanthin is 39 % of the deposition rate of β-apo-8-ester (Huyghebaert, 2004).

Table 11. **Zeaxanthin contents in yolks (mg kg-1) and deposition rates (%)**

Source	Dose	Yolk content (mg kg^{-1})	Deposition (%)	Reference
Beadlets	9 - 18 mg kg^{1*}	17 - 36	27.2 - 29.0	Marusich and Bauernfeind (1970a)
Beadlets	-	-	25.0	Hencken (1992)
Zeaxanthin product	-	-	20.0	Huyghebaert (2004)

* Recalculated from 1 + 2 mg hen^{-1} d^{-1}

Using a *Tagetes* product (12 % of total xanthophylls as zeaxanthin), Steinberg et al. (2000) described deposition rates for lutein and total xanthophylls (Table 10), those for xanthophylls being constantly lower than that for lutein. As values for the zeaxanthin deposition rate were not given, it can be concluded at least that the deposition rates for zeaxanthin were considerably lower than those of lutein (4–9 %). This is in contrast to the above-tabulated values (Table 11). This may be due to the differences in the bioavailability of zeaxanthin in the various products. A competitive inhibition of absorption of zeaxanthin by lutein is assumed considering the structural affinity of both compounds. Taking together the data published by Steinberg et al. (2000) and by Leeson and Caston (2004), zeaxanthin in egg yolk may reach a plateau of about 10 mg kg^{-1} yolk, which may be reduced by dietry lutein levels above 500 mg kg^{-1}.

Only one study on deposition of zeaxanthin to broiler skin is available. Hencken (1992) estimated a deposition rate of 1.7 %.

4.5. Metabolism of zeaxanthin

4.5.1. Poultry

Limited information is available concerning the absorption, distribution and fate of zeaxanthin in poultry. Zeaxanthin is predominantly absorbed in the ileum (Tyczkowski and Hamilton, 1986). Data concerning the metabolism in chickens and laying hens have been obtained from a study performed using [^{3}H]-(3R,3’R)-zeaxanthin (Schiedt, 1985; Mayer et al., 1982). The main features can be summarised as follows: (i) the absorption rate has not been investigated, (ii) the absorbed zeaxanthin is mainly metabolised in the liver, where esterified zeaxanthin was found, (iii) the deposition rate in the egg yolk was 25 % of the administered dose (multi-dose for four weeks, 16 mg kg^{-1} in the diet), (iv) free zeaxanthin represented 90 % of the whole radioactivity in the egg, (v) 5 to 10 % corresponded to a metabolite that was identified as 3 and 3’-oxozeaxanthin and a minor (3 %) oxidised metabolite identified as (6S,6’S)- ε,ε-caroten-3,3’-dione, (vi) the isomeric composition of zeaxanthin deposited in the egg yolk reflects that of the synthetic compound administered.

4.5.2. Humans

Zeaxanthin and lutein share common metabolic pathways, which allows the interconversion of both compounds but also give rise to monooxo- and dioxo- zeaxanthins/luteins (see 3.5.2).

4.6. Human intake estimates

Zeaxanthin is far less abundant in foodstuffs than lutein, although it is found especially in yellow corn (Gross, 1991), spinach and sweet red peppers (Granado et al., 1992). In Europe, the foods contributing to zeaxanthin intake vary per country, but the major foods are spinach, lettuce, broccoli, peas, yellow corn, sweet red pepper and egg yolk (O'Neil et al., 2001; Scott et al., 1996; Reed Mangels et al., 1993).

Based on the studies referred to in Section 3.6 (human intake of lutein), the overall dietary intake of zeaxanthin is estimated by the AFC Panel to be between 0.1 and 0.7 mg day^{-1}. The NDA Panel (EFSA, 2008) took 0.2–0.9 mg day^{-1} as the average zeaxanthin intake. The highest intake (95th percentile) of 1.8 mg day^{-1} was derived in an Italian study (Franceschi et al., 2000).

A field study with 41446 volunteers from five Spanish regions on the intake of lutein and zeaxanthin led to the conclusion that eggs and poultry meat do not add to the human intake of zeaxanthin (García-Closas, 2004).

In a recent field survey on commercial eggs, Schlatterer and Breithaupt (2006) determined 3.15 mg zeaxanthin kg^{-1} yolk in cage eggs and 10.2 mg kg^{-1} in organic eggs (free range).

4.7. Safety studies

JECFA has established a group ADI of 0–2 mg kg^{-1} bw day for lutein from *Tagetes erecta* and synthetic zeaxanthin (FAO/WHO, 2004). Although, the NDA Panel commented (EFSA, 2008) that the ‘toxicological data on zeaxanthin are not sufficient to derive an ADI’, for the reasons given in Section 3.7, the FEEDAP Panel retains the group ADI as an indicative value for the assessment.

The NDA Panel summarised the available data (EFSA, 2008). Most studies have been conducted before the current standards (OECD guidelines) were published.

The LD_{50} of zeaxanthin after a repeated dose administration for ten days was higher than 8000 mg kg^{-1} bw in mice and rats (after *i.p.* administration 840 and 1100 mg kg^{-1} bw, in mice and rats, respectively). The LD_{50} for a 10 % zeaxanthin formulation was higher than a dose corresponding to 800 mg zeaxanthin kg^{-1} bw.

4.7.1. Genotoxicity

The studies described for the genotoxicity of lutein in Section 3.6.2 may also relate to the genotoxicity of zeaxanthin, since the lutein samples tested may have contained a percentage of zeaxanthin although the concentrations tested were low.

The NDA Panel (EFSA, 2008) reported another six mutagenicity tests (Ames test, gene mutation using mammalian cells (V79/HGPRT-test), unscheduled DNA synthesis (UDS-test), chromosomal aberrations using human peripheral lymphocytes (two), micronucleus assay). The NDA Panel concluded that ‘no mutagenicity was observed in tests on gene mutations in bacteria and mammalians cells, in vitro tests on chromosomal aberration and an in vivo micronucleus test had some shortcomings, but did not provide evidence for genotoxicity.’

4.7.2. (Sub)chronic toxicity studies

The 13-week oral toxicity study in rats with lutein was performed with a lutein product derived from marigold flowers and containing lutein and zeaxanthin at concentrations of 79 and 5 %, respectively. No adverse effects were observed at the highest dose level of 260 mg lutein product kg^{-1} bw day^{-1}, amounting to about 200 mg lutein and 13 mg zeaxanthin kg^{-1} bw day^{-1} (Kruger et al,. 2002).

The NDA Panel reported three other 13-week feeding studies in rats (one of them was not considered further) and one in beagle dogs, in which a water soluble beadlet formulation with a zeaxanthin content of 9.3–9.4 % was used (EFSA, 2008). The NDA Panel concluded that those studies did not indicate adverse effects up to the highest doses tested (1000 mg kg^{-1} bw day^{-1} in rats, 422 mg kg^{-1} bw day^{-1} in beagle dogs).

The NDA Panel also assessed a 52-week feeding study with cynomolgus monkeys. In that study, the content and effect of zeaxanthin in the eyes was studied. The NDA Panel (EFSA, 2008) noted that '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 for crystal formation in the eyes' (20 mg zeaxanthin kg^{-1} bw day^{-1}). In haematology, clinical chemistry and urine analyses, organ weight determination and hystopathological examinations, no toxicologically relevant effects were noted.

4.7.3. Reproductive toxicity studies, including developmental toxicity

The NDA Panel (EFSA, 2008) assessed two developmental studies, one in rats (with doses up to 1000 mg kg^{-1} bw day^{-1} from day 7 to day 16 of gestation) and one in rabbits (with doses up to 400 mg kg^{-1} bw day^{-1} from day 7 to day 19 of gestation). The NDA Panel concluded that 'There were no indications of embryotoxic or teratogenic effects up to the highest dose' tested.

4.7.4. Human data

The NDA Panel reported no adverse effects in several studies on human subjects (EFSA, 2008). The daily intake was between 10 and 30 mg zeaxanthin for periods between 42 days up to four-six months.

4.8. Risk assessment

Zeaxanthin is not mutagenic and there is no evidence for genotoxity. In addition, no indications were found that zeaxanthin could be embryotoxic or teratogenic,

A 13-week toxicity study in rats using a marigold extract revealed a dose of zeaxanthin that was not toxic (13 mg kg^{-1} bw day^{-1}). A study in beagle dogs showed no toxic signs at the highest dose tested (422 mg zeaxanthin kg^{-1} bw), nor did two studies in rats at the highest dose tested (1000 mg zeaxanthin kg^{-1} bw). Those studies are in line with the JECFA group ADI of 0–2 mg kg^{-1} bw for lutein from *Tagetes erecta* and synthetic zeaxanthin.

In a one-year monkey study, no evidence for treatment-related adverse effects on the eyes, in particular on crystal formation, was found at 20 mg zeaxanthin kg^{-1} bw day^{-1}.

Several studies in human subjects did not reveal adverse effects for daily doses between 10 and 30 mg zeaxanthin.

The highest reported human exposure is 1.8 mg day^{-1}, corresponding to about 0.03 mg kg^{-1} bw. This is less than 2 % of the JECFA group ADI for lutein and zeaxanthin.

A worst case calculation is described by a daily intake of 100 g egg containing zeaxanthin at the highest observed level (about 10 mg kg^{-1}) in egg yolk, corresponding to about 2.7 mg kg^{-1} egg. This hypothetical assumption results in a potential human daily intake of 0.27 mg (0.005 mg kg^{-1} bw day^{-1}).

A realistic estimate of zeaxanthin intake from eggs can probably be derived from SCOOP consumption data (highest egg intake: 36 g day^{-1}) and the zeaxanthin contents of eggs collected in a field survey (10.2 mg kg^{-1} yolk (organic production), 3.15 mg kg^{-1} yolk (cage husbandry), Schlatterer and Breithaupt, 2006). The zeaxanthin intake from organic eggs would then

correspond to 0.1 mg day^{-1}, from cage eggs to 0.03 mg day^{-1}. The contribution of eggs to the maximum total human intake (1.8 mg) is then calculated to be between 2 (cage husbandry) and 6 % (organic production).

No data is available to asses the zeaxanthin intake from edible poultry tissues.

Considering that (i) the total intake of zeaxanthin is closely related to the lutein intake, (ii) lutein from food of animal origin is reported to contribute to less than 1 % of total intake, and (iii) the zeaxanthin content of eggs is about one third of lutein, the assessment of zeaxanthin contribution from food of animal origin to the total zeaxanthin exposure would follow the same principles as those established for lutein (see Section 3.8).

Taking into account the human zeaxanthin intake from all sources (as indication, about 2 % of JECFA group ADI), the contribution from food of animal origin (eggs and poultry tissues produced with zeaxanthin-containing diets) would represent a very small proportion of the total intake and varies with the consumption pattern in different countries. It does not require a particular safety assessment.

4.9. Environment

According to Directive 2001/79/EEC,[9] an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, the use of which will not alter the concentration or distribution in the environment. Zeaxanthin occurs abundantly in the terrestrial environment.

Given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers it unlikely that the use of zeaxanthin in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

4.10. Conclusions and recommendations

4.10.1. Conclusions

Zeaxanthin occurs in feedingstuff and additives in association with lutein at varying ratios (about 0.1–0.8:1). A separate assessment of the pigmenting efficiency of zeaxanthin under practical feeding conditions is therefore hardly possible. Zeaxanthin alone is effective in colouring yolk, its relative efficacy to lutein is reported with 5:1. In general, zeaxanthin allows more intensive yolk pigmentation than lutein.

A zeaxanthin supplementation is only seldom calculated as such but rather as total xanthophylls. Poultry diets rich in corn and corn products may contain about 5 mg zeaxanthin, supplemented diets about 8–12 mg zeaxanthin kg^{-1}. Those dietary levels may lead to egg yolk concentrations of 8–10 mg zeaxanthin kg^{-1}.

Zeaxanthin is predominantly absorbed in the ileum. Absorbed zeaxanthin is re-esterified in the liver. No cleavage of the isoprenoic chain occurs. Zeaxanthin is deposited in the egg predominantly as free zeaxanthin. Metabolisation is characterised by the oxidation of the hydroxyl groups. In humans, the interconversion of lutein and zeaxanthin is observed.

Data on the safety of zeaxanthin for the target animals are not available. However, given the widespread natural occurrence of the compound and considering the molecular structure of the carotenoid, the FEEDAP Panel does not see reasons for concern.

[9] OJ L 267, 06.10.2001, p. 1

Zeaxanthin is not mutagenic and there is no evidence for genotoxity. In addition, no indications were found that zeaxanthin could be embryotoxic or teratogenic. Repeated dose studies in rats and beagle dogs revealed NOAELs that were in line with the JECFA group ADI of 0–2 mg kg^{-1} bw for lutein from *Tagetes* erecta and synthetic zeaxanthin. From a one-year monkey study, it could be concluded that 20 mg zeaxanthin kg^{-1} bw day^{-1} does not cause adverse effects in the eyes. Several studies in human subjects did not find adverse effects for daily doses between 10 and 30 mg zeaxanthin.

A worst case calculation for zeaxanthin intake from eggs (daily intake of 100 g egg, 10 mg zeaxanthin kg^{-1} egg yolk) results in a potential human daily intake of 0.27 mg (0.005 mg kg^{-1} bw day^{-1}).

A realistic estimate of zeaxanthin intake from eggs is derived from SCOOP consumption data (high egg intake 36 g day^{-1}) and the zeaxanthin contents of eggs collected in a field survey.

Zeaxanthin intake from organic eggs would then correspond to 0.1 mg day^{-1}, from cage eggs 0.03 mg day^{-1}. The contribution of eggs to the maximum total human intake (1.8 mg) is then calculated to be between 2 % (cage husbandry) and 6 % (organic production).

No data is available to asses the zeaxanthin intake from edible poultry tissues.

Taking into account the human zeaxanthin intake from all sources, the contribution from food of animal origin (eggs and poultry tissues produced with zeaxanthin containing diets) would be a very small proportion of the total intake which varies with the consumption pattern in different countries. It does not require a particular safety assessment.

Taking into account the abundant occurrence of zexanthin in the terrestrial environment, the FEEDAP Panel considers it unlikely that the use of zeaxanthin in poultry feed at concentrations adequate for egg and skin colouring would affect the environment.

5. Concluding remarks and recommendations related to red and yellow carotenoids

5.1. Specifications

Specifications are given for the carotenoids/xanthophylls which are also authorised in the EU as food additives.

(i.) For the use of those carotenoids/xanthophylls as feed additives, the existing specifications appear insufficient because the percentage of total colouring matter in the additive (preparations, extracts) is not specified. The user of such additives who is obliged to consider the maximum content in feed must know the maximum concentration of the active substance (alternatively of total colouring matter) to avoid overdosage.

(ii.) The specification for capsanthin (paprika oleoresins) introduces by definition also capsorubin as active substance, although it is not expressis verbis authorised.

(iii.) Carotenoids/xanthophylls not authorised as food additives lack any specifications.

(iv.) The FEEDAP Panel recommends adjusting/supplementing the specifications taking into account the above remarks and considering also internationally recognised specifications.

(v.) Specifications should include identity and purity of the respective products. This information will also be necessary for the safety for the user, which can only be assessed on a product-specific basis.

5.2. Efficacy

All the carotenoids/xanthophylls assessed, except cryptoxanthin, are shown to be effective for the approved purposes in pigmenting products of animal origin: astaxanthin (and canthaxanthin) in colouring flesh, citranaxanthin in pigmenting the egg yolk, the others (including canthaxanthin) in colouring egg yolk and skin of chickens for fattening. They are deposited in tissues or products as such or as metabolites.

(i.) As an alternative to canthaxanthin, astaxanthin has already reached a dominant position in colouring the flesh of salmonids. Concerning egg yolk pigmentation, citranaxanthin is considered as an equivalent alternative, and to a minor extent capsanthin/capsorubin (paprika oleoresins). For skin colouring, canthaxanthin still appears as the dominant red-colouring pigment, the database to conclude on capsanthin/capsorubin being too scarce.

(ii.) β-cryptoxanthin is not considered effective in pigmenting poultry eggs and tissues.

5.3. Maximum content

The legislation sets the maximum contents for individual carotenoids and xanthophylls at 80 mg kg^{-1} of complete feedingstuffs (“alone or with the other carotenoids and xanthophylls”). The exception to this is astaxanthin (100 mg kg^{-1}, the maximum of astaxanthin with canthaxanthin is allowed provided that the total concentration of the mixture does not exceed 100 mg kg^{-1} in the complete feedingstuffs; for the astaxanthin-rich *Paracoccus carotinifaciens* the maximum content is expressed as the sum of astaxanthin, adonirobin and canthaxanthin).

(i.) The maximum content given by legislation for the individual carotenoids/xanthophylls (80 mg kg^{-1} diet) are not required for optimum pigmentation, except astaxanthin.

(ii.) The definition of the maximum content (‘together with the other…’) includes theoretically also -carotene, which is a carotenoid but a nutritional additive without colouring properties.

(iii.) The FEEDAP Panel recommends (i) excluding by definition -carotene from the colouring carotenoids/xanthophylls, (ii) introducing maximum contents for the individual carotenoids/xanthophylls supplemented by maximum contents for red and yellow colouring carotenoids/xanthophylls.

(iv.) The FEEDAP Panel reinforces its recommendation from Part II of the assessment, according to which the amount of each red or yellow carotenoid/xanthophyll should be calculated in proportion to the maximum content authorised when a mixture of red or yellow carotenoids/xanthophylls is used (see Section 5.7).

Due to the uncertainties in the safety assessment (see Section 5.6.2), the maximum content should consider (i) avoidance of any unnecessary exposure of the target animal and the consumer and (ii) limitation of the maximum content to a dose providing the desired effect.

The maximum contents proposed in the assessment are based on the effect of the individual use of each carotenoid. However, in feeding practice, yellow and red carotenoids are used together (see Section 1.6, Part I) in colouring eggs and skin to optimise the colour of the product. Considering this combined use of the carotenoids/xanthophylls would allow a further reduction of the maximum content proposed for the yellow carotenoids.

5.4. Safety for the target animal

Information on the tolerance of the target animals to carotenoids/xanthophylls is scarce. However, considering the natural occurrence of carotenoids/xanthophylls approved as colourants and the structural similarity of those substances, the FEEDAP Panel does not see reasons for concerns. It seems unnecessary to restrict the use of astaxanthin to a particular developmental stage.

5.5. Exposure assessment

Food products from animals treated with carotenoids/xanthophylls are not the only sources of those carotenoids/xanthophylls and in most cases are not the predominant ones. The databases to calculate exposure from all sources are incomplete. This is a source of uncertainty.

5.6. Safety aspects

In 2000, the SCF decided to withdraw the existing group ADI of 0–5 mg kg^{-1} bw $day^{-1}$for β-carotene and carotenoids. This was based on the adverse effects of β-carotene observed in human studies conducted in male smokers and the fact that the ADI relied on animal studies shown to lack relevance with regard to human risk assessment.

Since then, no ADI has been allocated on a European basis to carotenoids/xanthophylls, except for canthaxanthin. For canthaxanthin, an ADI of 0-0.03 mg kg^{-1} bw day^{-1} was established by the SCF (1997), derived from the NOAEL in electroretinographic studies in human volunteers.

5.6.1. The canthaxanthin effect on the eye

Crystalline deposits in the human retina were first described in the literature in 1982. As a result, the ADI was subsequently reduced to 0–0.5 mg kg^{-1} bw day^{-1} by the SCF, in 1989, and to 0–0.03 mg kg^{-1} bw day^{-1} by JECFA, in 1995, and by SCF, in 1997 (SCAN, 2002).

Comparable studies as for canthaxanthin are not available in literature for the structurally closely related carotenoids adonirobin (present in astaxantin-rich *Paracoccus carotinifaciens*) and astaxanthin. When assessing exposure to those related carotenoids, the FEEDAP Panel recommended adonirobin to be included in the canthaxanthin ADI (introduced in the legislation in 2008 by Regulation (EC) No 775/2008[10]).

Although there exists currently no evidence that astaxanthin (and adonirobin) would cause the same effect in the human retina, there are no data to support the opposite conclusion. A final safety evaluation of astaxanthin should be based on an ADI which could not be established without information regarding canthaxanthin-related effects in the retina.

5.6.2. β-carotene and lung cancer

At present, an ADI cannot be allocated to the carotenoids/xanthophylls due to the withdrawal of the previous group ADI for β-carotene, including carotenoids/xanthophylls, because of the relation between β-carotene and lung cancer in humans and the uncertain role of the other carotenoids (JECFA, 2006a; SCF, 2000).

As long as no other (structurally related to β-carotene) carotenoids/xanthopylls have been tested in a suitable animal model, the conclusion on consumer safety based on total exposure would retain a degree of uncertainty.

[10] OJ L 207, 5.8.2008, p. 5

The FEEDAP Panel recommends therefore the maximum content authorised for the individual carotenoids/xanthophylls be adjusted to the levels required to the desired effect in animal products.

5.6.3. User safety

No studies on the user safety for carotenoids/xanthophylls could be found. Because user safety can only be assessed on a product-specific basis (particularly data on dusting potential), user safety can be only fully assessed as required by Regulation (EC) No 429/2008, during the re-evaluation process.

5.6.4. Safety for the environment

All colouring agents under consideration are natural substances or with close structural relation (e.g. esters). Taking also into account the susceptibility to degradation of carotenoids, the FEEDAP Panel considers it unlikely that the use of these substances as feed additives will pose a risk for the environment. However, locally restricted scenarios might deserve particular consideration.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier submitted by FEFANA, June 2005 (Information regarding the safety of use of Carotenoids for the consumers of products from animal fed with feeds containing Carotenoids – Part ‘Yellow carotenoids’)

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