Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request from the Commission on the safety of use of colouring agents in animal nutrition

PART II. Capsanthin, Citranaxanthin, and Cryptoxanthin

(Question No. EFSA-2003-060)

Adopted on 12 September 2006

SUMMARY

Capsanthin [(3R,3’S,5’R)-3,3’-dihydroxy-β,kappa-caroten-6’-one] occurs naturally together with some other xanthophylls (zeaxanthin, lutein and capsorubin) and β-carotene in Capsicum fruits. Capsanthin is not available for the feed market as a pure substance but comes from paprika oleoresins, which contains also capsorubin and the other paprika carotenoids. Capsanthin/ capsorubin colours the egg yolk when fed to laying hens and the skin of broilers.

For laying hens, a level of 16 mg paprika carotenoids kg⁻¹ complete feed has been found to be effective in colouring egg yolks up to a yolk colour fan value of 13. The colouring capacity of capsanthin in relation to canthaxanthin is given as 1:2. While 8 mg canthaxanthin kg⁻¹ complete feed for laying hens is approved, 16 mg capsanthin kg⁻¹ layer feed could be considered enough as maximum content. This is also confirmed by studies with capsanthin on laying hens. Data on capsanthin or paprika xanthophylls for skin pigmentation is too scarce to formulate a conclusion. However, an upper level of 50 mg capsanthin, for poultry other than laying hens, appears to be enough for skin pigmentation, based on the maximum content approved for canthaxanthin and assuming the same relative efficacy of capsanthin as for laying hens. The existing definitions of the additive as capsanthin and as E160c (paprika oleoresins containing also capsorubin) in the current EU legislation are contradictory. It is therefore recommended to set the maximum contents for capsanthin alone and as a separate entry for the sum of capsanthin and capsorubin (E160c).

Data on the safety of capsanthin/capsorubin for the target animals are not available. However, the FEEDAP Panel does not see reasons for concern.

Based on the limited dataset available, no toxic effects have been reported in humans or in animals following oral exposure. Paprika is not genotoxic. The same conclusion has consequently been drawn for its constituents, capsanthin/capsorubin. An ADI or an UL has not been allocated for capsanthin/capsorubin.

The FEEDAP Panel concluded that there is insufficient data for a quantitative risk assessment. Consumption figures for Capsicum spp. as vegetable sources of capsanthin in human diet are not available for the EU Member States. A scenario calculated from the available data showed that daily intake of capsanthin/capsorubin by 100 g egg could amount to 90 µg capsanthin/capsorubin (16 mg paprika carotenoids kg⁻¹ diet) and to another 360 µg capsanthin (20 mg paprika xanthophylls kg⁻¹ diet) by 90 g skin, which is equivalent to a maximum of 21.4 (4.3 +17.1) mg of paprika oleoresins (E160c). On the basis
of this scenario, the FEEDAP Panel considers it likely that capsanthin/capsorubin derived from poultry fed paprika oleoresins at concentrations adequate for egg and skin colour will negligibly contribute to total human exposure.

Data on safety for the user was not available. Skin irritation reports are related to allergic patients only and do not allow conclusions on general irritation or sensitisation potential of the additive.

No data is available for a qualified assessment of the environmental impact of capsanthin/capsorubin used in poultry feed. However, given the oxidative susceptibility of the compound and its use in poultry feeding, the FEEDAP Panel considers that capsanthin/capsorubin presents a low to negligible risk for the environment.

**Citranaxanthin** (6'-methyl-6'-apo-β-caroten-6'-one), naturally occurring in the peel of citrus fruits and also available as synthetic product, acts as a vitamin A precursor in poultry (potency about two thirds of β-carotene).

Citranaxanthin is an effective additive for colouring eggs. Its relative efficacy to canthaxanthin in pigmenting egg yolks is 0.67. Nine mg citranaxanthin would provide a yolk colour fan value of slightly above 13. Regarding the maximum dose approved for canthaxanthin as well as deeper red pigmented eggs for pasta production, a maximum content of 12 mg citranaxanthin kg⁻¹ complete feed for laying hens appears to be enough. There is no need for an approval of higher concentrations.

The FEEDAP Panel does not see concern for the safety for laying hens, although data is not available.

Current data on the toxicity of citranaxanthin tested in laboratory animals do not raise concern for consumer safety. However, the available data on several aspects of toxicity, including metabolic fate and mutagenicity, are insufficient for the establishment of an ADI and a full assessment of both consumer and user safety. In contrast to other xanthophylls approved, it is likely that there is no considerable intake of citranaxanthin other than from eggs in human diet. More recent data on market eggs shows a daily citranaxanthin intake of about 0.15 mg by 100 g egg consumption. The FEEDAP Panel sees no urgent need for action but recommends a full re-evaluation of the compound according to current standards.

Citranaxanthin shows no inhalation toxicity and no irritancy for the eye.

No data is available for a qualified assessment of the environmental impact of citranaxanthin used in layer feed. However, given the oxidative susceptibility of the compound and its use in layer feeding, the FEEDAP Panel considers that citranaxanthin presents a low to negligible risk for the environment.

**Both isomers of cryptoxanthin, β- and α-cryptoxanthin** [(3Rβ,β-caroten-3-ol) and [(3'R,6'R)-β-, epsilon-caroten-3'-ol] are abundant in nature, especially in fruits. Their occurrence in fruits is so specific that β-cryptoxanthin in human serum can serve as a predictor of fruit intake. Natural β-cryptoxanthin sources for laying hens are feedingstuffs such as corn and alfalfa. β-cryptoxanthin is characterised by vitamin A activity.

The FEEDAP Panel could not find any information on the use of cryptoxanthin in pigmenting yolks or poultry tissues. There are serious doubts concerning the pigmenting capacity of β-cryptoxanthin because of its low intestinal absorption in poultry.

Considering the absence of commercially available β-cryptoxanthin for poultry diets and its questionable, if any, efficacy in pigmenting poultry eggs and tissues, the FEEDAP Panel sees no reason at present to maintain the approval of β-cryptoxanthin as a sensory additive.
Acute toxicity data could not be found. \(\beta\)-cryptoxanthin appears not to be mutagenic nor clastogenic. In three subchronic studies (one on mice and two on rats, for 21, 36, and 30 weeks, respectively) originally designed for testing anti-tumour effects of the substance, adverse effects of 13.7, 4.7, and 25 mg \(\beta\)-cryptoxanthin kg\(^{-1}\) bw were not observed. An ADI has not been allocated. In the European countries, there is considerable human intake of \(\beta\)-cryptoxanthin from vegetables, ranging from 0.05 mg to 1.36 mg day\(^{-1}\), depending on eating habit. In epidemiological studies, \(\beta\)-cryptoxanthin is predominantly considered as beneficial in preventing various diseases (cancer incidence, oxidative DNA damage and lipid peroxidation, early athero-sclerosis, and inflammatory polyarthitis). The FEEDAP Panel considers therefore \(\beta\)-cryptoxanthin as safe for the consumer.

The contribution of eggs to human \(\beta\)-cryptoxanthin intake varies. Recent data on market eggs indicate that a daily consumption of 100 g eggs supplies 0.02 mg \(\beta\)-cryptoxanthin.

As the product is not commercially available, no user safety data has been generated.

According to Directive 2001/79/EC, environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, as its use as a feed additive will not alter the concentration or distribution in the environment. As cryptoxanthin occurring abundantly in plants and in terrestrial environment, no environmental risk assessment is necessary.

Finally, the FEEDAP Panel recommends reconsidering the current practice of the European Commission to set a maximum content for these colouring substances “alone or with other carotenoids and xanthophylls”. This does not reflect indeed the practice in poultry feeding. Yellow and red pigments are considered separately in feed formulation, \(\beta\)-carotene can also be used as vitamin A source (nutritional additive). The FEEDAP Panel suggests therefore that when a mixture of red pigmenting xanthophylls is used, the amount of each xantophyll should be calculated in proportion to the maximum content authorised.

**Key words:** Carotenoids, capsanthin, capsorubin, citranaxanthin, cryptoxanthin, pigments, egg yolk colour, skin pigmentation, consumer safety, user safety
BACKGROUND

In its opinion of April 2002 on the use of canthaxanthin in feedingstuffs, the SCAN suggested that the required levels of canthaxanthin should be reviewed in order that human exposure to canthaxanthin may remain within the Acceptable Daily Intake (ADI) established for that compound. The lowering of 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 a report on an astaxanthin-rich product, the SCAN drew the attention of the European Commission to the fact that no risk assessment had ever been carried out and that no ADI had been established for carotenoids other than canthaxanthin.

TERMS OF REFERENCE

In the light of these opinions on 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’carotenic acid (E160f), lutein (E161b), cryptoxanthin (E161c), zeaxanthin (E161h), citranaxanthin (E161i), and astaxanthin (E161j) in feedingstuffs for laying hens, other poultry, salmon and trout on the basis of currently available scientific literature.

In its assessment, EFSA is requested to prioritise the substances which may be used as alternatives to canthaxanthin.
### Table 1. Annex Entry

<table>
<thead>
<tr>
<th>EC No.</th>
<th>Additive</th>
<th>Chemical formula, description</th>
<th>Species or category of animal</th>
<th>Maximum age</th>
<th>Minimum content</th>
<th>Maximum content</th>
<th>Other provisions</th>
<th>End of period of authorisation</th>
</tr>
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<tr>
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<td>Colourants including pigments</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1. Carotenoids and xanthophylls</td>
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</tr>
<tr>
<td>E 160c</td>
<td>Capsanthin</td>
<td>C₄₀H₅₆O₃</td>
<td>Poultry</td>
<td>-</td>
<td>-</td>
<td>80 (alone or with the other carotenoids and xanthophylls)</td>
<td>-</td>
<td>Without a time limit</td>
</tr>
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<td>E 160e</td>
<td>Beta-apo-8’-carotenal</td>
<td>C₂₅H₄₀O</td>
<td>Poultry</td>
<td>-</td>
<td>-</td>
<td>80 (alone or with the other carotenoids and xanthophylls)</td>
<td>-</td>
<td>Without a time limit</td>
</tr>
<tr>
<td>E 160f</td>
<td>Ethyl ester of beta-apo-8’-carotenoic acid</td>
<td>C₃₂H₄₄O₂</td>
<td>Poultry</td>
<td>-</td>
<td>-</td>
<td>80 (alone or with the other carotenoids and xanthophylls)</td>
<td>-</td>
<td>Without a time limit</td>
</tr>
<tr>
<td>E 161b</td>
<td>Lutein</td>
<td>C₄₀H₅₆O₂</td>
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<td>-</td>
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<td>Without a time limit</td>
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<td>Cryptoxanthin</td>
<td>C₄₀H₅₆O</td>
<td>Poultry</td>
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<td>-</td>
<td>80 (alone or with the other carotenoids and xanthophylls)</td>
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<td>Without a time limit</td>
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<td>Zeaxanthin</td>
<td>C₄₀H₅₆O₂</td>
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<td>-</td>
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<td>E 161i</td>
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<td>C₃₃H₄₄O</td>
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<td>Without a time limit</td>
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<td>Astaxanthin</td>
<td>C₄₀H₅₅O₄</td>
<td>Salmon, trout</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>Use only permitted from the age of 6 months onwards.</td>
<td>Without a time limit</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>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.</td>
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<tr>
<td></td>
<td>Ornamental fish</td>
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<td>-</td>
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<td>Without a time limit</td>
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Assessment of Colouring Agents in Animal Nutrition

Following the Commission request to conduct a safety assessment and prioritise the carotenoids which may be used as alternatives of canthaxanthin, the FEEDAP Panel focused in a first step on general principles and the red-colouring carotenoid Asthaxanthin (EFSA, 2005). The following is the second of a series of documents dealing with the assessment of carotenoids that has been prepared by the FEEDAP Panel and deals with Capsanthin, Citranaxanthin and Cryptoxanthin.

1. Capsanthin (E160c)

Capsanthin is listed in the Community Register of Feed Additives according to Regulation (EC) No 1831/2003\(^1\) under the sub-classification carotenoids and xanthophylls. It is an approved sensory additive for poultry.\(^2\) Its single entry consists of two definitions: as E160c, according to the specifications of the European Union,\(^3\) which is Paprika extract, and as capsanthin, with the molecular formula C\(_{40}\)H\(_{56}\)O\(_3\). The maximum content is 80 mg kg\(^{-1}\) complete feed, alone or in combination with other carotenoids and xanthophylls.

1.1. Specification of E160c

According to the specifications of the European Union, the colouring compound E160c is Paprika extract (capsanthin, capsorubin, known also under the synonym of paprika oleoresin) obtained by solvent extraction of the natural strains of paprika (Capsicum annuum L.), which consists of the ground fruit pods, with or without seeds, and contains the major colouring principles of this substance (capsanthin and capsorubin). A wide variety of other coloured compounds is known to be present.

Paprika extract should not contain less than 7.0% of total carotenoids. The content of capsanthin/capsorubin should not be less than 30% of total carotenoids and capsaicin not more than 250 mg kg\(^{-1}\). The EU specification also reports information on the following contaminants: 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 40 mg kg\(^{-1}\)).

Based on the above data and the legal status, Paprika extract can be added to a maximum amount of 1.14 g kg\(^{-1}\) feed, equal to 80 mg carotenoids and xanthophylls and to 24 mg capsanthin/capsorubin.

Paprika oleoresin is produced from any Capsicum\(^4\) variety, but mostly excluding “chilli peppers” to create a product with wider applications in the food industry. Normally, extracts of dried fruits of Capsicum annuum are prepared by dehydration, solvent extraction, saponification and stabilisation and contain a mixture of different xanthophylls, mainly capsanthin/capsorubin, lutein and zeaxanthin (Nys, 2000). Since in the analysis of

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\(^1\) [http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/index_en.htm](http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/index_en.htm) (last update 2/05/06)


\(^3\) OJ L 226, 22.9.1995 (Directive 95/45/EC laying down specific purity criteria concerning colours for use in foodstuffs)

\(^4\) Capsicum terminology is confusing. Pepper, chili, chile, chilli, aji, paprika, and Capsicum are used interchangeably for plants in the genus Capsicum. Capsicum investigators use chili, pepper, or aji, as vernacular terms. Capsicum is reserved for taxonomic discussion. Capsicum species are used fresh or dried, whole or ground, and alone or in combination with other flavouring agents. Paprika (sweet bell peppers, pimento) is derived from Capsicum annum L (plants with 10 to 30 mg capsaicinoids kg\(^{-1}\)). Chili pepper is produced from Capsicum frutescens L (plants with 30 to 600 mg capsaicinoids kg\(^{-1}\), red chili pepper from plants with 600 to 13,000 mg capsaicinoids kg\(^{-1}\))
xanthophylls of paprika oleoresins and derived products it is difficult to distinguish between capsanthin and capsorubin, the combined contents of capsanthin and capsorubin are reported in most experiments. Following a fractionated extraction with supercritical carbon dioxide, paprika oil and \( \beta \)-carotene are removed during the first extraction step, allowing for second stage oleoresin extracts (Jaren-Galan et al., 1999) with a high pigment concentration (200% relative to the standard extraction) and a red:yellow pigment ratio of 1.8 (as compared to 1.3 in the standard extraction).

### 1.1.1. Carotenoids and xanthophylls in Capsicum varieties

The total carotenoid pigments of red paprika ripe fruits (Capsicum annuum var. lycopersiciforme rubrum) was about 1.3 g 100 g\(^{-1}\) dry matter and composed as follows: capsanthin 37%, zeaxanthin 8%, cucurbitaxanthin A 7%, capsorubin 3.2%, and \( \beta \)-carotene 9%. The remaining part was composed of capsanthin 5,6-epoxide, capsanthin 3,6-epoxide, 5,6-diepikarpoxanthin, violaxanthin, antherexanthin, \( \beta \)-cryptoxanthin, and several cis-isomers and furanoid oxides (Deli et al., 2001). 11 apocarotenoids present in Capsicum annuum L. were assumed to be the oxidative cleavage products of C(40) carotenoids such as capsanthin (Maoka et al., 2001a). According to Hornero-Mendez and Minguez-Mosquera (2000) the Capsicum annuum content of free capsanthin plus capsorubin is about 24%, the ratio of both pigments depending on the ripening status of the fruit. Partially and totally esterified capsanthin/capsorubin amounts to 31% and 44%, respectively. In the same study, pepper fruit carotenoids were experimentally oxidized with a method using a free radical initiator and the rate of oxidation was reported (Perez-Galvez and Minguez-Mosquera, 2002). Capsorubin was degraded more slowly, followed by zeaxanthin, capsanthin, and \( \beta \)-carotene.

### 1.2. General characteristics of capsanthin and capsorubin

Capsanthin \([3R,3'S,5'R]-3,3'-dihydroxy-\( \beta \),kappa-caroten-6'-one\], CAS number 68917-78-2, has many commonly used synonyms like \( \beta \)-kappa-carotene, paprika extract, paprika oleoresin, capsanthin/capsorubin. The molecular formula of capsanthin is C\(_{40}\)H\(_{56}\)O\(_3\) and the molar mass is 584.85 g mol\(^{-1}\). The molecular structure of capsanthin, which has three chiral carbon atoms in the ring structures, is given in Figure 1. Capsanthin has two hydroxyl (OH) groups, one on each terminal ring, located at the 3 and 3' positions of the six and five member carbon rings, respectively (Figure 1). Capxanthin exists in a free (alcohol) or esterified form. A methyl group is located at the 5' position of the five carbon ring. The three chiral centers in the capsanthin molecule can exist either in R- or S- form and thus, there is in theory a total of eight isomers. Capsanthin has several double bonds in the linear portion of the molecule, each of which can potentially exist in the Z- or E- form. The thermodynamically most stable form of the molecule is all-E (all-trans) capsanthin. No Z-isomer has been reported in any position.

Capsanthin is hydrophobic and forms aggregates or adheres non-specifically to structural surfaces. It is practically insoluble in water but partially soluble in ethanol. The octanol/water partition value for capsanthin is not available. Capsanthin has a characteristic maximum absorption wavelength at 470 nm in hexane.
Capsanthin

Capsorubin

Figure 1. Molecular structures of capsanthin and capsorubin - The asymmetric carbon atoms are indicated by numbers 3, 3' and 5'.

Capsorubin [(3S,3'S,5R,5'R)-3,3'-dihydroxy-kappa,kappa-carotene-6,6'-dione] is a constituent of paprika oleoresin (Figure 1). The molecular formula is C_{40}H_{56}O_{4} and the molecular weight is 600.85 g. Capsorubin exhibits chemical characteristics similar to those of capsanthin.

1.2.1. Analytical methods

The CRL reports that no ISO and CEN methods could be found as official analytical method for the determination of cryptoxanthin.

Methods for the isolation and purification of carotenoids from natural sources, including capsanthin and capsorubin, are available based on HPLC and LC/MS. (Deli et al., 2001, Weissenberg et al., 1997, Breithaupt et al., 2003).

1.3. Capsanthin in poultry feeding

For the practical use in animal diets, capsanthin is not available as pure substance. Capsanthin for feeding exists only as paprika extract.

1.3.1. Sources and use

The European Feed Manufactures Federation (FEFAC, 2004) made available some non quantitative market data on the use of capsanthin, covering responses from nine EU Member States. According to these data, capsanthin is used by the majority of feed manufacturers in only one Member State, by about half of the feed manufacturers in another Member State and in a small proportion in five Member States. In two Member States, it is not used. A combination of capsanthin with lutein, zeaxanthin and canthaxanthin is reported to be used in five Member States.

Normal capsanthin/capsorubin supplementation levels to layer diets cannot be given, as the pigment is not commonly used in egg production in Europe. However, paprika extract plays a certain role in organic egg production.
1.3.2. Effects on egg yolk colour

Grashorn et al. (2001) tested two preparations of paprika oleoresins and confirmed a linear increase in yolk colour due to addition of paprika oleoresins. Capsanthin/capsorubin had to be fed in concentrations two to three times higher than canthaxanthin to reach the same yolk colour, measured by DSM-YCF (yolk color fan).\(^5\)

For achieving an egg yolk colour of 12-13 (DSM-YCF scale), 8 to 16 mg total carotenoids from paprika oleoresins have to be supplemented to corn/soybean diets (Table 2). Lai et al. (1996) found a linear increase in the deposition of carotenoids from paprika oleoresins or saponified paprika oleoresins in yolk when supplementing up to 16 mg paprika carotenoids kg\(^{-1}\) diet.

Table 2. Effect of esterified and free carotenoids of paprika oleoresins on yolk colour and carotenoid content of yolks (Lai et al., 1996)

| Carotenoid source | Paprika carotenoids* (mg kg\(^{-1}\) diet) | DSM-YCF | Total carotenoids** (mg per egg***)
<table>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>4.6</td>
<td>0.087</td>
</tr>
<tr>
<td>Paprika oleoresin</td>
<td>4</td>
<td>9.3</td>
<td>0.128</td>
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<tr>
<td></td>
<td>8</td>
<td>11.2</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>13.5</td>
<td>0.219</td>
</tr>
<tr>
<td>Saponified paprika oleoresin</td>
<td>2</td>
<td>6.7</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.3</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11.2</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>13.0</td>
<td>0.201</td>
</tr>
</tbody>
</table>

* Diets with 4, 8 and 16 mg paprika carotenoids may contain 1.9 mg, 4.8 mg and 10.9 mg capsanthin, respectively

** Total carotenoids: sum of lutein, zeaxanthin and capsanthin/capsorubin

** Standardised egg weight 60 g, yolk percentage 27%

In contrast to the data of Hamilton et al. (1990), which demonstrated that the saponified carotenoids from paprika oleoresins were deposited in the egg yolk twice as efficiently as those from unsaponified, Lai et al. (1996) could not show significant differences between the two preparations in colouring egg yolk.

Antony et al. (2004) found DSM-YCF values of about 12, already with a supplementation of only 2.3-2.8 mg trans-capsanthin from saponified paprika oleoresins kg\(^{-1}\) complete diet containing 10.4-11.2 mg total carotenoids kg\(^{-1}\).

Another study reports that hens fed 7.5 mg yellow xanthophylls extracted from Tagetes and 4 mg red xanthophylls from Capsicum kg\(^{-1}\) diet, had shown egg yolks with DSM-YCF values of 11.7. In addition, this study shows that Capsicum extracts had a linear effect on the DSM-YCF values and that synthetic carotenoids (2.5 mg apo-carotenoic ester and 1.5 mg canthaxanthin kg\(^{-1}\)) gave a DSM-YCF value of 12.5. The authors concluded that at least three times more xanthophylls from natural sources are necessary to obtain the same egg yolk pigmentation as compared with the synthetic ones (Santos-Bocanegra et al., 2004).

1.3.3. Effects on tissue colour

To the knowledge of the FEEDAP Panel, few references are available on the use of capsanthin for pigmenting tissues in poultry meat production. Erkek et al. (1993) supplemented a low xanthophyll diet (10 mg kg\(^{-1}\)) with 2.5% paprika meal, which resulted in

---

\(^5\) Roche Yolk Colour Fan (RYCF) nowadays called DSM-YCF
a total xanthophyll content of 30 mg kg⁻¹ diet. The a* (redness) and b* (yellowness) values obtained for skin were lower than for the group fed a diet supplemented with canthaxanthin (30 mg total xanthophylls kg⁻¹), which indicates a lower pigmenting efficiency of capsanthin/capsorubin.

### 1.3.4. Deposition of capsanthin/capsorubin in egg and tissues

Deposition rates given for capsanthin vary highly according to different literature data (6-29%). The reason for this may be that the sources used for paprika oleoresins were markedly different in their purity and colouring abilities and varied in their capsanthin content (Table 3).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Deposition (%)</th>
<th>Reference</th>
<th>Deposition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>El Boushy and Raterink, 1992</td>
<td>6</td>
<td>Steinberg et al., 2000</td>
<td>7.3</td>
</tr>
<tr>
<td>Huyghebaert, 1993a</td>
<td>8</td>
<td>Lai et al., 1996</td>
<td>17</td>
</tr>
<tr>
<td>González et al., 1999</td>
<td>18-25</td>
<td>Hamilton et al., 1990</td>
<td>29</td>
</tr>
</tbody>
</table>

The yolk deposition yield of capsanthin in relation to canthaxanthin is given as 1:3 (Terashima and Tsurumaki, 1988) to 1:5 (Huyghebaert, 1993b) and 1:2 for colouring capacity (Huyghebaert, 1993b; Beardsworth and Hernandez, 2004).

The capsanthin/capsorubin in egg yolk may amount to a maximum of 32-53 µg per egg yolk of a standardised egg, calculated from the data of Lai et al. (1996) at DSM-YCF values between 11 and 13 (8-16 mg paprika carotenoids). Precise data is not available.

Another estimation can be derived from the assumptions that: (i) DSM-YCF values of 11-13 require 8-16 mg total carotenoids from paprika oleoresins kg⁻¹ diet (Lai et al., 1996); (ii) total carotenoids of paprika oleoresins contain at least 30% capsanthin/capsorubin (specification of 160c); (iii) mean deposition rate of capsanthin/capsorubin is 15% (average of Table 3), and; (iv) standardised egg weight 60 g, yolk percentage 27%. This calculation leads to 6-12 µg capsanthin/capsorubin per standardised egg (10-20 µg 100 g⁻¹ egg). If the same calculation is based on the highest deposition rate reported (29%), capsanthin/capsorubin in the standardised egg would be 12-24 µg (20-40 µg 100 g⁻¹ egg). The difference to the above estimation may be due to different carotenoids contents and purities of paprika oleoresins.

Erkek et al. (1993) determined a content of 400 µg capsanthin per 100 g skin when supplementing diets with 20 mg paprika xanthophylls kg⁻¹. No deposition rate could be calculated from the reported data.

### 1.4. Metabolism of Capsanthin

Free and esterified capsanthin are absorbed by the chicken leading to similar plasma free capsanthin concentrations (Breithaupt et al., 2003) which is consistent with the suggestion that carotenoid ester hydrolysis is indispensable prior to absorption (Bowen et al., 1993; Furr and Clark, 1997; Wingerath et al., 1995).

No data are available concerning the metabolic fate of capsanthin in poultry or laboratory animals. After feeding laying hens with synthetic (3S,5R,3'S,5'R)-capsorubin, 14 fully characterized metabolites of almost identical chromophore, but with diverse polarities...
ranging from monosulfate to fatty acid monoesters, were identified (review from Schiedt, 1998).

1.4.1. Absorption of paprika carotenoids in humans

Oshima et al. (1997) administered capsanthin rich paprika juice for one week (equivalent to three doses of 5.4 μmol capsanthin day\(^{-1}\) ~ 3.2 mg day\(^{-1}\)) to four male human volunteers. Plasma capsanthin level reached a plateau between day 2 and day 7 and was not detectable after 16 days. Capsanthin was distributed in the plasma lipoproteins (VLDL: 13%, LDL: 44%, HDL: 43%). Etoh et al. (2000) identified in the blood plasma of male human volunteers, after ingestion of paprika juice, capsanthin, capsanthone, cucurbitaxanthin A, 11 cis-capsanthin, lutein and zeaxanthin. In another study (Perez-Galvez et al., 2003), nine volunteers ingested after overnight fasting a single dose of paprika oleoresin containing 6.4 mg zeaxanthin, 4.2 mg \(\beta\)-cryptoxanthin, 6.2 mg \(\beta\)-carotene, 35.0 mg capsanthin and 2.0 mg capsorubin. From the major carotenoids in paprika oleoresin, only zeaxanthin, \(\beta\)-cryptoxanthin and \(\beta\)-carotene were detectable in considerable amounts. The authors concluded that the bioavailability of capsanthin and capsorubin from paprika oleoresins shown to be very low.

1.5. Human exposure

With regard to capsanthin in the diet, only data on paprika as the major dietary source are available. Concentration of capsanthin in paprika ranges from 20 mg to 400 mg/100 g dry matter, depending on the stage of ripening in paprika (Deli et al., 2001; Topuz and Ozdemir, 2003). Data on human paprika consumption in the EU Member States is not available.

1.6. Safety studies

Fruit and spice containing capsanthin and capsorubin are natural components of the diet. In some cultures (Mexican and Indian), daily intakes of 2.5 to 5 g of red pepper have been estimated (BIBRA 1987b; Srinivasan et al., 1980).

Capsanthin has been mentioned as one of the various natural carotenoids that may have beneficial health effects (Nishino et al., 2002; Maoka et al., 2001(b); Narisawa et al., 2000). Paprika oleoresins are the basis for the food colour additive capsanthin (E160c) for which no ADI was allocated. In 1990, JECFA did not establish an ADI as the use of paprika oleoresin is “self limiting as a spice extract” (JECFA 1990). Later on, JECFA stressed that this statement only refers to the use of the additive as a spice and that its use as a colouring agent was not evaluated (JECFA 2001). There is no reason to expect any adverse effect if exposure lies within the normal use of paprika as a food item. Safe doses may be derived from the estimated intake of capsanthin in human diet. The Nordic working group evaluating food additives in Europe (TemaNord, 2002) concluded that:

"The use of paprika extract as a food colour has not been evaluated by SCF or JECFA and no toxicological data exist on this colour. However, as paprika is a normal constituent of food as a spice, there is no reason to expect undesirable side effects from its use as a food colour. Especially so as the specifications exclude the content of capsaicin (EU max 250 mg kg\(^{-1}\) paprika extract (EU, 1995) and JECFA max 5 g kg\(^{-1}\) (JECFA, 1992). Capsaicin is the constituent, which gives strong paprika its pungent taste and which has been reported to possess toxic properties. There are significant differences between the EU specification and the JECFA specification, the former being the most restrictive. The EU specification defines the preparation sold and used as a food additive.” Thus, only low levels of the severely irritant,
pharmacologically active and possibly mutagenic capsaicin (BIBRA, 1987b) are generally present in the paprika based colouring.”

1.6.1. Acute Toxicity
The acute oral toxicity in mice was reported to be low, with an LD50 for natural paprika colour exceeding 11.25 g kg⁻¹ bw (Noda et al., 1984; Hallagan et al., 1995). At the dose of 11.25 g kg⁻¹ bw, body weight gain was slightly suppressed, which was not observed at a dose of 5.0 or 7.5 g kg⁻¹ bw (Noda et al., 1984).

1.6.2. Genotoxicity
BIBRA concluded that any mutagenicity associated with paprika fruit or spice is due, at least in part, to components other than capsorubin and capsanthin (BIBRA 1987b).

A study on the mutagenicity of commercial natural food colours, including capsanthin, based on the Ames test with TA 100 and TA 98 (with and without S9) was published (Yasui et al., 1982). Paprika extract at 10 mg per plate was negative in all tests. A study on the effects of paprika extract on chromosome morphology of mammalian cells in vitro was conducted by Goodpasture and Arrighu (1976). Water solubilized powdered paprika and water soluble filtered paprika extracts added to cell cultures induced some alterations in chromosome morphology, notably chromosome condensation, indications of chromosome banding, chromosome breakage, fragmentation and disintegration, mitotic arrest, formation of micronuclei and reduction in nucleic acid synthesis, which were related to the overall toxicity of the samples.

On the basis of the evaluation of results abstracted from literature, Ishidate et al. (1988) concluded that capsanthin (2 mg mL⁻¹) as well as paprika extract (2 mg mL⁻¹) were negative in tests for clastogenicity in mammalian cell cultures. Ishidate et al. (1984) performed Salmonella/microsome tests (Ames tests) and chromosome aberration tests in vitro, using a hamster fibroblast cell line on a large series of food additives, including paprika dye, which tested negative at a dose of 0.25 mg mL⁻¹ in both tests.

Results of the effect of food additives in the rec-assay, using spores of Bacillus subtilis strains H17 Rec+ and M45 Rec-, supplemented with S9 homogenates for metabolic activation, were reported by Ueno et al. (1983). Paprika colour at 5 and 25 mg per disk was found to be negative in this test.

Hallagan et al. (1995) concluded that a weight-of-evidence analysis indicates that paprika is not genotoxic.

A recent study investigated the potential genotoxicity of pure capsaicin (a constituent of paprika oleoresins) in bacterial mutation, chromosome aberration and rodent micronucleus tests. The aim of the study was to clarify some previous conflicting reports on the potential genotoxicity of capsaicin (Proudlock et al., 2004), known to be present at low level in capsanthin preparations (up to not more than 250 mg kg⁻¹ according to EU specifications). The results of the study confirm the absence of genotoxic activity of high purity capsaicin in the bacterial mutation and chromosome aberration tests. Furthermore, no evidence for cytotoxicity or genotoxicity was observed in the rat bone marrow micronucleus test. The authors concluded that pure capsaicin is not active in a series of standard genotoxicity assays and that the previously reported in vitro genotoxic activity of capsaicin is probably due to mutagenic impurities.
1.6.3. (Sub-) chronic Toxicity Studies

Two studies were available. Sambaiah and Satyanarayana (1982) could not detect significant changes in body weight or liver weight, carcass composition or liver lipids after feeding rats with about 5 g paprika spice kg\(^{-1}\) bw day\(^{-1}\) for eight weeks.

Narisawa et al. (2000) studied the capsanthin mediated prevention of N-methylnitrosourea-induced colon cancer. F344 rats received up to 10 mg kg\(^{-1}\) bw capsanthin solution for 30 weeks. Although these studies were not designed to investigate adverse effects, such effects were not reported.

1.6.4. Reproductive Toxicity Studies, including developmental toxicity

No data were available.

1.6.5. Human data

No data on oral exposure is available. A BIBRA working group evaluating toxicity profiles of capsanthin and capsorubin (BIBRA 1987b) reported that a low incidence of local reactions in dermatitis patients was observed in skin tests with paprika. Three out of 336 dermatitis patients reacted to paprika powder in a 24 h patch test (Niinimaki, 1984). Skin scratch tests with paprika powder were also positive in 59 out of 894 patients susceptible to allergenic agents (atopic patients), but no reactions were seen in 362 normal individual (Niinimaki and Hannuksela, 1981). Sastre et al. (1996) reported a case of development of IgE antibodies specific to paprika dust in a person who was previously diagnosed with allergy to coconut, banana and kiwi. However, no cases have been reported after intake of food containing paprika extract (TemaNord, 2002).

1.7. Risk Assessment

There is not enough data on capsanthin/capsorubin available to perform a precise risk assessment. In the European poultry industry, capsanthin/capsorubin plays a minor role among the red pigmenting xanthophylls. It is mainly used (as paprika oleoresin) in organic poultry (egg) production.

Common literature gives the total carotenoid content in dried paprika of 0.1 to 0.5%, of which 30-60% are capsanthin/capsorubin. Dried paprika may therefore contain between 0.03 and 0.30% capsanthin/capsorubin. Other references (Deli et al., 2001; Topuz and Ozdemir, 2003) give the concentration of capsanthin in dried paprika of 0.02 to 0.40%. Human consumption data are not available.

Based on the limited dataset available, no toxic effects have been reported in humans or in animals following oral exposure. Paprika is not genotoxic. The same conclusion has consequently been drawn for its constituents, capsanthin/capsorubin. An ADI or an UL has not been allocated for capsanthin/capsorubin.

A worst case scenario calculation\(^6\) shows that 100 g egg may contain a maximum of 90 µg capsanthin/capsorubin (Lai et al. (1996); 16 mg paprika carotenoids kg\(^{-1}\) diet). This corresponds to a human intake of 4.3 mg paprika oleoresins (E160c).

Concerning capsanthin content in skin, only one reference was available (Erkek et al., 1993). A scenario calculation shows that 90 g skin would contain 360 µg capsanthin (20 mg paprika xanthophylls kg\(^{-1}\) diet), which is equivalent to 17.1 mg paprika oleoresins (E160c).

\(^6\) OJ L 267/1, 6.10.2001 (Directive 2001/79/EC)
Although consumption figures for *Capsicum annuum* and *Capsicum frutescens*, fresh or dried, and their preparations as vegetable sources of capsanthin in human diet are not available for EU Member States, the FEEDAP Panel, on the basis of the above scenarios, considers it likely that capsanthin/capsorubin derived from poultry fed paprika oleoresins at concentrations adequate for egg and skin colour will negligibly contribute to total human exposure.

### 1.8. Environment

No data are available for a qualified assessment of the environmental impact of capsanthin/capsorubin used in poultry feed.

According to Directive 2001/79/EC, environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, as its use will not alter the concentration or distribution in the environment. Capsanthin and capsorubin are substances naturally occurring in plants and the terrestrial environment.

Given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers it unlikely that the use of paprika oleoresins in poultry feed at concentrations adequate for egg and skin colour will have an impact on environment.

### 1.9. Conclusions and recommendations

#### 1.9.1. Conclusions

Capsanthin occurs naturally together with some other xanthophylls (zeaxanthin, lutein and capsorubin) and β-carotene in *Capsicum* fruits. Capsanthin (not available on the feed market as a pure substance) and capsanthin/capsorubin from paprika oleoresins colour the egg yolk when fed to laying hens, and the skin when fed to broilers.

The effective feed concentration for colouring egg yolks up to a DSM-YCF value of 13 is about 16 mg paprika carotenoids kg⁻¹ complete feed for laying hens.

The colouring capacity of capsanthin in relation to canthaxanthin is given as 1:2. While eight mg canthaxanthin kg⁻¹ complete feed for laying hens is approved, 16 mg capsanthin may suffice as upper feed level for laying hens. This is also confirmed by studies with capsanthin on laying hens.

Data for capsanthin or paprika xanthophylls for skin pigmentation is too scarce to conclude on a maximum content. Only on the basis of the maximum content approved for canthaxanthin and the assumption of the same relative efficacy of capsanthin compared to canthaxanthin as for laying hens, an upper level of 50 mg capsanthin for poultry other than laying hens appears sufficient.

Data on the safety of capsanthin/capsorubin for the target animals are not available. However, the FEEDAP Panel does not see reasons for concern.

Based on the limited dataset available, no toxic effects have been reported in humans or in animals following oral exposure. Paprika is not genotoxic. The same conclusion has consequently been drawn for its constituents, capsanthin/capsorubin. An ADI or an UL has not been allocated for capsanthin/capsorubin.

The FEEDAP Panel concluded that there is insufficient data for a quantitative risk assessment. Consumption figures for *Capsicum annuum* and *Capsicum frutescens*, fresh or dried, and their preparations (e.g. spices) as vegetable sources of capsanthin in human diet are not available for EU Member States.
A scenario calculated on the basis of available data shows that the daily intake of capsanthin/capsorubin by 100 g egg may amount to 90 µg capsanthin/capsorubin (16 mg paprika carotenoids kg⁻¹ diet) and to another 360 µg capsanthin (20 mg paprika xanthophylls kg⁻¹ diet) by 90 g skin, which is equivalent to a maximum of 21.4 mg (4.3 + 17.1) paprika oleoresins (E160c). Based on this scenario, the FEEDAP Panel considers it likely that capsanthin/capsorubin derived from poultry fed paprika oleoresins at concentrations adequate for egg and skin colour will negligibly contribute to total human exposure.

Data for the safety for the user are not available. Skin irritation reports are related to allergic patients only and do not allow to conclude on the general irritation or sensitization potential of the additive.

No data is available for a qualified assessment of the environmental impact of capsanthin/capsorubin used in layer feed. However, given the oxidative susceptibility of the compound and its use in layer feeding, the FEEDAP Panel considers that capsanthin/capsorubin presents a low to negligible risk to the environment.

1.9.2. Recommendations

The existing definitions in the current EU legislation of the additive capsanthin/capsorubin as capsanthin or E160c are contradictory. The existing approval for paprika oleoresins includes capsorubin in addition to capsanthin. It is therefore recommended to set the maximum contents for capsanthin alone and, as a separate entry, the sum of capsanthin and capsorubin (E160c).

The FEEDAP Panel therefore recommends setting the maximum contents as 16 mg kg⁻¹ complete feed for laying hens and 50 mg capsanthin kg⁻¹ for poultry other than laying hens for capsanthin alone as well as for the sum of capsanthin/capsorubin.

The current practice of the European Commission to set a maximum content for these colouring substances “alone or with other carotenoids and xanthophylls” does not reflect the practice in poultry feeding. Yellow and red pigments are considered separately in feed formulation, β-carotene can also be used as vitamin A source (nutritional additive). The FEEDAP Panel suggests that when a mixture of red pigmenting xanthophylls is used, the amount of each xantophyll should be calculated in proportion to the maximum content authorised.

2. Citranaxanthin (E161i)

Citranaxanthin is listed in the Community Register of Feed Additives according to Regulation (EC) No 1831/2003 under the sub-classification carotenoids and xanthophylls. It is an approved sensory additive for laying hens. It shows two legal definitions: as E161i and as citranaxanthin with the molecular formula C₃₃H₄₄O. The maximum content is 80 mg kg⁻¹ complete feed, alone or with other carotenoids and xanthophylls.

2.1. Specification of E161i

Although citranaxanthin has an E-number, its use is not allowed as a food additive in the EU. Therefore, no specification can be found in the food additives legislation.

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7  http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/index_en.htm (last update 2/05/06)
2.2. General characteristics

Citranaxanthin occurs naturally in the peel of citrus fruits. The molecular formula of citranaxanthin (6'-methyl-6'-apo-β-caroten-6'-one), CAS number 3604-90-8, is C₃₃H₄₄O and its molar mass, 456.71 g mol⁻¹. Melting point and octanol/water partition of citranaxanthin are unknown. A solution of citranaxanthin has a characteristic maximum absorption wavelength ranging from 478 to 482 nm in chloroform. Citranaxanthin exists as deep violet crystals that are sensitive to oxygen and light. It is a hydrophobic compound which forms aggregates or adheres non-specifically to structural surfaces. It is insoluble in water, very slightly soluble in ethanol, slightly soluble in vegetable oil and soluble in chloroform.

Citranaxanthin has no chiral carbon atom (Figure 2). Citranaxanthin has several double bonds in the linear portion of the molecule, each of which can potentially exist in the Z or E form. The thermodynamically most stable form of the molecule is all-E (all-trans) citranaxanthin. Commercialised citranaxanthin is a synthetic product and exibits the all-E isomer structure.

![Molecular structure of citranaxanthin](image)

2.2.1. Analytical methods

The CRL reports that no ISO and CEN methods could be found as official analytical method for the determination of citranaxanthin.

Presumably, most of the methods developed for other carotenoids will work for this particular compound. Some apo-β-carotenals were determined from rat plasma samples using reverse phase HPLC (Schweigert et al. 2000). Schlatterer and Breithaupt (2006) published a HPLC-diode array detection method where peak identification was ascertained by LC-(APCI)/MS analysis. The method allows the simultaneous detection of eight xanthophylls, including citranaxanthin, in the egg yolk. LOQ for citranaxanthin was 50, LOD 25 µg 100 g⁻¹.

2.3. Citranaxanthin in poultry feeding

Citranaxanthin is used, as is the case for canthaxanthin, as red pigment for colouring egg yolks. Citranaxanthin was first isolated from citrus peel. A synthetic form has been available for many years with a pigmenting efficiency for yolks of 0.67 compared to canthaxanthin, regardless of the dosage (Huyghebaert, 1993b). This means that one unit canthaxanthin has to be replaced by one and a half units of citranaxanthin to obtain the same red colour intensity for yolks (Marusich and Bauernfeind, 1981; Sidibé, 2001; Beardsworth and Hernandez, 2004). Citranaxanthin is not approved for use in meat-type poultry.

Data on the safety of citranaxanthin for laying hen are not available. However, given its history of use, the FEEDAP Panel does not see any reason for concern.
2.3.1. Sources and use

The European Feed Manufacturers Federation (FEFAC) made available some market data on the use of citranaxanthin, covering responses from nine EU Member States (FEFAC, 2004). According to these data, citranaxanthin is used by the majority of feed manufacturers in one Member State, by about half of them in another Member State, and in a small proportion of the feed manufacturers in two Member States. In four Member States, citranaxanthin is not used (data is not available for one country). A combination of citranaxanthin with lutein, zeaxanthin, canthaxanthin and ethyl ester β-apo-8’carotenoid acid has been reported in five Member States.

2.3.2. Egg yolk colour

Hughes (1985) and Sidibé (2001) reported a linear increase in yolk colour in relation to the dietary content of citranaxanthin. For each 1 mg kg⁻¹ diet increase in dietary citranaxanthin, yolk colour increased by 0.94 scores (Sidibé, 2001). The dose-dependent effect of citranaxanthin was not influenced when substituting yellow corn pigments by ethyl ester of β-apo-8-carotenoic acid (Table 4).

Table 4. Yolk colour and deposition of citranaxanthin in yolks; measurement on day 24 of feeding (Sidibé, 2001)

<table>
<thead>
<tr>
<th>Citranaxanthin mg kg⁻¹ diet</th>
<th>Corn %</th>
<th>ethyl ester of β-apo-8-carotenoic acid mg kg⁻¹ diet</th>
<th>DSM-YCF</th>
<th>Citranaxanthin in yolk mg/egg*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>60</td>
<td>-</td>
<td>10.4</td>
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<td>40</td>
<td>1.3</td>
<td>13.5</td>
<td>0.146</td>
</tr>
</tbody>
</table>

* egg weight 60 g, yolk percentage 27 %

2.3.3. Deposition of citranaxanthin in the egg

Deposition rates found in literature are difficult to summarize and to compare, since many different factors had been included in the experiments and not all necessary information is provided to calculate deposition rates with enough accuracy from the measured contents in egg yolks. As Table 4 shows, the average content of citranaxanthin in the yolk of a standard egg may vary between 50 and 170 µg, depending on the supplementation level. An egg yolk with a colour score of 13 DSM-YCF may contain about 160 µg citranaxanthin while an egg yolk with a colour score of 10 may contain 50 µg citranaxanthin.

Calculation of deposition rates for citranaxanthin indicates values comprised between 13 and 15% with only slight differences between studies (Table 5).
Opinion on Carotenoids. PART II. Capsanthin, Citranaxanthin, and Cryptoxanthin

Table 5. Deposition rates (%) of citranaxanthin to egg yolks as derived from literature (Values are partially derived from calculations of the FEEDAP Panel)

<table>
<thead>
<tr>
<th>Percentage (%)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>13</td>
<td>Hoppe and Wiesche (1988)</td>
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<tr>
<td>14</td>
<td>El Boushy and Raterink (1992)</td>
</tr>
<tr>
<td>15</td>
<td>Huyghebaert (1993a)</td>
</tr>
<tr>
<td>15</td>
<td>Hencken (1992)</td>
</tr>
<tr>
<td>15</td>
<td>Sidibé (2001)</td>
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</table>

2.4. Metabolism of Citranaxanthin

No new data have been published concerning the metabolic fate of citranaxanthin since its last assessment by JECFA (1987), which indicated that: i) in rat, 95-98% of the administered dose was recovered in the faeces after three days, the urinary excretion being very limited (0.2% after eight days); ii) in poultry, 50% of the administered dose was absorbed, of which two thirds were converted to vitamin A by retro-aldol cleavage of the polyenic chain, and one third was deposited in the fat of various tissues, including skin and egg yolk. No indication was given on the nature of the excreted material and, consequently, on the extent of the eventual biotransformation of the compound (Morgenthaler, 1979; Morgenthaler, 1978; Crina, 1974a and Crina 1974b).

2.5. Human exposure

A recent study (Schlatterer and Breithaupt, 2006) on commercial eggs of different origins (ecological, free range, barn and cages) in Germany revealed a mean citranaxanthin concentration of 560 ± 231 µg 100 g⁻¹ egg yolk (free range, two batches; barn, one batch). Sidibé (2001) found about 150 µg citranxanthin 100 g⁻¹ egg at 9 mg citranaxanthin kg⁻¹ diet. Data from marketed eggs indicate values of 151 ± 63 µg 100 g⁻¹ egg.

2.6. Safety studies

Citranaxanthin was evaluated in 1987 by the FAO/WHO joint expert committee on food additives (JECFA) (JECFA, 1987). It was concluded that;

“If the substance were to be used as a direct food colouring agent, the data were not sufficiently comprehensive for evaluation (e.g. only one lifetime feeding study was available).”

And also that:

“In the case of use as an animal feed additive, an evaluation could not be performed because the data base did not include sufficient information on the nature of residues to be found in animal-derived foodstuffs and because there was no information concerning the use levels that would constitute good animal husbandry practice.”

The FEEDAP Panel has reviewed the original data, which has been included in the following sections.

2.6.1. Acute Toxicity

An unpublished report tested the acute oral toxicity of citranaxanthin dry powder in Sprague-Dawley rats (BASF, 1972b). The rats were orally dosed with 1 600, 3 200 or 6 400 mg citranxanthin kg⁻¹ bw. No mortalities were observed, although dyspnoea was reported at the two highest doses. No abnormalities were found in the internal organs on gross pathological examination. It was concluded that the LD₅₀ was higher than 6400 mg kg⁻¹ bw.
A similar study in six mongrel dogs of varying ages (three males and three females) using single oral doses of 10 000, 12 600 and 15 900 mg kg\(^{-1}\) bw. No mortality was observed. At necropsy, 14 days after exposure, no abnormalities were found upon gross pathological examination (BASF, 1976).

### 2.6.2. Genotoxicity

No data are available. However, the structure of the molecule does not suggest to present genotoxic properties.

### 2.6.3. (Sub-) chronic Toxicity Studies

In an oral toxicity study, citranaxanthin was administered in diet to groups of 13 to 15 male and female Sprague-Dawley rats at dose levels of 0, 10, 20, 50, or 100 mg kg\(^{-1}\) day\(^{-1}\) for one month. Growth rates and feed consumption were reported not to be significantly different between treated and control groups during the test period. No toxic symptoms or abnormalities in urinalysis, biochemical values, blood analysis, wet organ weights, or histopathological changes were observed (Kawase et al., 1972).

The JECFA monograph (JECFA, 1987) describes the results of an unpublished study in which four groups of 20 or 30 male and female Sprague-Dawley rats were maintained over a period of 91 days on a diet containing 25 000, 50 000, or 100 000 mg kg\(^{-1}\) 10% citranaxanthin dry powder (equivalent to 2 500, 5 000, and 10 000 mg kg\(^{-1}\) active ingredient and intakes of approximately 125-250, 250-500 and 500-1000 mg kg bw\(^{-1}\) day\(^{-1}\)). The monograph states that:

“Citranaxanthin was tolerated without externally recognizable toxic symptoms and without impairment of feed ingestion or growth over a period of 91 days. A significant increase in total serum lipids was observed in both males and females during the treatment period. GPT values were temporarily increased after 8 weeks, but not after 12 weeks. The absolute and relative weights of the liver and kidneys showed a significant increase when compared with those of the control group. All the increases were reversible in the post-observation period, except that the increase in kidney weight did not revert completely. No salient pathological changes were observed in any groups. A few cases of enlargement of the liver proved after histological investigation to be hyperaemia, in some cases diffuse and in other cases patchy. Accumulated changes in the cylinders containing protein in the distal tubule sections of the kidneys were found in all groups (Hempel et al., 1973).”

The JECFA monograph (JECFA 1987) also reports two long-term studies on citranaxanthin toxicity, one with rats and one with dogs, and these can be summarised as done by JECFA.

Groups of 25 male and 25 female Sprague-Dawley rats were fed for two years with graded levels of 1000, 3300, or 10,000 mg kg\(^{-1}\) 8.6% citranaxanthin dry powder (equivalent to 86, 284, and 860 mg kg\(^{-1}\) active ingredient and intakes of approximately 4.3-8.6, 14.2-28.4 and 43-86 mg kg bw\(^{-1}\) day\(^{-1}\)). Another group was administered 9000 mg kg\(^{-1}\) dry powder without active ingredient. A control group remained untreated. After six months, the highest concentration of 10,000 mg kg\(^{-1}\) was raised to 20,000 mg kg\(^{-1}\) (1720 mg kg\(^{-1}\) active ingredient and an intake of approximately 86-172 mg kg bw\(^{-1}\) day\(^{-1}\)) and the concentration of the placebo was raised to 18,000 mg kg\(^{-1}\) for the remaining period of the experiment. There were no signs of incompatibility in any group throughout the experiment. External appearance, behaviour, weight gain, food and drinking water intake, haematology, clinical chemistry, urinalysis, weight of organs and their autopsy did not reveal any influence of treatment. There were no histological changes in organs or tissues. The number and kind of tumours or mortality did not differ among groups (Leuschner et al., 1976b).
A 180-day feeding trial with five groups of eight beagle dogs (four males and four females) was performed with graded levels of 1000, 3300, or 10,000 mg kg\(^{-1}\) 8.6% citranaxanthin dry powder (equivalent to 86, 284, and 860 mg kg\(^{-1}\) active ingredient and intakes of approximately 2.2, 7.1 and 21.5 mg kg bw\(^{-1}\) day\(^{-1}\)). Another group was administered dry powder without active ingredient. A control group remained untreated. In all groups with citranaxanthin, the faeces had a remarkable red-brown colour. At the highest level, the faeces were more liquid than normal. Feed intake decreased in a dose-dependent manner due to palatability problems. The dry powder group without citranaxanthin also showed a decreased feed intake. Appearance, behaviour, weight gain, haematology, clinical chemistry, electrocardiography, urinalysis, weight of organs, and their autopsy did not reveal any influence of treatment. Discolouration of internal organs was observed but there were no pathological or histological changes in organs or tissues (Leuschner et al., 1975).

2.6.4. Reproductive Toxicity Studies, including Developmental Toxicity

The JECFA monograph (JECFA, 1987) describes the unpublished results of a three-generation reproduction study with groups of 20 male and 20 female Sprague-Dawley rats (Leuschner et al., 1976a). In this study, animals were administered 1000, 3300, or 10,000 mg citranaxanthin kg\(^{-1}\) (8.6% dry powder, equivalent to 86, 284, and 860 mg active ingredient kg\(^{-1}\) and intakes of approximately 4.3-8.6, 14.2-28.4 and 43-86 mg kg\(^{-1}\) bw day\(^{-1}\)). Another group was administered 9000 mg dry powder without active ingredient kg\(^{-1}\). A control group remained untreated. Treatment of both males and females started seven weeks before breeding with the parent generation and continued during mating, pregnancy, and rearing periods. Fertility and reproduction performance were not influenced in any generation (F\(_0\), F\(_1\), or F\(_2\)) or group. Mating, pregnancy, litter size, birth weights and rearing were within normal limits. The indices of fertility, pregnancy, viability, and lactation did not differ between control and treatment groups. Animals of the F\(_2\)-generation were investigated for malformations; none were observed. Behaviour, appearance, feed and water intake and weight gain were not affected by treatment in any generation or group. No macroscopic pathological effects were observed in parents or their offspring. The final macroscopic examination of F\(_2\)-generation animals at nine weeks of age revealed no changes due to treatment. Comparisons of organ weights and histological examinations showed no differences among groups (Leuschner et al., 1976a).

Data of an unpublished study on the prenatal toxicity of citranaxanthin in Wistar rats after oral administration were also available (BASF, 1975). The test substance was administered as an oily suspension to 23-25 pregnant female rats/group per stomach tube at doses of 0 (control, vehicle only), 100, 400 and 1000 mg of the oily suspension containing 11.7% citranaxanthin from day 6 to day 15 post coitum, leading to an intake of 11.7, 46.8 or 117 mg citranaxanthin kg\(^{-1}\) bw day\(^{-1}\). On day 20, all females were sacrificed and assessed by gross pathology. The foetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and/or skeletal findings. Reddish-brown discolorisation of faeces and subcutis was observed in all groups receiving citranaxanthin, which disappeared upon cessation of treatment. In the two highest dose groups, a slight but statistically significant increase in absolute and relative liver weight was observed compared to control. No substance-related adverse effects were observed in the dams and there were no effects on gastrointestinal parameters, and no signs of developmental toxicity or teratogenicity.

2.6.5. Human data

There are no data giving precise or approximate information on intake of citranaxanthin from foodstuff other than eggs, but it is probably negligible.
2.6.5.1. User safety

In an inhalation study, twelve rats were exposed for eight hours to crystalline citranaxanthin in air at a concentration of 0.29 mg L\(^{-1}\) of air. No death occurred and no toxic symptoms were observed, except for slight mucous membrane irritation (BASF, 1972a).

In a special study on mucous membrane irritation, 50 mg crystalline citranaxanthin was applied to the eyes of rabbits. No irritation was seen. After one hour, the eyes had a red appearance; after 24 hours and eight days these symptoms were not observed (BASF, 1972a).

2.7. Risk Assessment

Current data on the toxicity of citranaxanthin tested in laboratory animals do not raise concern for consumer safety. However, the available data on several aspects of toxicity, including metabolic fate and mutagenicity, are insufficient for the establishment of an ADI and a full assessment of both consumer and user safety. In contrast to other xanthophylls approved, it is likely that there is no considerable intake of citranaxanthin other than from eggs in human diet. Recent data (see 2.5) indicates that the intake of 100 g eggs daily (a worst case situation) contributes to an exposure to about 0.15 mg citranaxanthin. The FEEDAP Panel sees no urgent need for action but recommends a full re-evaluation of the compound according to current standards.

2.8. Environment

No data are available for a qualified assessment of the environmental impact of citranaxanthin used in poultry feed. In poultry, 50% of the dietary dose is excreted. According to Directive 2001/79/EC, environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, as its use will not alter its concentration or distribution in the environment. Citranaxanthin occurs naturally in plants.

Given the oxidative susceptibility of carotenoids, the FEEDAP Panel considers it unlikely that the use of citranaxanthin in layer feeding at concentrations adequate for egg colour will have an impact on environment.

2.9. Conclusions and recommendations

2.9.1. Conclusions

Citranaxanthin is an effective additive in colouring eggs. Its relative efficacy compared to canthaxanthin in pigmented egg yolks is 0.67. Nine mg citranaxanthin kg\(^{-1}\) layer feed would provide a yolk with a DSM-YCF value slightly above 13. Regarding the maximum dose approved for canthaxanthin as well as the pigmented eggs used for pasta production, a maximum content of 12 mg citranaxanthin kg\(^{-1}\) complete feed for laying hens appears sufficient. There is no need for an approval of higher concentrations.

Data on the safety of citranaxanthin for laying hen are not available. However, given its history of use, the FEEDAP Panel does not see any reason for concern.

Current data on the toxicity of citranaxanthin tested in laboratory animals do not raise concern for consumer safety. However, the available data on several aspects of toxicity, including metabolic fate and mutagenicity, are insufficient for the establishment of an ADI and a full assessment of both consumer and user safety. In contrast to other xanthophylls approved, it is likely that there is no considerable intake of citranaxanthin other than from eggs in human diet. Recent data (see 2.5) indicates that the intake of 100 g eggs daily (a
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worst case situation) contributes to an exposure to about 0.15 mg citranaxanthin. The FEEDAP Panel sees no urgent need for action but recommends a full re-evaluation of the compound according to current standards.

Citranaxanthin shows no inhalation toxicity and no irritancy for the eye.

No data is available for a qualified assessment of the environmental impact of citranaxanthin used in layer feed. However, given the oxidative susceptibility of the compound and its use in layer feeding, the FEEDAP Panel considers that citranaxanthin presents a low to negligible risk to the environment.

2.9.2. Recommendations

The current practice of the European Commission to set a maximum content for citranaxanthin “alone or with other carotenoids and xanthophylls” does not reflect the practice in poultry feeding. Yellow and red pigments are considered separately in feed formulation, \( \beta \)-carotene can also be used as vitamin A source (nutritional additive). The FEEDAP Panel suggests that when a mixture of red pigmenting xantophylls is used, the amount of each xantophyll should be calculated in proportion to the maximum content authorised.

3. Cryptoxanthin (E161c)

Cryptoxanthin is listed in the Community Register of Feed Additives according to Regulation (EC) No 1831/2003\(^9\) under the sub-classification carotenoids and xanthophylls. It is an approved sensory additive for poultry.\(^{10}\) It presents two legal definitions: as 161c and as cryptoxanthin with the molecular formula \( \text{C}_{40}\text{H}_{56}\text{O} \). The maximum content is 80 mg kg\(^{-1}\) complete feed, alone or with other carotenoids and xanthophylls.

3.1. Specifications

Although cryptoxanthin has an E-number, its use as a food additive is not allowed in the EU, therefore it has no specification in the food additives legislation.\(^{11}\)

3.2. General characteristics

Cryptoxanthin is an abundant xanthophyll in nature. It is found mostly in fruits such as orange (also orange peel), tangerine, papaya and persimmon, petals and berries of \( \text{Physalis} \) species (Mullaca, or cape gooseberry). It was also isolated from \( \text{Cucurbita pepo} \) (Pumpkin) and is present in yellow maize, peach, microalgae (e.g. \( \text{Chlorella pyrenoidosa} \)), and clams (e.g. \( \text{Corbicula} \) \( \text{japonica}, \text{Corbicula} \) sandai, and \( \text{Corbicula} \) sp.).

Cryptoxanthin \( [(3\text{R}-\beta,\beta\text{-caroten-3-ol}) \text{ or } [(3'\text{R},6'\text{R})-\beta,\epsilon\text{-caroten-3'-ol}] \) has many commonly used synonyms like cryptoxanthin and \( \beta \)-caroten-3-ol. The molecular formula of cryptoxanthin is \( \text{C}_{40}\text{H}_{56}\text{O} \) and the molar mass is 552.881 g mol\(^{-1}\). It exists in two isomeric forms: \( \beta \)-cryptoxanthin (CAS number 472-70-8) and \( \alpha \)-cryptoxanthin (CAS 24480-38-4). Melting point of \( \beta \)-cryptoxanthin is 160°C and log P (octanol/water partition) 16.08. Melting point and log P values of \( \alpha \)-cryptoxanthin are unknown. Characteristic maximum absorption wavelengths for \( \beta \)-cryptoxanthin are 428, 450 and 478 nm in ethanol, 435, 463

\(^9\) http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/index_en.htm (last update 2/05/06)
\(^{10}\) OJL 270, 14.12.1970 and C50, 25.2.2004
\(^{11}\) OJL 226, 22.9.1995 (Directive 95/45/EC laying down specific purity criteria concerning colours for use in foodstuffs)
and 489 nm in benzene. The corresponding values for $\alpha$-cryptoxanthin are 423, 446 and 473 nm (ethanol) and 433, 459 and 488 nm (benzene).

\[ \text{\alpha}-\text{cryptoxanthin} \]

\[ \text{\beta}-\text{cryptoxanthin} \]

**Figure 3. Molecular structures of \text{\alpha}-\text{cryptoxanthin} and \text{\beta}-\text{cryptoxanthin}**

$\beta$-cryptoxanthin has one chiral atom (at the 3 position of the six carbon ring). The different location of a carbon double bond in the six member ring structures of the $\alpha$-cryptoxanthin results in two chiral centers. $\alpha$- and $\beta$-cryptoxanthin molecules have several double bonds in the linear portion of the molecule, each of which can potentially exist in the Z or E form. The thermodynamically most stable form of the molecule is all-E (all-trans) cryptoxanthin.

Cryptoxanthin has one hydroxyl (OH) group attached on the terminal ring. This group can exist as free alcohol or in an esterified form.

### 3.2.1. Analytical methods

The CRL reports that no ISO and CEN methods could be found as official analytical method for the determination of cryptoxanthin.

Modern methods for the detection and quantification of $\alpha$- and $\beta$-cryptoxanthin, simultaneously with other carotenoids from natural sources and serum are available (Mercadante and Rodriguez-Amaya, 2001; Panfili et al. 2004; Hao et al. 2005; Rajendran et al. 2005).

Schlatterer and Breithaupt (2006) published a method for carotenoid detection in egg yolk (HPLC and LC-(APCI) MS using a C30 phase) a LOQ for $\beta$-cryptoxanthin of 52.7, and a LOD of 26.4 µg 100 g⁻¹.

### 3.3. Cryptoxanthin in poultry feeding

Although Huyghebaert (1993a) stated that cryptoxanthin is available as a synthetic carotenoid, the FEEDAP Panel could not found any information on the use of cryptoxanthin in pigmenting yolks or poultry tissues. This may be due to the fact that cryptoxanthin is

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poorly absorbed in the digestive tract (Tyczkowski and Hamilton, 1986), resulting in low tissue contents of this carotenoid.

3.3.1. Sources and use

Synthetic cryptoxanthin is not available on the feed market. The European Feed Manufacturers Federation (FEFAC) made available some market data on the use of cryptoxanthin, covering responses from six EU Member States (FEFAC, 2004). According to data, cryptoxanthin is used by feed manufacturers in a small proportion in only one Member State. But such information is questionable as β-cryptoxanthin is not commercially available.

3.3.2. Occurrence of β−cryptoxanthin in eggs

A recent study (Schlatterer and Breithaupt, 2006) on commercial eggs of different origins (ecological husbandry, two batches; cages, one batch) in Germany revealed a mean β-cryptoxanthin concentration of 70.9 µg 100 g−1 egg yolk (presumably from natural sources).

3.4. Metabolism of Cryptoxanthin

Cryptoxanthin is poorly absorbed in chicken (Tyczkowski and Hamilton, 1986). It has been shown that free and esterified cryptoxanthin administered to adult human have comparable bioavailability and lead to similar plasma levels of the free form. This is consistent with the suggestion that ester hydrolysis is indispensable prior to absorption of carotenoid esters (Bowen et al., 1993; Furr and Clark, 1997; Wingerath et al., 1995).

No data is available concerning the metabolic fate of cryptoxanthin in poultry or laboratory animals. β-cryptoxanthin is a precursor of vitamin A.

3.5. Human exposure assessment

Food sources of cryptoxanthin are fruits, especially products such as orange juice, and vegetables, such as paprika, where it is present mainly as cryptoxanthin esters (Breithaupt and Bamedi 2001, Irwig, et al., 2002). In a recent study, Schlatterer and Breithaupt (2005) determined the carotenoid fraction in citrus fruits, which is inter alia dominated by structural cryptoxanthin isomers as β-cryptoxanthin and zeinoxanthin. Both xanthophylls were identified in saponified citrus fruit extracts based on their comparison to reference compounds extracted from corn and their typical fragmentation pattern in LC-(APCI)MS analyses. α-cryptoxanthin, another structural cryptoxanthin isomer usually found in carrot leaves, was not identified in the citrus fruits studied. In Finland, low levels are present in eggs (Ollilainen et al., 1989). Table 6 gives the mean β-cryptoxanthin concentrations for some food products.
Table 6. Average values of $\beta$-cryptoxanthin (mg kg$^{-1}$ food) in food products

<table>
<thead>
<tr>
<th>Food type</th>
<th>$\beta$-cryptoxanthin</th>
<th>Food type</th>
<th>$\beta$-cryptoxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>0.10</td>
<td>Nectarine</td>
<td>0.30</td>
</tr>
<tr>
<td>Avocado</td>
<td>0.26</td>
<td>Orange</td>
<td>2.26</td>
</tr>
<tr>
<td>Beansprouts</td>
<td>0.20</td>
<td>Orange (Mandarin)</td>
<td>3.09</td>
</tr>
<tr>
<td>Blackberry</td>
<td>0.00</td>
<td>Orange (juice)</td>
<td>7.01</td>
</tr>
<tr>
<td>Broccoli (cooked)</td>
<td>0.24</td>
<td>Peach</td>
<td>0.70</td>
</tr>
<tr>
<td>Cherries</td>
<td>0.05</td>
<td>Pear</td>
<td>0.03</td>
</tr>
<tr>
<td>Fruit salad (home made)</td>
<td>0.50</td>
<td>Red pepper (raw)</td>
<td>2.50</td>
</tr>
<tr>
<td>Grapefruit (white/yellow)</td>
<td>0.03</td>
<td>Red pepper cooked</td>
<td>3.70</td>
</tr>
<tr>
<td>Grapefruit (pink)</td>
<td>0.00</td>
<td>Pineapple</td>
<td>0.02</td>
</tr>
<tr>
<td>Japanese meddler</td>
<td>6.60</td>
<td>Pumpkin</td>
<td>0.60</td>
</tr>
<tr>
<td>Lemon</td>
<td>0.14</td>
<td>Spring greens</td>
<td>0.23</td>
</tr>
<tr>
<td>Mango (ripe/raw)</td>
<td>0.54</td>
<td>Water melon (peeled ripe)</td>
<td>0.62</td>
</tr>
<tr>
<td>Marrow (cooked/peeled)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Al Delaimy et al. (2005) performed a study meant to assess the association between individual plasma carotenoid levels ($\alpha$-carotene, $\beta$-carotene, lycopene, $\beta$-cryptoxanthin, lutein, zeaxanthin) and fruit and vegetable intakes. Data was collected through a calibrated food questionnaire (FQ) and 24-h dietary recall records (24HDR) in nine different European countries with diverse populations and widely varying intakes of plant foods. A stratified random sub-sample of 3089 men and women from nine countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) was included. Selected participants provided blood samples as well as dietary and other lifestyle information for the period of time extending from 1992 to 2000. $\beta$-cryptoxanthin was most strongly correlated with total fruits (FQ $r = 0.52$, 24HDR $r = 0.39$), lycopene with tomato and tomato products (FQ $r = 0.38$, 24HDR $r = 0.25$), and $\alpha$-carotene with intake of root vegetables ($r = 0.39$) and of total carrots ($r = 0.38$) for FQ only. Based on diet measured by FQ, and adjusting for possible confounding factors by body mass index (BMI), age, gender, smoking status, alcohol intake, and energy intake, the strongest predictors of individual plasma carotenoid levels were fruits ($R^{(partial)(2)} = 17.2\%$) for $\beta$-cryptoxanthin, total carrots ($R^{(partial)(2)} = 13.4\%$) and root vegetables ($R^{(partial)(2)} = 13.3\%$) for $\alpha$-carotene, and tomato products ($R^{(partial)(2)} = 13.8\%$) for lycopene.

Rajendran et al. (2005) found in Taiwanese human serum, among other carotenoids, all-trans-$\alpha$-cryptoxanthin (55.7-188.2 ng mL$^{-1}$) and all-trans-$\beta$-cryptoxanthin (43.1-134.5 ng mL$^{-1}$).

A comparative study assessing the carotenoid uptake in five European countries, based on a food frequency questionnaire (FFQ), showed that in all countries, cryptoxanthin was primarily obtained from citrus fruit. Daily intake was highest in Spain (1.36 mg day$^{-1}$) and lowest in France, with 0.45 mg day$^{-1}$. Highest interquartile range for Spain was 2.16 mg day$^{-1}$ (O'Neill, et al., 2001). In Germany, the average intake of cryptoxanthin is 0.05 mg day$^{-1}$ (Pelz et al., 1998). The uptake data corresponds to data found in the USA (Tangney et al., 2004). On the basis of these data sets, mean $\beta$-cryptoxanthin intake in adults from different countries has been determined (Table 7). The comparison of the data from specific European country studies suggests that the FFQ and carotenoid database described in the present paper can be used for comparative dietary intake studies in Europe. The results show that there are discrepancies among Europe countries in the specific intake of some carotenoids due to the consumption of different food products (O'Neill et al., 2001).
### Table 7. β-cryptoxanthin intake in different countries (mg day⁻¹)

<table>
<thead>
<tr>
<th>Country</th>
<th>Pelz et al., 1998</th>
<th>Granado et al., 1996</th>
<th>O’Neill et al., 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA: (dietary recall), 19-50 years female, n=1102</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA: FFQ, 43-85 years adults, n=2152</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany: Food samples, n=39</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany: Consumption protocol n=12308, female</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany: Consumption protocol n=10901, male</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>0.40</td>
<td>1.36; 0.74 – 2.16</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td></td>
<td>0.45; 0.17 – 0.88</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td>0.99; 0.32 – 1.64</td>
<td></td>
</tr>
<tr>
<td>Republic of Ireland</td>
<td></td>
<td>0.78; 0.40 – 1.44</td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td></td>
<td>0.97; 0.50 – 1.75</td>
<td></td>
</tr>
</tbody>
</table>

Paprika, oranges, and orange-juice are the most important cryptoxanthin sources in Germany (Pelz et al., 1998). Quantitative estimations are given in Table 8.

### Table 8. Data on sources of β-cryptoxanthin for the German population (Pelz et al., 1998)

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Food Item</th>
<th>Percentage (%)</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paprika (red)</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Eggs *</td>
<td>12</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>Orange juice</td>
<td>8</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>Oranges</td>
<td>7</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Fruit preservatives</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Fruits in general</td>
<td>9</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Vegetables in general</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

*Annotation of FEEDAP Panel: Cryptoxanthin in eggs originates probably from corn and alfalfa.

In baby food, varying concentrations of β-cryptoxanthin were found at levels of, in average, 220–400 µg kg⁻¹. 57% of the juices contained β-cryptoxanthin. Concentrations ranged from 2–44 µg L⁻¹ (Österreichischer Ernährungsbericht, 2003).

### 3.6. Safety studies

In 1987, BIBRA published a toxicity profile on xanthophylls, including cryptoxanthin (BIBRA 1987a) and reported that no toxicity studies had been identified. They also concluded that no specific data could be found on genotoxicity or carcinogenicity. The SCF (SCF, 1975) accepted the use of xanthophylls prepared from natural foods by physical processes, and did not feel that the establishment of an oral acceptable daily intake for man was needed.

Since the product is not commercially available, no user safety data has been generated.

#### 3.6.1. Acute Toxicity

There is no data available.
3.6.2. Genotoxicity

Mutagenicity studies in which \( \beta \)-cryptoxanthin was tested focussed on the antimutagenic and in vivo anticlastogenic effects of series of carotenoids (He and Campbell 1990; Rauscher et al., 1998).

It has been reported that a cryptoxanthin-rich orange juice extract proved to be able to inhibit the mutagenicity of aflatoxin B\(_1\) in *Salmonella typhimurium* TA 100 and TA 98 (He and Campbell 1990). Incubations performed to determine the effects of carotenoids alone on the spontaneous reversion frequency of TA 98 and TA 100, showed no changes in the number of spontaneous revertants at \( 100 \mu g \) of carotenoid per plate.

In the study of Rauscher et al. (1998), it appeared that \( \beta \)-cryptoxanthin was active in inhibition of aflatoxin B\(_1\), benzo[a]pyrene- and cyclophosphamide-, but not 2-amino-3-methylimidazole[4,5-f] quinoline (IQ)-induced mutagenicity in the histidine deficient strains of *Salmonella typhimurium* TA98, TA 98NR and/or TA100 (Ames test). No mutagenic activities could be detected at concentrations up to \( 100 \mu g \) \( \beta \)-cryptoxanthin per plate in the control incubations, where carotenoids were tested for mutagenic activities in the absence of model mutagens (Rauscher et al., 1998).

In the same study, the influence of \( \beta \)-cryptoxanthin on the clastogenic effect of benzo[a]pyrene and cyclophosphamide in mice was investigated. In the in vivo situation at a single p.o. dose of 180 mg kg\(^{-1}\) bw, given in 200 \( \mu l \) corn oil, \( \beta \)-cryptoxanthin was effective in protection against both clastogens. Negative control groups dosed with \( \beta \)-cryptoxanthin alone showed no clastogenic activity.

3.6.3. (Sub-)chronic Toxicity Studies

The effects of \( \beta \)-cryptoxanthin in specific experimental animal studies have been reported. Although these studies have not been designed to assess the safety of \( \beta \)-cryptoxanthin, they have not revealed adverse effects. These studies can be summarised as follows:

A study was conducted by Kohno et al. (2001) exposing male A/J mice for 21 weeks to a mandarin juice and drinking water containing 3.9 mg \( \beta \)-cryptoxanthin and 100 mg hesperidin in 100 g sample. Assuming a body weight of 20-30 g and a daily consumption of 3-7 mL, the intake amounts to approximately 3.9 to 13.7 mg \( \beta \)-cryptoxanthin kg\(^{-1}\) bw day\(^{-1}\). The control group received drinking water without additions. At the end of the experiment, organs were examined histopathologically. All animals remained healthy throughout the experimental period. Body, liver, and relative liver weights of the mandarin juice exposed animals was similar to that of the control group. Mice tolerated the treatment with mandarin juice containing 3.9 mg \( \beta \)-cryptoxanthin and 100 mg hesperidin in 100 g sample without any adverse effects.

The suppression of azoxymethane-induced colon carcinogenesis in male F344 rats exposed to similar \( \beta \)-cryptoxanthin and hesperidin-rich mandarin juices, was reported by Tanaka et al. (2000). Azoxymethane-exposed rats received mandarin juice containing 3.9 mg \( \beta \)-cryptoxanthin and 100 mg hesperidin per 100 g juice as drinking water for 36 weeks. Assuming a body weight of 250 to 300 g and a daily consumption of 20-30 mL, the intake amounts to approximately 2.6 to 4.7 mg \( \beta \)-cryptoxanthin kg\(^{-1}\) bw day\(^{-1}\). There was no effect of mandarin juice on body weight or liver weight of rats.

Previous reports on chemoprevention by \( \beta \)-cryptoxanthin against N-methylnitrosourea-induced colon carcinogenesis in F344 rats are also available (Narisawa et al., 1999). Four groups of 25 rats each received N-methylnitrosourea (2 mg three times a week intrarectal, for five weeks), and were fed a diet supplemented with 0 mg kg\(^{-1}\) (control), 1, 5 or 25 mg kg\(^{-1}\) \( \beta \)-cryptoxanthin for 30 weeks. No adverse effects were observed.
3.6.4. Reproductive Toxicity Studies, including developmental toxicity

There is no data available.

3.6.5. Human data

Epidemiological studies on β-cryptoxanthin intake and human health effects generally report no effects (Nomura et al., 1997, Kang et al., 2003) or beneficial effects of increased β-cryptoxanthin intake or plasma levels. Beneficial effects include inverse association between β-cryptoxanthin intake levels and cancer incidences (Batieha et al., 1993; Michaud et al., 2000; Comstock et al., 1997, Nishino et al., 2000; Sato et al., 2002; Abnet et al., 2003; Goodman et al., 2003; Männistö et al., 2004), between β-cryptoxanthin plasma levels and oxidative DNA damage and lipid peroxidation (Haegle et al., 2000), or between plasma levels of β-cryptoxanthin and early atherosclerosis (Dwyer et al., 2004) and inflammatory polyarthitis (Pattison et al., 2005).

3.7. Risk Assessment

Most observations are available for β-cryptoxanthin compared to α-cryptoxanthin. Acute toxicity data could not be found. β-cryptoxanthin appears to be not mutagenic and not clastogenic. However, complete genotoxicity could not be assessed due to the lack of data. In three subchronic studies (one on mice and two on rats, for 21, 36, and 30 weeks, respectively), originally designed for testing anti-tumor effects of the substance, adverse effects were not observed at doses of 13.7, 4.7, and 25 mg β-cryptoxanthin kg⁻¹ body weight. An ADI has not been allocated.

In European countries, the intake of β-cryptoxanthin by humans from vegetables is considerable, varying between 0.05 and 1.36 (upper quartile 2.14) mg day⁻¹ according to the eating habit. It is predominantly considered in epidemiological studies as beneficial in preventing various diseases (cancer incidence, oxidative DNA damage and lipid peroxidation, early atherosclerosis, and inflammatory polyarthitis). The FEEDAP Panel therefore considers β-cryptoxanthin as safe for the consumer.

There is a varying contribution of eggs to the β-cryptoxanthin intake of humans. In countries with a low β-cryptoxanthin intake, the relative contribution is obviously high. But this is not the result of the transfer of β-cryptoxanthin as a feed additive into the egg but as a constituent of feedingstuffs like corn and alfalfa. More recent data shows that intake of 100 g eggs daily will contribute with about 0.02 mg of β-cryptoxanthin.

As the product is not commercially available, no user safety data has been generated.

3.8. Environment

According to Directive 2001/79/EC, an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, as its use as a feed additive will not alter its concentration or distribution in the environment. As cryptoxanthin occurs abundantly in plants and the terrestrial environment, no environmental risk assessment is necessary.
3.9. Conclusions and recommendations

3.9.1. Conclusions

The FEEDAP Panel could not find any information on the use of cryptoxanthin in pigmenting yolks or poultry tissues. There are serious doubts about the pigmenting capacity of \(\beta\)-cryptoxanthin because of its low intestinal absorption in poultry.

Considering the absence of commercially available \(\beta\)-cryptoxanthin for poultry diets and its questionable, if any, efficacy in pigmenting poultry eggs and tissues, the FEEDAP Panel sees no reason to maintain the approval of \(\beta\)-cryptoxanthin as a sensory additive.

Acute toxicity data could not be found. \(\beta\)-cryptoxanthin appears not to be mutagenic and not clastogenic. In three subchronic studies (one on mice and two on rats for 21, 36, and 30 weeks, respectively) originally designed for testing anti-tumour effects of the substance, adverse effects of 13.7, 4.7, and 25 mg \(\beta\)-cryptoxanthin kg\(^{-1}\) body weight were not observed. An ADI has not been allocated.

In European countries, the intake of \(\beta\)-cryptoxanthin by humans, from vegetables, is considerable, varying between 0.05 and 1.36 (upper quartile 2.14) mg day\(^{-1}\), depending on the eating habit. It is predominantly considered in epidemiological studies as beneficial in preventing various diseases. The FEEDAP Panel therefore considers \(\beta\)-cryptoxanthin as safe for the consumer.

There is a varying contribution of eggs to the \(\beta\)-cryptoxanthin intake of humans. In countries with a low \(\beta\)-cryptoxanthin intake, the relative contribution is obviously higher. But this is not the result of the transfer of \(\beta\)-cryptoxanthin as a feed additive into the egg but as a constituent of conventional feedingstuffs like corn and alfalfa. Most recent data on market eggs show a daily \(\beta\)-cryptoxanthin intake of about 0.02 mg by 100 g egg consumption.

As the product is not commercially available, no user safety data has been generated.

According to Directive 2001/79/EC, an environmental risk assessment is not considered necessary if the active ingredient of the feed additive is a natural/physiological substance, as its use as a feed additive will not alter its concentration or distribution in the environment. As cryptoxanthin occurs abundantly in plants and the terrestrial environment, no environmental risk assessment is necessary.

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**Documentation provided to EFSA**

1. Dossier submitted by FEFANA, January 2005 (Risk Assessment Red Carotenoids – Astaxanthin and Capsaxanthin/Capsorubin)
2. Dossiers submitted by BASF, November 2004
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