SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of lutein (E 161b) as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of lutein (E 161b). Lutein has been previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2006. JECFA established a group Acceptable Daily Intake (ADI) of 0-2 mg/kg body weight (bw) for lutein from *Tagetes erecta* and zeaxanthin. The SCF could not establish an ADI, but concluded that xanthophylls prepared from natural foods by physical processes are acceptable for use in food. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. New studies included a 90-day study in rats in which no adverse effects were reported up to dose levels of 400 mg/kg bw/day. However, the Panel noted that, compared to the standard regulatory studies, the study is too limited to identify a NOAEL for the safety evaluation of lutein. The Panel concluded, based on the NOAEL of 200 mg/kg bw/day (the highest dose level tested) in a 90-day rat study, the absence of developmental toxicity at dose levels up to 1000 mg/kg bw/day (the highest dose level tested), the fact that lutein is not genotoxic, the fact that in 90-day studies no effects on reproductive organs were observed, and the fact that lutein is a normal constituent of the diet, that an ADI can be derived. Given the absence of a multigeneration reproductive toxicity study and of chronic toxicity/carcinogenicity studies the Panel applies an uncertainty factor of 200 and establishes an ADI of 1 mg/kg bw/day. The Panel noted that this ADI refers to lutein derived from *Tagetes erecta* containing at least 80% carotenoids consisting of lutein and zeaxanthin (79 and 5% respectively). The Panel concluded that at the current levels of use Tier 3 intake estimates are above the ADI at the upper end of the range.

KEY WORDS

Lutein, E 161b, CAS Registry Number 127-40-2, food colour, EINECS number 204-840-0.

1 On request from the European Commission, Question No EFSA-Q-2008-787, adopted on 7 July 2010.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources (ANS) added to Food was asked to provide a scientific opinion re-evaluating the safety of lutein (E 161b) when used as a food colour.

Lutein (E 161b) is a natural carotenoid dye authorised as a food additive in the EU (E 161b) and previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2006. JECFA established a group Acceptable Daily Intake (ADI) of 0-2 mg/kg body weight (bw) for lutein from *Tagetes erecta* and zeaxanthin. The SCF could not establish an ADI, but concluded that xanthophylls prepared from natural foods by physical processes are acceptable for use in food (SCF, 1975).

Specifications have been defined in the EU legislation Directive 2008/128/EC and JECFA. EU specifications for lutein only describe 4% of the commercial product. The Panel concluded that the existing specifications need to be extended to include the material not accounted for.

The Panel noted that separate specifications are available for lutein from *Tagetes erecta* in the JECFA but not in the EU specifications and that the JECFA specifications on lutein from *Tagetes erecta* are higher with respect to lutein content (> 70%) than the EU specifications (> 4%).

Furthermore, the EU specifications and the JECFA specifications (both for mixed carotenoids and for lutein from *Tagetes erecta*) on the purity, differ with respect to solvent residues, metals, moisture, ash, zeaxanthin and waxes.

The JECFA ADI was based on a No Observed Adverse Effect Level (NOAEL) of 200 mg/kg bw/day (the highest dose level tested) derived from a 90-day study in rats. The lutein tested in this study was a lutein product derived from marigold flowers. A compositional analysis of the test product identified 97% of the components and indicated that at least 80% of the product consisted of the carotenoids lutein and zeaxanthin (79 and 5% respectively). The remaining content consisted of waxes, palmitic acid/palmitate, potassium and water.

The Panel noted that in a more recent 90-day study in rats no adverse effects were reported up to dose levels of 400 mg/kg bw/day. However, the Panel noted that the study included only 5 animals/sex/group and that only a limited number of tissues were examined. Therefore, the Panel considered that, compared to the standard regulatory studies, the study is too limited to identify a NOAEL for the safety evaluation of lutein.

From animal and human studies it can be concluded that after a single oral dose, absorption of lutein results in peak concentrations at about 3-4 hours in rats, at 12 hours in cows and at 2-16 hours in humans. Low tissue concentrations of radioactivity indicate that lutein and/or its metabolites do not accumulate. Highest concentrations were found in liver and gastrointestinal mucosa. In most studies, lutein is reported to be mainly excreted via the faeces. In humans, the main sites of lutein storage are the adipose tissue and the liver. The eye in general and the retina (fovea) in particular, contain high concentrations of lutein and zeaxanthin.

Little is known about the metabolism or degradation of lutein, but several metabolites have been detected in human serum. Lutein can exist in equilibrium with zeaxanthin. Lutein has only a minimal, if any, provitamin A effect.

No multigeneration reproduction studies are available. In a developmental toxicity study, no adverse effects were observed and therefore the NOAEL was defined as 1000 mg/kg bw/day, the highest dose tested. The Panel noted that no effects on reproductive organs were observed in any of the available oral 90-day studies.

A number of *in vitro* (bacterial reverse mutation and chromosomal aberration) and *in vivo* (micronucleus formation, Comet assay) genotoxicity studies are available for lutein. The Panel concluded that based on these studies there is no concern with respect to genotoxicity for lutein.

No chronic toxicity/carcinogenicity studies are available for lutein.

Studies in mice, cats and dogs have shown that lutein may stimulate both cell-mediated and humoral immune responses. In a cat study immune stimulating effects were observed at dose levels of 0.7 mg lutein/kg bw/day and higher. The Panel considered that immunostimulating and immunomodulating effects of lutein have not been demonstrated in a robust and reproducible way, which could enable them to be used as pivotal studies for risk assessment.

The Panel concluded, based on the NOAEL of 200 mg/kg bw/day (the highest dose level tested) in a 90-day rat study, the absence of developmental toxicity at dose levels up to 1000 mg/kg bw/day (the highest dose level tested), the fact that lutein is not genotoxic, the fact that in 90-day studies no effects on reproductive organs were observed, and the fact that lutein is a normal constituent of the diet, that an ADI can be derived. Given the absence of a multigeneration reproductive toxicity study and of chronic toxicity/carcinogenicity studies the Panel applies an uncertainty factor of 200 and establishes an ADI of 1 mg/kg bw/day.

The Panel noted that this ADI refers to lutein derived from *Tagetes erecta* containing at least 80% carotenoids consisting of lutein and zeaxanthin (79 and 5% respectively). According to specifications provided by NATCOL this may refer to the lutein with high concentrations of total saponified carotenoids at levels of at least 80% (cf. JECFA specifications for *lutein from Tagetes erecta*). The ADI does not refer to lutein preparations of lower purity or from other sources.

The Panel also noted that other preparations of lutein are also on the market, i.e. lutein with low concentrations of total carotenoids at levels of ~5-12%, and lutein with high concentrations of total carotenoids extracted and present as esters at levels of at least 60%. The Panel concluded that the toxicological data base available on these preparations is too limited to conclude that the ADI also applies to these preparations.

Tier 3 intake estimates, based on the maximum use levels from the NATCOL usage survey, ranged from 0.6-2.2 mg/kg bw/day. High level intakes ranged from 0.7-5.7 mg/kg bw/day. Therefore, at the current use levels, the ADI of 1 mg/kg bw/day will be exceeded due to the use of lutein as a food colour at the upper end of the range. Furthermore, EFSA (2006) indicated that overall the dietary intake of lutein as such is estimated to be between 0.8 and 2.5 mg/day, equivalent to 0.01 – 0.04 mg/kg bw/day for a 60 kg person, indicating that the worst case scenario for intake of lutein used as a food colour in combination with its average intake from other dietary sources does exceed the ADI of 1 mg/kg bw/day.

The Panel concluded that at the current levels of use Tier 3 intake estimates are above the ADI of 1 mg/kg bw/day at the upper end of the range.

The Panel concluded that the average intake for adults from the regular diet amounts to 1-4 % of the ADI of 1 mg/kg bw/day. High level intakes from the regular diet would amount to 28% of this ADI for chidren (assuming an intake of lutein present in food of 7 mg/day and a body weight of 25 kg, equal to 0.28 mg/kg bw/day).

The Panel concluded that the existing specifications need to be extended to include the material not accounted for and to match the material tested in the toxicological studies.

The Panel noted that the JECFA specifications for lead are $\leqq 5$ or ≤ 3 mg/kg whereas the EC specification is $\leqq$10 mg/kg.

The Panel noted that, if available, the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

According to the framework Directive 89/107/EEC[4] on food additives, the Scientific Committee on Food (SCF) should be consulted before the adoption of provisions likely to affect public health, such as the drawing up of lists of additives and the conditions for their use. Accordingly, all food additives, prior to their authorization, have been evaluated for their safety by the SCF or by its successor, the European Food Safety Authority (EFSA).

Directive 89/107/EEC as well as Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives[5] which will apply as from 20 January 2010, require that food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. In addition Regulation (EC) No 1333/2008 requires that all food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA.

In accordance with Regulation (EC) No 1333/2008, the Commission should, after consultation with EFSA, set up by 20 January 2010 an evaluation programme for EFSA to re-evaluate the safety of the permitted food additives. That programme will define the needs and the order of priorities according to which the approved food additives are to be examined.

Food colours were among the first additives to be evaluated therefore, many of the evaluations are old. For some of these colours new studies have become available and the results of these studies should be included in the evaluation. Therefore, food colours should be evaluated with priority. The order of priorities for the re-evaluation of the remaining permitted food additives will be set in the Regulation for the re-evaluation program.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission asks the European Food Safety Authority to start a systematic re-evaluation of all authorised food additives and to issue scientific opinions on these additives, taking into account that colours as a group should be given the highest priority for the reasons outlined above.

[4] OJ L 40, 11.2.1989, p. 27
[5] OJ L 354, 31.12.2008, p. 16.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of lutein (E 161b) when used as a food colour.

Lutein is a natural carotenoid dye authorised as a food additive in the EU (E 161b) and previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2006.

The E-number E 161b refers to three different preparations including lutein, mixed carotenoids and lutein from *Tagetes erecta*.

The term '*carotenoids*' is the generic name for a class of hydrocarbons consisting of '*carotenes*' (non-oxygenated hydrocarbon forms) and '*xanthophylls*' (oxygenated hydrocarbon forms). The main chain of the carotenoid molecule consists of eight isoprenoid units joined in a manner that the arrangement of isoprenoid units is reversed at the centre of the molecule.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substance

Lutein (E 161b) is a natural carotenoid dye with the formula $C_{40}H_{56}O_2$. Its chemical name has been described as 4',5'-didehydro-5',6'-dihydro-β,β-carotene-3,3'-diol . The CAS number is 127-40-2.

The structural formula of lutein is given in Figure 1:

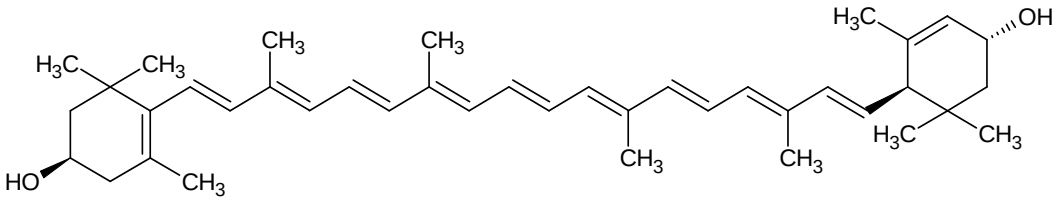

Figure 1: Structural formula of lutein

At least 15 synonyms for lutein are described (ChemIDplus, via Internet, 2008), including for example: 3,3'-dihydroxy-d-carotene, xanthophylls, mixed carotenoids: 3,3'-dihydroxy-d-carotene; β,ε-carotene-3,3'-diol, 3R, 3'R, 6'R-β,ε-carotene-3,3'-diol, all-*trans*-lutein, vegetable lutein, vegetable luteol, Bo-Xan.

Lutein is insoluble in water and soluble in fats and in fat solvents.

The following lutein preparations are all synonyms for lutein E 161b.

Lutein (Directive 2008/128/EC) (E 161b):

The main colouring principle of lutein consists of carotenoids of which lutein (oxidised carotenoid) and its fatty acid esters account for the major part. Variable amounts of carotenes are also present. Lutein may contain fats, oils and waxes naturally occurring in the plant material (Directive 2008/128/EC[6]).

Mixed carotenoids (JECFA, 2006b) (E 161b):

The main colouring principle consists of carotenoids of which lutein accounts for the major part. Variable amounts of neoxanthin, violaxanthin and β-carotene are also present. Mixed carotenoids may contain fats, oils and waxes naturally occurring in the plant material. Vegetable oils may be added for standardising purposes.

The Panel noted that the EU authorised also a food colour named 'mixed carotenes' (E 160a (i)). The main colouring principle of this food colour consists of carotenoids of which β-carotene (and not lutein) accounts for the major part.

Lutein from Tagetes erecta L. (JECFA, 2006b) (E 161b):

Lutein from *Tagetes erecta L.* is a purified extract of xanthophylls obtained from marigold (*Tagetes erecta L.*) flowers, saponified with potassium hydroxide in either methanol or propylene glycol. The resulting crystalline material contains lutein, and minor components including other carotenoids and waxes.

2.2. Specifications

Specifications for lutein (E 161b) have been defined in the EU legislation (Directive 2008/128/EC) and by JECFA (JECFA, 2006b) (Table 1).

'Mixed carotenoids' and 'xanthophylls' are included in these specifications as synonyms for lutein. The Commission Directive specifications also cover lutein from *Tagetes erecta*. In contrast, JECFA has separate specifications for 'mixed carotenoids' and for 'lutein from *Tagetes erecta*'. Information for all three specifications (lutein (Directive 2008/128/EC)), mixed carotenoids (JECFA, 2006b) and lutein from *Tagetes erecta* (JECFA, 2006b) is presented in Table 1.

The Panel noted that the term "marigold" may be used to denote *Tagetes erecta*, but that this common name may be used for other species including for example *Calendula officinalis*, and that for this reason the term "marigold" should not be used in the specifications.

Lutein (Directive 2008/128/EC) (E 161b):

Content of total colouring matter not less than 4% calculated as lutein. Total "colouring matter" is expressed as "total carotenoids". The Panel noted that this implies that the actual level of lutein can be even less than 4%.

The Panel has been informed by the Natural Food Colours Association (NATCOL) that the following products are marketed under the current EU specifications:

a) Lutein with low concentrations of total carotenoids at levels of ~5-12%.

b) Lutein with high concentrations of total carotenoids extracted and present as esters at levels of at least 60%.

[6] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63.

c) Lutein with high concentrations of total saponified carotenoids at levels of at least 80% (cf. JECFA specifications for lutein from *Tagetes erecta*).

Zeaxanthin occurs together with lutein. In the three products described zeaxanthin is present as follows:

a) In low-lutein concentrated *Tagetes* extracts zeaxanthin is present at approximately 4% of the total carotenoids content. A typical extract contains around 10-12% lutein (total carotenoids) including 0.4-0.5% zeaxanthin.

b) The high concentration esterified lutein extract contains carotenoids in the ester form. Less than 7% of the carotenoid esters are zeaxanthin esters (usually between 3-5% on a batch-by-batch basis). The conversion factor 1.86 gives the free carotenoids (after hydrolysis e.g. by esterases present in body tissues).

c) The high concentration saponified lutein extract contains at least 9% of zeaxanthin (cf. JECFA specifications for *lutein from Tagetes erecta*)

If a proportional limit for zeaxanthin in all lutein extracts is envisaged, a maximum level of 10% (of total carotenoids) may be representative.

***Mixed carotenoids* (JECFA, 2006b) *(E 161b)*:**

Total colouring matter (as lutein) 'not less than declared'.

***Lutein from Tagetes erecta* (JECFA, 2006b) *(E 161b)*:**

According to the specifications of (JECFA, 2006b), lutein from *Tagetes erecta* should contain not less than 80% total carotenoids, not less than 70% lutein.

Table 1: Specifications for lutein (E 161b) according to Commission Directive 2008/128/EC and JECFA (JECFA, 2006b)

Purity	Commission Directive 2008/128/EC (lutein)	JECFA (2006b) (mixed carotenoids)	JECFA (2006b) (lutein from *T. erecta*)
Solvent residues:			
- Acetone	≤ 50 mg/kg, singly or in combination	≤ 50 mg/kg, singly or in combination	≤ 10 mg/kg
- Methanol			
- Ethanol			
- Propan-2-ol			
- Hexane			≤ 50 mg/kg
- Methyl ethyl ketone		≤ 10 mg/kg	
- Dichloromethane	≤ 10 mg/kg	≤ 10 mg/kg	
- Propylene glycol	-	-	≤ 1000 mg/kg
Moisture	-	-	≤ 1.0%
Ash	-	-	≤ 1.0%
Zeaxanthin	-	-	≤ 9.0%
Waxes	-	-	≤ 14.0%
Arsenic	≤ 3 mg/kg	-	-
Lead	≤ 10 mg/kg	≤ 5 mg/kg	≤ 3 mg/kg
Mercury	≤ 1 mg/kg	-	-
Cadmium	≤ 1 mg/kg	-	-
Heavy metals (as Pb)	≤ 40 mg/kg	-	-

The Panel noted that neither the EU nor the JECFA specifications provide information on the % of subsidiary colouring matter.

In several studies, described in the previous evaluations and available in more recent literature, details are provided on the source and the purity of the lutein product tested. In most (specified) cases, the lutein content is > 70%. In the current EU specifications, a lutein content of > 4% is required. Therefore, the materials tested in the available studies could differ substantially from the food colour preparations that are on the market.

The Panel noted that the JECFA specifications for lead are ≤ 5 or ≤ 3 mg/kg whereas the EC specification is ≤ 10 mg/kg.

Aluminium lake: According to the information provided by NATCOL to EFSA, only a few aluminium lake versions of natural colours are technically possible (carmine, curcumin, chlorophyllin, copper chlorophyllin). The aluminium lake version of lutein is apparently not technically possible.

The Panel noted that, if available, the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

2.3. Manufacturing process

Lutein* (*Directive 2008/128/EC) (E 161b):

Lutein is obtained by solvent extraction of the natural strains of edible fruits and plants, grass, lucerne (alfalfa) and *Tagetes erecta.* Only the following solvents may be used for the extraction: methanol, ethanol, propan-2-ol, hexane, acetone, methyl ethyl ketone, dichloromethane and carbon dioxide.

Upon request, NATCOL indicated that the principal solvent used for preparation of the major products on the market is hexane and that in addition manufacturers reported the use of the following more polar solvents: isopropyl alcohol (propan-2-ol), acetone, methanol and ethanol. Dichloromethane can be present as a carry over from chlorophyll extract if the lutein is derived from a green leaf (grass). One manufacturer reported also the use of propylene glycol.

The main sources for the manufacturing of lutein are *Tagetes erecta* flowers (mainly from India, Peru, Mexico). Lutein of higher concentration is produced exclusively from this source. Lower concentrated lutein is also extracted from other sources which primarily are used as a source for the food colour chlorophylls and chlorophyllins (grass, nettle, lucerne, spinach).

Mixed carotenoids* (JECFA, 2006b) *(E 161b):

Mixed carotenoids (of which lutein accounts for the major part) are obtained by solvent extraction of alfalfa, removal of chlorophylls through saponification and subsequent purification of the carotenoids by solvent extraction. Only the following solvents may be used for the extraction: methanol, ethanol, propan-2-ol, hexane, acetone, dichloromethane and methyl ethyl ketone (JECFA, 2006).

Upon request NATCOL indicated that the principal solvent used for preparation of the major products on the market is hexane and that in addition manufacturers reported the use of the following more polar solvents: isopropyl alcohol (propan-2-ol), acetone, methanol and ethanol. Dichloromethane can be present as a carry over from chlorophyll extract if the lutein is derived from a green leaf (grass). One manufacturer reported also the use of propylene glycol.

***Lutein from Tagetes erecta* (JECFA, 2006b) *(E 161b)*:**

Lutein from *Tagetes erecta* is a purified extract of xanthophylls obtained from marigold oleoresin. The oleoresin is prepared from hexane extracts of marigold flowers, saponified with potassium hydroxide in either methanol or propylene glycol (JECFA, 2006b).

2.4. Methods of analysis in foods

The content of lutein can be analysed by HPLC using conditions specified in (JECFA, 2006b). This method however, appears to be applicable for colour samples rather than for lutein in food.

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 (EFSA, 2009).

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

2.5. Reaction and fate in foods, stability

The stability of lutein in two liquid enteral nutrition products (milk-based) has been analysed. The lutein content in these products was analysed every three months, during a 12-month period and results showed that the levels remained stable within ± 10%. As lutein is sensitive to oxygen and light, it should be stored in sealed containers and in the dark (EFSA, 2006).

2.6. Case of need and proposed uses

Currently, lutein is an authorised natural food colour in the EU, with maximal allowed use levels of 50 to 500 mg/kg food for various foodstuffs. Lutein is also allowed in beverages at levels up to 200 mg/l. Table 2 summarises those beverages and foodstuffs that are permitted to contain lutein up to specified maximum levels set by EC legislation (94/36/EC[7]). In Council Directive 94/36/EC it is stated that the maximum levels indicated refer to the quantities of colouring principle contained in the colouring preparation (article 2, paragraph 6). According to Directive 2008/128/EC the total lutein content of the additive is not less than 4%. This implies that maximum levels of the colouring preparation (i.e. E 161b) in foodstuffs can be up to 25 times higher than the levels mentioned in Table 2, which apply specifically to the colouring principle.

Table 2: Maximum permitted usage levels of lutein in beverages and foodstuffs according to Council Directive 94/36/EC

Beverages	**Maximum level (mg/l)**
Non-alcoholic flavoured drinks Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum level (mg/kg)**
Complete formula for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Jam, jellies and marmalades and other similar fruit preparations including low calorie	100

[7] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 273, 10.9.94, p.13

products Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins	
Edible ices Desserts including flavoured milk products	150
Candied fruits and vegetables, Mostarda di frutta Preserves of red fruits Fine bakery wares Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Confectionery Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	*Quantum satis*

In a report by Tennant (2007), data are provided from a food additive usage survey conducted in 2006-2007. All manufacturers of natural food colours who are members of NATCOL were asked to provide information about the formulation of the products and also on the amount of each formulation used in each food. This allowed the concentration of pigment in each food to be established. A range of lutein concentrations was allowed because different use levels correspond to different products (e.g. mild colouring required for some food products versus very intense colours for others). Since it is necessary to identify one lutein level for use in intake estimates, manufacturers were also asked to identify a 'typical' colour use level. This was to avoid the need to use the maximum use level that corresponds to the most intense colours in all applications. Four companies provided usage data for lutein covering 10 food applications. The collated results of the usage survey for lutein are tabulated below (Table 3). Typical use levels represent values provided by contributors except in cases where no typical value was provided, in which case the upper limit of the range was used.

Table 3: Outcome of NATCOL usage survey for lutein

Beverages	**Range (mg pigment**/l)**	**Maximum reported use level* (mg pigment/l)**
Non-alcoholic flavoured drinks	1-85	85
Foodstuffs	**Range (mg pigment/kg)**	**Maximum reported use level* (mg pigment/kg)**
Confectionery	3-300	300
Decorations & coatings	2-500	500
Fine bakery wares (egg, Viennoiserie, biscuits, cakes, wafers)	2-100	100
Edible ices	5-100	100
Desserts including flavoured milk products	1-15	15
Sauces, seasonings (e.g. curry powder, tandoori), pickles, relished, chutney, piccalilli	10-500	500
Snacks: dry, savoury potato, cereal or starch-based snack products: extruded or expanded savoury snack products	15-60	60

Savoury snack products and savoury coated nuts	15-60	60
Edible cheese rind and edible chasings	60-120	120
Soups	50	50
Meat and fish analogues based on vegetable protein	1.4-4.2	5

*All dosages ready-to-eat.

**'pigment' refers to the colouring principle (i.e. lutein) and not to the food colour preparation that is specified as E 161b.

2.7. Information on existing authorisations and evaluations

Lutein has been previously evaluated by the SCF in 1975 and JECFA in 2004 and 2006.

The SCF indicated that no specific biological data were available (1975). Therefore, the SCF could not establish an Acceptable Daily Intake (ADI), but nevertheless recommended that xanthophylls prepared from natural foods by physical processes be accepted for use as colouring matters in food without further investigation. For the purposes of the Council Directive 62/2645/EEC[8] , the SCF suggested that the acceptable natural xanthophylls be defined as including the 3-hydroxy- and 3,3-dihydroxy-neoxanthin, neochrome, and the fatty acid esters of these compounds present in natural foods.

In 1977, the SCF concluded that no ADI could be established for antheraxanthin oleoresin (hexane extract of the flower petals of Aztec Marigold (=*Tagetes erecta*)) and that antheraxanthin oleoresin is not toxicologically acceptable for use in food.

JECFA established a group ADI of 0-2 mg/kg body weight (bw) for lutein from *Tagetes erecta* and for zeaxanthin (JECFA, 2004; 2006). The ADI was derived from a 90-day study in rats (Kruger et al., 2002; Pfannkuch et al., 2000a; Pfannkuch et al., 2001a). The ADI was based on the NOAEL of this study identified to be 200 mg/kg bw/day (the highest dose tested) and an uncertainty factor of 100. Although the ADI was based on the results of a short-term study, JECFA concluded on the basis of the supporting data and lack of effects at much higher doses in some other studies (e.g. a study of developmental toxicity) that an uncertainty factor of 100 was appropriate. Zeaxanthin was included in the ADI in view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin. The group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications. According to the specifications of JECFA (JECFA, 2006b), lutein from *Tagetes erecta* should contain 'not less than 80% total carotenoids, not less than 70% lutein'. However, according to the 'mixed carotenoids' specifications of JECFA (2006b), total colouring matter (as lutein) should be 'not less than declared'. The Panel noted that it is not completely clear how the JECFA group ADI relates to these 'mixed carotenoids' specifications.

EFSA's Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) evaluated lutein for use in the manufacture of Foods prepared for Particular Nutritional Uses (PARNUTs) (EFSA 2006). The product evaluated had the same specifications as the approved food additive. The petitioner's proposed use of lutein was for Foods for Special Medical Purposes (FSMPs) at levels that would give rise to daily intakes of 0.5–2 mg lutein/day. This is within the range of a regular dietary intake. Given that lutein extract from the natural strains of edible fruits and plants, grass, Lucerne and *Tagetes erecta* is already permitted as a food additive, the AFC Panel concluded that the use of lutein in FSMPs is not of safety concern under the proposed use levels which are in the range of the regular dietary intake of lutein, provided that the preparation is in compliance with the existing EU specifications for the food additive. The Panel was not able to evaluate the general use of lutein in PARNUTS, since no information was provided on proposed uses and use levels other than for FSMPs (EFSA, 2006).

[8] Council Directive 62/2645/EECof 23 October 1962 on the approximation of the rules of the Member States concerning the colouring matters authorized for use in foodstuffs intended for human consumption. OJ 115, 11.11.1962, p. 2645–2654.

EFSA's Panel on Dietetic Products, Nutrition and Allergies issued an opinion on the safety, bioavailability and suitability of lutein for the particular nutritional use by infants and young children (EFSA, 2008b). The Panel considered that the information provided in the dossier does not raise concerns about the safety of lutein in infant formulae at the levels achieved through the natural content of ingredients nor at the level of use (concentration of added lutein 250µg/l) proposed by the applicant for infant formulae with a low natural lutein content (about 20 µg/l or lower).

EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety of use of colouring agents, including lutein, in animal nutrition (EFSA, 2009). It was stated that data on the safety of lutein for the target animals are not available, but that 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. It was also concluded that 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, and that it does not require a particular safety assessment.

2.8. Dietary exposure

Dietary intake via use as a food colour

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the scientific cooperation (SCOOP) Task 4.2 (EC, 1998), to estimate additives' intakes. For each successive Tier, this involved a further refinement of intakes. The approach goes from the conservative estimates that form the first Tier of screening, to progressively more realistic estimates that form the Second and Third Tiers (Appendix A).

2.8.1. Crude estimates (Budget method)

The dietary exposure to lutein from the maximum permitted use levels was estimated using the Budget method (Tier 1), with the assumptions described in the report of the SCOOP Task 4.2 (EC, 1998).

In the case of lutein, the maximum permitted use level considered for beverages was 200 mg/l. The maximum permitted level considered for solid foods was 500 mg/kg (Table 2).

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In effect, even though lutein may be used in a variety of solid foods that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed solid foods with the same colour added. In the case of beverages, uses are reported for a limited number of beverages; however, some of these may constitute a significant proportion of liquid intake (i.e., non-alcoholic flavoured drinks) with consumer loyalty to a single brand (and therefore to a specific colour) often being high for this category of product. The 25% proportion was therefore considered adequate also for beverages (EC, 1998). This assumes that a typical adult, weighing 60 kg, consumes daily 1.5 litres of beverages and 375 g of solid foods, containing lutein. The theoretical maximum daily exposure for adults would therefore be:

(200 x 0.1 x 0.25) + (500 x 0.025 x 0.25) = 5 + 3.12 = 8.1 mg/kg bw/day.

For children, the level of lutein considered in beverages was 100 mg/l (after exclusion of alcoholic drinks), and in solid food 500 mg/kg. The proportion of 25% used, for beverages, was recognised to be inadequate for children, as the corresponding consumption rate of 375 ml/day could easily be exceeded by young children. This conclusion was derived from UK data on consumption of soft drinks by children aged less than 5 years, where the 97.5th percentile of consumption was between 70 and 80 ml/kg bw/day and a proportion factor of 100% for beverages was recommended for children in the SCOOP Task 4.2 (EC, 1998). This assumes that a typical 3-year old child, weighing 15 kg, consumes daily 1.5 litres of beverages and 94 g of solid foods, containing lutein.

The overall theoretical maximum daily exposure in children would therefore be:

(100 x 0.1 x 1) + (500 x 0.025 x 0.25) = 10 + 3.12 = 13.1 mg/kg bw/day.

It was noted that lutein may be used *quantum satis* in edible cheese rinds and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, it was excluded from the Budget calculation, since it is not expected to influence the outcome of this exposure calculation to any relevant extent.

2.8.2. Refined estimates

Refined exposure estimates have been performed for Tier 2 using maximum permitted use levels presented in Table 2 and maximum practical used levels presented in Table 3 to deal with the specific cases of *quantum satis* authorization for edible cheese rinds and edible casings, and for Tier 3 using the maximum reported use levels presented in Table 3, for children and the adult population.

Exposure estimates for children (1-10 years old) have been performed by the Panel, based on detailed individual food consumption data from eight European countries provided by the EXPOCHI consortium (Belgium, France, the Netherlands, Spain, Czech Republic, Italy, Finland and Germany) for Tier 2 and Tier 3. As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant, 2006).

Since the UK population is considered to be one of the highest consumers of soft drinks in Europe and as estimates were provided on more refined adult food consumption data, in comparison to those available to the Panel (e.g. EFSA Concise European Food Consumption Database, which gives access to aggregate food categories consumed in 15 European countries), the Panel decided to select the UK population as representative of the EU consumers for the lutein intake estimates for adults.

Estimates of lutein exposure from the UK adult population (>18 years old) have been made by the Panel with the use of the detailed individual food consumption data (UK NDNS, 2000-2001) available from the UNESDA report (Tennant, 2006).

Table 4 summarises the anticipated exposure of children and adults to lutein.

Tier 2

In the case of lutein, when considering MPLs of use (Tier 2), the mean dietary exposure of UK children aged 1.5 to 4.5 years and weighing 15 kg, was 3.0 mg/kg bw/day and 7.2 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks (55%), confectionery (13%) and desserts, including flavoured milk products (12%).

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg) considered by the EXPOCHI consortium ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to lutein (>10% in all countries, these contributions differed per country), were soft drinks (up to 56%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (up to 48%), and desserts, including flavoured milk products (up to 53%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for up to 44% of exposure in six countries.

Estimates reported for the UK adult population give a mean dietary exposure to lutein of 0.8 mg/kg bw/day, and of 2.0 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure to lutein (>10%) were soft drinks (47%).

Tier 3

Further data suggest that current use levels of lutein in some food categories are lower than the MPLs. Therefore, it was decided that concentration data made available to the Panel by NATCOL surveys, would be used to refine the estimate of dietary exposure to lutein (Tier 3).

When considering the maximum reported use levels from Table 3, the mean dietary exposure of UK children, aged 1.5 to 4.5 years and weighing 15 kg, was 2.2 mg/kg bw/day and 5.7 mg/kg bw/day for high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) for UK pre-school children were soft drinks (55%), confectionery (13%) and desserts including flavoured milk products (12%).

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg), considered by the EXPOCHI consortium, ranged from 0.2 to 2.2 mg/kg bw/day, and from 0.7 to 5.7 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to lutein (>10% in all countries), were soft drinks (up to 66%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (up to 46%) and desserts including flavoured milk products (up to 23%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for up to 61% of exposure in four countries.

Estimates reported for the UK adult population give a mean dietary exposure to lutein of 0.6 mg/kg bw/day and of 1.6 mg/kg bw/day for the high level (97.5th percentile) consumers of soft drinks. The main contributors to the total anticipated exposure (>10%) were soft drinks (60%), confectionary (10%) and sauces, seasonings, pickle, relishes, chutney, piccalilli (12%).

Table 4: Summary of anticipated exposure to lutein using tiered approach (EC, 2001) in children and adult populations

	Adult UK population (>18 years old)	**Pre-school UK children** (1.5 - 4.5 years old, 15 kg body weight)	**Children EXPOCHI population (1-10 years old, 25-30 kg body weight)**
	mg/kg bw/day		
Tier 1. Budget method	8.1	13.1	
Tier 2. Maximum Permitted Level			
• Mean exposure	0.8	3.0	0.5-3.4
• Exposure 95th *or 97.5th percentile	3.2	7.2	1.2-7.2
Tier 3. Maximum reported use levels			
• Mean exposure	0.6	2.2	0.2-2.2
• Exposure 95th *or 97.5th percentile	1.6	5.7	0.7-5.7

* For EU children, estimates are based on the EXPOCHI report, which gives the 95th percentile intake.
** For UK, estimates are based on the UNESDA report which gives the 97.5th percentile intake from beverages plus per capita average from the rest of diet (Tennant, 2006).

Dietary intake via other sources

Lutein is present in green (leafy) vegetables such as spinach, lettuce, broccoli, kale and Brussels sprouts, but also in maize, carrots, green peppers and peas. In Europe the foods contributing to lutein intake vary per country, but the major foods are spinach, lettuce, broccoli, peas and egg yolk (O'Neil et al. 2001; Scott et al. 1996; Reed Mangels et al. 1993, Holden et al., 1999).

TemaNord (2002), JECFA (2006) and EFSA (2006) have provided several dietary intake estimates for lutein. These intake estimates are also listed in Table 5. Although not completely clear from the available summaries, these intake estimates (besides part of the data described by DSM (2004) and Kruger (2002)) are most likely based solely on the intake of foods that naturally contain lutein. The foods to which the food colour lutein is added according to the user survey (Table 5) in general do not

naturally contain lutein and are therefore probably not included in the intake assessments mentioned below.

TemaNord (2002) provided some information on the intake of lutein. Based on the concentration of lutein in different vegetables and a household budget survey of vegetables and fruits, the average daily intake of lutein from natural sources in Denmark is estimated to be less than 1 mg/day with large individual variations (Strube and Dragsted, 1999).

In the EFSA opinion (2006), exposure to lutein is described as follows:

"Typical carotenoid intakes from the diets of healthy adults and children have been quantified in many studies. O'Neill et al. (2001) compared the carotenoid intakes in five European countries of individuals between 25 and 45 years of age. Lutein plus zeaxanthin intake was estimated by a food frequency questionnaire (FFQ) and carotenoid database and was reported to vary per country, ranging on average between 1.14 and 4.34 mg per day (O'Neil et al. 2001). The authors state that this food frequency questionnaire method probably overestimates the intake (O'Neil et al., 2001).

In a British study conducted with females aged 50-65 year the average daily consumption of lutein-rich food, was reported to result in a daily lutein intake of 0.89 mg/day per person (Scott et al., 1996). For Canadian adults, age 18 to 65 years, mean lutein intakes between 1.1 and 1.5 mg lutein/day were reported (Johnson-Down et al., 2002). In studies from the United States conducted with males (Forman et al., 1993) or pre-menopausal females (Yong et al., 1994) assessment of food frequency questionnaires and food diaries revealed a lutein intake of approximately 2.1 to 2.5 and 1.9 to 2.4 mg/day per person respectively. Caroll et al. (1997) reported dietary intakes of lutein plus zeaxanthin; revisiting the original publication revealed that reported dietary intakes for males and females aged 24 - 45 year and > 65 year ranged from 0.8 - 2.6 mg/person.

EFSA (2006) indicated that overall the dietary intake of lutein as such is estimated to be on average between 0.8 and 2.5 mg/day (0.01-0.04 mg/kg bw/day for adults).

JECFA (2006) provided the following information:

"Dietary recall data from 1102 adult women participating in the 1986 Continuing Survey of Food Intake by Individuals indicate mean intakes of lutein/zeaxanthin of 1.3 mg/day (Chug-Ahuja et al., 1993). Food frequency data from 8341 adults participating in the 1992 National Health Interview Survey indicate that mean intakes of lutein for men were 2.2 mg/day and for women 1.9 mg/day (Nebeling et al., 1997). The Nutritional Factors in Eye Disease Study reported mean dietary intakes of lutein/zeaxanthin of 0.7–0.8 mg/day (VandenLangenberg et al., 1996). In a pooled analysis of seven cohort studies designed to assess the effect of dietary carotenoids on risk of lung cancer, intakes of lutein/zeaxanthin were energy-adjusted using the predicted intake of 2100 kcal/day for men and 1600 kcal/day for women (Mannisto et al., 2004). Food consumption was assessed at baseline using a validated dietary questionnaire for each study population. For these seven populations, the mean intake of lutein/zeaxanthin for men and women combined was 3.7 mg/day (range, 1 - 6 mg/day) (not specified for men and women separately).

The estimated mean and 90th percentile consumption of lutein and zeaxanthin in a survey of sample foods were 1.71 and 3.01 mg/day respectively in the United States of America (USA) (DSM, 2004). Simulations considering proposed food use levels in the total population of the USA resulted in estimated mean and 90th percentile intake of lutein by all users of 7.3 and 13.4 mg/day, respectively (DSM, 2004). Kruger et al. (2002) estimated the intake of lutein/zeaxanthin in the USA using dietary records. The mean and 90th percentile intakes of lutein/zeaxanthin were 3.83 and 7.29 mg/day respectively, and the foreseen mean and 90th percentile intakes of a crystalline lutein product were 0.91 and 1.77 mg/day, respectively. Intake of lutein in 1543 Canadians (aged 18 - 65 years), estimated by 24-hour recall, was 1.41 and 0.57 mg/day (mean and median, respectively) (Johnson-Down, 2002). Intake of lutein in 76 women (aged 50–65 years) from the United Kingdom (UK), estimated by the determination of both food intake and concentrations of lutein was 0.92 mg/day (Scott et al., 1996).

Formulations of lutein/zeaxanthin are also available as dietary supplements, but according to JECFA (2006) there are no reliable estimates of intake from these sources.

In short, JECFA (2006) summarised that data on dietary intake from a number of studies in North America and the UK indicate that the average intake of lutein from natural sources is in the range of 1 - 2 mg/day (approximately 0.01–0.03 mg/kg bw/day). Simulations considering proposed levels of use of lutein as a food ingredient resulted in an estimated mean and 90th percentile intake of lutein plus zeaxanthin of approximately 7 and approximately 13 mg/day, respectively.

Table 5: Dietary intake estimates of lutein from other (i.e. not food colour) sources as referred to in EFSA (2006) and JECFA (2006)

	Population	Method	Average intake	Country	Reference
Lutein + Zeaxanthin	25-45 years	Food Frequency Questionnaire (FFQ) and carotenoid database	1.14-4.34 mg/day	Five European countries	O'Neill et al., 2001
Lutein	Women aged 50-65 years	Determination of both food intake and concentration of lutein	0.9 mg/day	UK	Scott et al., 1996
Lutein	18-65 years	24-hours food recall	1.1-1.5 mg/day (median 0.57)	Canada	Johnson-Down et al., 2002
Lutein	Males	FFQ + diaries	2.1-2.5	USA	Forman et al., 1993
Lutein	Premenopausal females	FFQ + diaries	1.9-2.4	USA	Yong et al., 1994
Lutein + Zeaxanthin	Males 24-45 years Females 24-45 years Males > 65 years Females > 65 years	FFQ + diaries	1.0-2.3 0.8-1.9 0.9-2.6 1.0-2.1	Ireland	Caroll et al., 1997
Lutein + Zeaxanthin	Adult women	Dietary recall	1.3	USA	Chug-Ahuja et al., 1993
Lutein	Men Women	FFQ	2.2 1.9	USA	Nebeling et al., 1997
Lutein + Zeaxanthin	Adults (43-85 year)	FFQ + carotenoid food composition database	0.7-0.8	USA	VandenLangenberg et al., 1996
Lutein + Zeaxanthin	Men and women	Dietary questionnaire	3.7 (range 1-6 mg/day)	Various countries	Mannisto et al., 2004
Lutein + Zeaxanthin	-	Survey of sample foods	1.71 (90th percentile 3.01)	USA	DSM, 2004
Lutein + Zeaxanthin*	All users	Simulations considering proposed food levels	7.3 (90th percentile 13.4)	USA	DSM, 2004
Lutein + Zeaxanthin		Food consumption data and carotenoid levels in food	3.83 (90th percentile 7.29)	USA	Kruger et al., 2002
Crystalline lutein		Food consumption data and	0.91 (90th percentile	USA	Kruger et al., 2002

	Population	Method	Average intake	Country	Reference
product*		anticipated lutein concentrations	1.77)		

* These intake estimates do not refer to lutein naturally present in food but to lutein used as a food additive.

In its opinion on the safety of use of colouring agents in animal nutrition (EFSA, 2009) the Panel on Additives and Products or Substances in Animal Feed (FEEDAP) estimated that the contribution to human exposure of lutein from eggs from hens fed diets containing lutein would represent a very small proportion of the total intake and varies with the consumption pattern in different countries.

The combined exposure from lutein naturally present in food and from its use as food colour, assuming for the former an average intake of 2.5 mg/day for both children (equal to 0.1 mg/kg bw/day) and adults (equal to 0.04 mg/kg bw/day), and using for the latter the anticipated exposure estimates from Tier 3 in table 4 is estimated to be in the range from 0.3-2.3 mg/kg bw/day for children and 0.64 mg/kg bw/day for adults on average. For the combined exposure at the 95th percentile, assuming an intake of lutein naturally present in food of 7 mg/day for both children (equal to 0.28 mg/kg bw/day) and adults (equal to 0.12 mg/kg bw/day), the range was estimated from 1.0-6.0 mg/kg bw/day for children and 1.7 mg/kg bw/day for adults.

3. Biological and toxicological data

Lutein has been previously evaluated by the SCF in 1975, by JECFA in 2006 and TemaNord in 2002. The present opinion briefly reports the major studies evaluated in these opinions and describes the additionally reported new literature data in some more detail.

The studies described in this section were mostly performed between 1990 and 2008. For several studies it is indicated that they complied with OECD guidelines and/or Good Laboratory Practice (GLP) principles. For the other studies, it is not clear whether they complied with these guidelines.

The Panel noted that in most studies where the lutein content of the test material is specified, the lutein content is > 70%. In the current EU specifications, a lutein content of > 4% is requested. Therefore, the substances tested in the available studies could differ substantially from the specifications provided for the present food colour preparation.

3.1. Absorption, distribution, metabolism and excretion

Several studies on toxicokinetics were described by EFSA (2006) and by JECFA (2006).

Animal data

Mice

Groups of 36 BALB/c mice received diets containing an extract of marigold petals for up to 28 days, corresponding to approximately 0, 75, 150, 300 or 600 mg lutein/kg bw/day and 0, 1, 2, 4, or 8 mg zeaxanthin/kg bw/day, respectively. On days 0, 3, 7, 14, 21 and 28, six mice per group were killed. Body, liver and spleen weights did not differ between the groups throughout the experiment. Plasma concentrations of lutein and zeaxanthin reached a maximum of about 3 µmol/l in all treatment groups by day 3 of dosing (the first time-point examined after the start of dosing) and did not differ between groups thereafter. At day 3, the concentrations of lutein and zeaxanthin in the liver and spleen were increased. Further increases, although small, in these concentrations were observed up to day 28. The liver was considered to be the major storage organ for lutein and zeaxanthin (Park et al., 1998).

Rats

Absorption, distribution, excretion and plasma kinetics of [^{14}C]-lutein (data from an unpublished report evaluated by JECFA; position of label not given in JECFA opinion) given as a single oral dose of 2 mg/kg bw were investigated in groups of three female RoRo SPF rats per time-point. The [^{14}C]-lutein was diluted with non-radioactive lutein purified from marigold petals, and was formulated as a beadlet containing an emulsion of gelatin and vegetable oil. Lutein was rapidly absorbed from the intestinal tract, resulting in peak plasma concentrations within 4 hours after dosing. About 80% of the radioactivity was recovered from the faeces and 11% from the urine, within 96 hours after dosing. Of the total amount of radioactivity excreted, 80% was recovered already within 24 hours. Low tissue concentrations of radioactivity indicated that lutein and/or its metabolites did not accumulate. With the exception of the intestinal tract, kidneys and liver, radioactivity was present in all tissues at all times at <0.01% of the administered dose. Total residual radioactivity in the carcass plus dissected tissues was negligible (0.23% of the administered dose). The absorption was estimated to have been 11.3% (Wendt et al., 2000). As the molecular weight of lutein is > 500 g/mol, this estimate may be too low, because biliary elimination was not accounted for. In addition, 10% of the dose was not accounted for.

Pharmacokinetics and tissue distribution of [^{14}C]-lutein (data from an unpublished report evaluated by JECFA; position of label not given in JECFA opinion) were investigated in Wistar rats (5/sex/group), following a single dose of 2 or 20 mg/kg bw administered by gavage. The rats had been maintained on a diet containing 2 or 20 mg/kg bw/day non-radioactive lutein for 2 weeks. The non-radioactive lutein was added to the diet as a beadlet formulation. Absorption of the [^{14}C]-lutein was rapid, with peak plasma concentrations reached within 3-4 hours at either dose. The pharmacokinetics of lutein were not linear. A ten-fold increase in dose resulted in an increase of approximately two-fold in the maximum plasma concentration of radioactivity. At 4 hours after dosing, the majority of tissues had been exposed to low levels of radioactive lutein, maximum tissue concentrations having been reached at this time-point. Highest lutein concentrations were found in the liver and gastrointestinal mucosa. Elimination from plasma was not complete after 48 hours, but concentrations of lutein at 96 hours after dosing were below the limit of detection in all tissues examined except the liver. There was no evidence for accumulation of lutein in any tissue examined. Most radioactive lutein was eliminated in the faeces (> 90% and > 65% of the administered dose for males and females, respectively) within 48 hours of dosing, with urinary and biliary excretion accounting for < 6% and < 2% respectively, of the administered dose. There was negligible (< 0.1%) recovery of radioactivity from expired air. Excretion was slightly more prolonged in females than males. Increasing the dose had minimal effects on the absorption or the rates and routes of excretion (Froescheis et al., 2001).

Plasma and liver concentrations of lutein were assessed as part of a 4-week study of toxicity, which complied with GLP. Wistar rats (6/sex/dose) received crystalline lutein (extracted from marigold petals) formulated as beadlets at dietary doses of 0, 2, 6, 20, 60, 200 or 600 mg/kg bw/day. There was a dose-dependent, almost linear increase in plasma concentrations of lutein. A ten-fold increase in dose between 20 and 200 mg/kg bw/day resulted in a two- to three-fold increase in plasma concentrations. Plasma steady state conditions were reached by day 3 (plasma concentrations were below the limit of detection at the lowest dose, and there were insufficient data at the next higher dose of 6 mg/kg bw/day). Liver tissue determinations revealed a dose-dependent increase in concentrations of lutein, and suggested that saturation was reached at 200 mg/kg bw/day. There were no sex-relevant differences in plasma concentrations, but the liver content of lutein was 1.5- to 3-fold higher in females than in males in the three groups receiving the highest doses (Buser et al., 1999; Simpson, 1999).

Male weanling Fischer rats (15/group) were maintained on diets supplemented with lutein for eight weeks. Lutein (extracted from marigolds) was formulated with 2.2% vitamin E (α- and γ- tocopherols) in beadlets delivering lutein at doses of 0, 2.1 4.3, 8.6, 17.8 or 34.7 mg/day. The apparent absorption of lutein was estimated to range from 28.7% at the highest dose to 43.1% at the lowest dose, based on intake and the faecal excretion of lutein. The limited absorption at higher intakes was reportedly due, in part, to factors such as solubility (i.e. limited capacity for micellar incorporation). There were significantly increased plasma concentrations of lutein in animals fed the two higher doses, and

increases in liver and spleen concentrations of lutein with increasing dietary intake. The relative distribution of lutein between the liver and spleen was approximately 95 and 5%, respectively, with no lutein detected in the heart, lungs, kidneys, testes or brain, the only other organs examined (Jenkins et al., 2000).

Cows

Six calves were fed milk replacer for one week, and were then given a single oral dose of 20 mg (about 0.4 mg/kg bw) of crystalline lutein from marigold petals (containing small amounts of zeaxanthin) in olive oil. The calves showed increased plasma concentrations of lutein that peaked at 12 hours after dosing, declined rapidly thereafter, and levelled out at approximately 72 hours (Bierer et al., 1995).

Human data

Absorption, distribution, elimination

After uptake into mucosal cells, carotenoids are incorporated into chylomicrons and released into the lymphatics. The carotenoids within the chylomicrons are transported to the liver where they are distributed between the lipoprotein fractions. A significant fraction of the xanthophylls is carried in the blood stream by high-density lipoprotein (HDL) (Romanchik et al., 1995). Carotenoids are present in variable amounts in many tissues such as kidneys, buccal mucosal cells and adrenal glands, but the main sites of storage are adipose tissue and the liver (Parker, 1996). The eye in general and the retina in particular, contain high concentrations of zeaxanthin and lutein which are responsible for the colouration of the *macula lutea* (yellow spot) (Bone et al., 1993; Landrum and Bone, 2001).

Xanthophylls may be ingested in free or esterified forms. Absorption of carotenoids by mucosal cells is believed to occur by passive diffusion, rather than via active transport (Hollander and Ruble, 1978). Before absorption, the esters are hydrolysed by pancreatic esterases and lipases such that only the free forms are found in the circulation (Wingerath et al., 1995). Once released from the food matrix as a lipid emulsion, these compounds must be solubilised within micelles in the gastrointestinal tract to permit absorption by mucosal cells (Erdman et al., 1993). The transfer of carotenoids from lipid emulsion droplets to mixed micelles depends on their hydrophobicity, as well as pH and concentration of bile acid. Other carotenoids do not affect the transfer of lutein (Tyssandier et al., 2001). The xanthophylls are preferentially solubilised on the surface of lipid emulsion droplets and micelles. This facilitates the transfer of compounds like lutein and zeaxanthin from the lipid droplets to the aqueous phase. Xanthophylls are more readily incorporated into micelles than other carotenoids (Borel et al., 1996; Garrett et al., 1999; Garrett et al., 2000).

The presence of fat in the small intestine stimulates the secretion of bile acids from the gall bladder and improves the absorption of carotenoids by increasing the size and stability of micelles, thus allowing a greater amount of carotenoids to be solubilised (Hollander and Ruble, 1978). The amount of dietary fat required to ensure absorption of carotenoids seems to be low (3-5 g/meal), although it depends on the physico-chemical characteristics of the carotenoids ingested. In one experiment, the plasma concentration of lutein added as esters, was about 100% higher when lutein was consumed with 35 g of fat than with 3 g of fat (van het Hof et al., 2000). The low amount of fat may have limited the solubilisation of lutein esters and/or the release of esterases and lipases (Roodenburg et al., 2000). Bioavailability of carotenoids is also affected by the absorbability of the dietary fat (Borel et al., 1998). Sterol and stanol esters apparently have no effect on absorption of lutein (Raeini-Sarjaz et al., 2002). Egg yolk is a source of zeaxanthin and lutein. The lipid matrix of the egg yolk, containing cholesterol, triacylglycerols and phospholipids, provides a vehicle for the efficient absorption of xanthophylls (Handelman et al., 1999).

The availability of lutein from a diet of mixed vegetables was 67% relative to that from a diet supplemented with crystalline lutein (van het Hof et al., 1999a). The relative bioavailability of lutein from various spinach products ranged from 45-54%, as compared to bioavailability of that from

supplements containing 6.6 mg of lutein plus 9.8 mg of β-carotene (Castenmiller *et* al., 1999). The plasma concentration of lutein was increased by about 14% when spinach was consumed chopped rather than whole (van het Hof et al., 1999b). The matrices of formulated natural or synthetic carotenoids (e.g. water dispersible beadlets, crystalline powders, oils suspensions etc.) and whether the compounds are esterified or non-esterified may affect availability (Swanson et al., 1996; Boileau et al., 1999). The presence of dietary fibre may (at least partly) explain the low availability of carotenoids from plant foods. Fibre may interfere with micelle formation by partitioning bile salts and fat in the gel phase of the fibre. Riedl et al. (1999) tested the effects of pectin, guar, alginate, cellulose or wheat bran on the availability of lutein in six healthy female volunteers. All fibres significantly (40-74%) reduced the plasma concentrations of lutein. However, in another study pectin had no effect on serum concentrations of lutein after administration of a diet supplemented with liquefied spinach (Castenmiller et al., 1999).

Interactions between carotenoids may also decrease absorption (Gärtner et al., 1996). Competitive inhibition may occur at the level of micellar incorporation, intestinal uptake and/or lymphatic transport. Simultaneous ingestion of various carotenoids may induce an antioxidant-sparing effect in the intestinal tract, resulting in increased levels of uptake of the protected carotenoids. Even in the presence of large amounts of β-carotene, chylomicrons preferentially take up xanthophylls rather than β-carotene from the intestinal lumen (Gärtner et al., 1996). An inhibitory effect of dietary lutein on the absorption of β-carotene has been observed when the carotenoids were measured in plasma lipoproteins (Van den Berg, 1998; Van den Berg and Van Vliet, 1998). Healthy volunteers were given single oral doses of 15 mg of lutein derived from marigold extract either alone or together with 15 mg of β-carotene derived from palm oil. The inclusion of β-carotene reduced the area under the curve (AUC) of concentration–time for lutein to 54-61% of that for lutein administered alone (Kostic et al., 1995). In the same study, while lutein appeared to slow the initial absorption of β-carotene, lutein did not have any significant effect on the plasma concentration of β-carotene at the main peak or on the AUC value for β-carotene. Indeed, lutein enhanced the AUC value for β-carotene in subjects whose AUC value for β-carotene only was the lowest. In a similar study to investigate the interactions between β-carotene and dietary lutein, healthy male subjects on controlled diets were given capsules containing purified β-carotene at one of two high daily doses (12 or 30 mg/day, corresponding to 0.2 or 0.5 mg/kg bw/day) for six weeks. Plasma concentrations of lutein in the group receiving β-carotene were decreased compared with baseline and were significantly lower than the levels reported in control groups given a placebo (Micozzi et al., 1992). Another study showed that the post-prandial appearance of vegetable-borne carotenoids in chylomicrons is competitive, but that this did not affect the plasma concentrations of the carotenoids after three weeks of feeding (Tyssandier, et al., 2002). Van den Berg (1999) has concluded that, in general, long-term supplementation with β-carotene has limited or no effect on plasma or serum concentrations of other carotenoids. However, in the α-Tocopherol and β-Carotene Cancer Prevention Study Group (ATBC) Study (ATBC, 1994), a total of 29 133 male Finnish smokers aged 50–69 years were given daily supplements of 20 mg of β-carotene (0.3 mg/kg bw/day) for an average of 6.7 years. Significantly decreased serum concentrations of lutein (no changes in serum concentrations of zeaxanthin) were observed in comparison with groups that did not receive supplements containing β-carotene (Albanes et al., 1997).

In contrast to the interactions observed between lutein and β-carotene during absorption, supplementation with lycopene (5 mg/day from whole tomatoes, tomato juice or gel capsules containing tomato oleoresin) reportedly had no effect on the plasma concentrations of lutein or zeaxanthin in a 6-week intervention study in 22 healthy female volunteers (Böhm and Bitsch, 1999).

Also the isomeric form (*cis* versus *trans*) of the carotenoids may affect their absorption. Lutein and zeaxanthin occur in nature predominantly in the all-*trans* configuration. However, small amounts of *cis* isomers of each carotenoid have been isolated from human serum (Krinsky et al., 1990; Khachik et al., 1999). Also in macaques and squirrel monkeys, higher proportions of the 13-*cis* isomer were found in plasma compared to the diet (Snodderly et al., 1990). It is not known whether the presence of *cis* isomers in human serum is exclusively due to their selective uptake and absorption from the diet, or

whether they are the product of *in vivo* isomerisation of all-*trans* lutein/zeaxanthin in the presence of gastric acids.

A number of non-dietary factors also affect the availability of carotenoids, including exposure to tobacco smoke, alcohol consumption, intestinal parasites, malabsorption diseases, liver and kidney diseases, hormone status, poor intake of iron, zinc and protein, gastric pH and hyperthyroidism (Albanes et al., 1997; Alberg, 2002; Patrick, 2000; Williams et al., 1998).

Bioavailability of lutein from marigold extracts was investigated in Type 1 diabetics and healthy controls. After 15 days depletion of carotenoids, subjects ingested 15 mg of lutein, and plasma and lipoprotein lutein response was monitored until 48 hours after ingestion. The AUC and maximal concentrations of lutein in serum and triglyceride-rich lipoprotein (TRL) were similar in diabetics and controls. The peak plasma lutein concentration occurred nine hours after ingestion, and the peak concentration of TRL lutein occurred eight hours post-dosing (Granado et al., 2002).

JECFA (2006) indicated that concentrations of lutein and zeaxanthin in serum and tissues are quite variable and increase with increased intake either from dietary sources or from supplements (Boileau et al., 1999; Hammond et al., 1997; Landrum et al., 1997a, 1997b; Carroll et al., 1999; Müller et al., 1999; Tucker et al., 1999; Berendschot et al., 2000; Johnson et al., 2000; Curran-Celantano et al., 2001; Olmedilla et al., 2001; Schalch et al., 2001; Bone et al., 2003). Also after administration of a single lutein supplement, considerable inter-individual variability was observed in the bioavailability, based on plasma concentrations of lutein (Kostic et al., 1995; Burri and Neidlinger, 2000; Torbergsen and Collins, 2000).

Lower serum concentrations of lutein and zeaxanthin are generally associated with male gender, smoking, younger age, lower HDL cholesterol levels, greater consumption of alcoholic beverages and higher body mass index (Brady et al., 1996).

Eighteen subjects ingested lutein either as diester or unesterified at 2 doses (0.5 or 0.67 µmol lutein/kg bw, equivalent to about 20 and 25 mg lutein) and serum lutein response was monitored. The AUC of serum lutein was significantly higher (+ 62%) after lutein diesters than after the unesterified formulation (Bowen et al., 2002).

In a multi-centre, placebo-controlled supplementation study, 400 healthy male and female volunteers from five different European countries aged 25-45 years received a daily lutein supplement containing 15 mg lutein from marigold extracts for 20 weeks. A five-fold increase (by approximately 0.95 µmol/l) of plasma lutein concentrations was observed, which reached a plateau after four weeks of supplementation (Olmedilla et al., 2002).

Subjects received 10 mg lutein derived from marigold daily during 12 weeks. Mean plasma lutein concentration increased from 0.18 to 0.90 µmol/l within the first four weeks and stayed at this level during the supplementation period (Berendschot et al., 2000).

Healthy volunteers (175) were supplemented with 15 mg lutein or placebo for three months. In the lutein group, plasma lutein concentrations increased from 0.22 ± 0.12 to 0.94 ± 0.13 µmol/l and LDL lutein concentrations increased from 43 ± 1.10 to 121 ± 1.32 ng/mg LDL cholesterol (Hininger et al., 2001).

The relative bioavailability of food-derived lutein was investigated in four human subjects (one male, three females) using deuterium-labeled lutein from intrinsically labelled spinach or collard green. Area under the curve analysis integrated over 29 days yielded serum lutein responses of 128, 145, 149 and 262 µg/day/mg dietary lutein following an acute dose of 15.4, 18.8, 18.8 and 29.8 mg lutein, respectively (Lienau et al., 2003).

EFSA (2006) referred to a pilot study conducted by industry and reported by the petitioner (no details on reference provided). A pilot-study was performed, investigating the effect of supplementing clinical

nutrition with a carotenoid mix, containing lutein, on plasma carotenoid concentrations. Five patients requiring long-term enteral tube feeding (> 3 months) and receiving at least 50% of their energy requirements from clinical nutrition were included. They were changed from a tube feed without carotenoids to a tube feed supplemented with the carotenoid mix (containing 0.41 mg lutein/1500 kcal) and received this supplementation for three months. Before supplementation and after 3 months of supplementation, blood samples were drawn and plasma carotenoid concentrations were determined. Mean lutein intake was 531 ± 94 µg/day during the supplementation period. At baseline all patients had lutein concentrations below the normal range (normal range was 0.078-0.442 µmol/l). Although plasma lutein concentrations increased, they were still below the normal range after supplementation. Plasma lutein concentrations were 0.049 ± 0.004 µmol/l at baseline and increased significantly (p=0.007) to 0.076 ± 0.008 µmol/l after three months of supplementation.

Human volunteers (four men and four women/group) were daily supplemented with capsules containing either 4.1 mg of crystalline lutein (with 0.34 mg of zeaxanthin) or 20.5 mg lutein (with 1.7 mg of zeaxanthin) for 42 days. Plasma concentrations of lutein and zeaxanthin were measured for a further 25 days after the dosing phase. Steady-state concentrations of xanthophylls were reached between days 38 to 43 (0.06 µmol/l and 0.13 µmol/l for the lowest and highest doses, respectively). Dose-normalised incremental maximum and average steady-state concentrations of lutein and zeaxanthin were found to be comparable, indicating that they have similar bioavailability. The elimination half-life was calculated to be approximately 5–7 days for either compound (Cohn et al., 2001).

Absorption of lutein was measured in the triacylglycerol-rich fraction of the blood of three men and three women fed with a standard meal after an overnight fast and given lutein at a dose of 31.2 mg (equal to about 0.4 mg/kg bw). Peak concentrations of lutein were observed two hours after dosing (O'Neill and Thurnham, 1998).

Serum levels of lutein were measured in eight adults (males and females) given single doses of 0.5 µmol/kg bw (equivalent to about 0.3 mg/kg bw) of crystalline lutein from marigold extract in corn oil. A mean peak serum concentration of about 0.7 µmol/l was reached at 16 hours after dosing, followed by a moderate decline to about 50% of the peak in the subsequent 120 hours and then a slow decline to baseline levels at 440 hours (Kostic et al., 1995).

Similarly, plasma concentrations of radioactive lutein from an algal source measured in four women given 3 mg of [^{13}C]-lutein (equivalent to 0.05 mg/kg bw) showed a mean peak concentration of about 0.007 µmol/l that was reached between 11 and 16 hours after dosing, followed by a moderate decline to about 50% of the peak concentration in the subsequent 100 hours and then a slow decline to baseline levels within about 500 hours (Yao et al., 2000).

Sixteen healthy volunteers were administered 4 or 20 mg lutein from marigold petals (equivalent to about 0.1 and 0.3 mg/kg bw/day) for 42 days. Plasma concentrations of lutein were observed to be about three and eight times greater than those in untreated controls. Twenty-five days after the end of the dosing, plasma concentrations were nearly at baseline levels for the low dose group, but were still noticeably greater than baseline in the high-dose group. In the low dose group, plasma concentrations of lutein increased up to day 42, but many subjects in the high dose group showed peak concentrations at earlier time-points (Schalch et al., 2001).

Three males received 10 mg lutein/day for a total of 18 days (equivalent to 0.2 mg/kg bw/day) and showed four- to five-fold increases in plasma concentrations of lutein compared with baseline by day seven of dosing (Khachik et al., 1995a).

Five male and three female subjects received 15 mg of lutein/day for seven days (equivalent to 0.25 mg/kg bw/day). The lutein was in capsule form and contained 80% all-*trans*-lutein and 20% *cis*-lutein. By day seven, plasma concentrations of lutein had increased two- to three-fold compared with baseline levels, decreasing to near-baseline levels after three weeks of wash-out (Torbergsen and Collins, 2000).

In humans, the administration of lutein or lutein esters extracted from marigold petals at doses of 0.2 to 0.5 mg/kg bw has been shown to result in accumulation of lutein in the macula, as evidenced by an increase in the macular pigment density (Berendschot et al., 2000; Duncan et al., 2002; Landrum et al., 1997a, b).

Subjects were supplemented lutein from frozen spinach at levels corresponding to 19 µmol lutein/day for up to 15 weeks. A significant increase in lutein concentrations in serum, buccal mucosal cells, adipose tissue and macular pigment density was observed. Serum lutein concentrations increased from 0.37 ± 0.05 to 0.67 ± 0.11 µmol/l during 15 weeks, but this concentration reached a plateau after four weeks of supplementation. The lutein concentrations in buccal mucosa cells increased from 4.45 ± 0.86 pmol/mg protein to a peak concentration of 10.30 ± 1.85 pmol/mg protein after 12 weeks. Lutein concentrations decreased again thereafter to 5.95 ± 1.73 pmol/mg protein after 15 weeks of supplementation. Lutein concentrations in adipose tissue increased from 0.23 ± 0.07 to 0.47 ± 0.08 µmol/mg dry weight after eight weeks of supplementation. From 8 to 15 weeks of supplementation lutein concentrations in adipose tissue decreased slightly to 0.38 ± 0.11 µmol/mg dry weight. Macular pigment density showed a gradual increase from 0.399 ± 0.045 to 0.469 ± 0.059 during 15 weeks of supplementation and reached a steady state after 12 weeks (Johnson et al., 2000).

The kinetics of carotenoid depletion and elimination have been investigated in 19 healthy women (Burri et al., 2001) and 12 healthy men (Rock et al., 1992) fed controlled low-carotenoid diets for approximately 10 and 13 weeks, respectively. In females, the decline in serum concentrations of all investigated carotenoids (including lutein and zeaxanthin) during depletion followed apparent first-order kinetics. The authors analysed these data and derived plasma half-lives for lutein and zeaxanthin of 76 and 38 days, respectively (Burri et al., 2001). In addition, JECFA indicated that a steady-state level for lutein in plasma was reached after about 30 days, and that this would indicate an elimination half-life of about six days (JECFA, 2006). This estimation of the plasma half-life of lutein by JECFA is not justified from the data in the original paper. In males (Rock et al., 1992), there were significant decreases in concentrations of all carotenoids, including lutein and zeaxanthin up to days 14-15, followed by a slower decline to days 63-64, that may be indicative of two pools, with one pool having a more rapid rate of turnover. Concentrations of lutein and zeaxanthin in the final sample of plasma (days 63-64) averaged 40% of the initial concentration, and the mean plasma depletion half-life for lutein and zeaxanthin (combined) was estimated by the authors to be between 33 and 61 days (Rock et al., 1992).

A non-statistically significant trend toward decreased concentrations of lutein in plasma and bile in patients with biliary and pancreatic diseases compared with controls was observed in a study of 41 patients and 14 healthy controls. However, it is clear, that carotenoids, including lutein/zeaxanthin, undergo appreciable biliary secretion. In addition, it was noted that biliary concentrations of carotenoids reflect plasma concentrations in both normal and pathological conditions. Interference with biliary secretion did not result in plasma retention of carotenoids (Leo et al., 1995).

Altogether the human studies reveal that lutein is bioavailable.

Metabolism

Little is known about the metabolism or degradation of lutein. Several lutein and zeaxanthin metabolites have been identified in human serum and are formed by chemical rather than enzymatic reactions (reviewed by Khachik et al., 1995a). Oxidation, reduction and double bond migration of the end groups of lutein are the main chemical reactions. Lutein and zeaxanthin can exist in equilibrium. Allylic oxidation of lutein at C3 results in the formation of oxolutein B that can exist in equilibrium with lutein and 3'-epilutein through reduction reactions. 3'-Epilutein and zeaxanthin can also exist in equilibrium through reversible double-bond migration. Acid-catalysed dehydration is another reaction of carotenoids with 3-hydroxy-ε endgroups. Lutein is believed to undergo dehydration in stomach acid to form 3-hydroxy-3',4'-didehydro-β,γ-carotene and 3'-hydroxy-2',3'-didehydro-β,ε-carotene (an-hydroluteins) both of which have been isolated from serum. In addition to their presence in human

serum, these metabolites as well as lutein itself have also been detected in breast milk as well as in retinal extracts (Khachik et al., 1995b; Khachik et al., 1997a,b,c). In human breast milk lutein was present at concentrations in the range of 37.0 to 58.1 nmol/l (n=3 different lactating females) with the ratio between the lutein concentration in serum compared to that in milk amounting to 5.4 to 8.5 (Khachik et al. 1997b).

Xanthophylls are precursors of retinol. However, they have been shown to have little or no activity as substrates of β-carotene-15,15'-dioxygenase, (i.e. no provitamin A activity) although they are able to inhibit the conversion of β-carotene to retinol (Ershov et al., 1993; Van Vliet et al., 1996; Grolier et al., 1997). However, in a rat model, Weiser and Kormann (1993) showed that the xanthophylls have small but significant provitamin A activity (4–5% of the activity of β-carotene), probably via a vitamin A-sparing effect.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA (2006) described the result of one acute oral toxicity study. The median lethal dose (LD_{50}) for orally-administered lutein (purified plant extract containing 70–85% lutein) in rats was estimated to be > 2000 mg/kg bw (Pfannkuch et al., 1999).

Since the JECFA (2006) evaluation, an additional acute oral toxicity study is available. Female Wistar rats (10/group) were given 1, 2 or 4 g/kg bw lutein or lutein ester (both isolated from marigold flowers) by oral gavage in four equal portions in 2 ml sunflower oil at 2-hour intervals. The 4 g/kg bw dose was given by oral gavage in six equal portions in 2 ml sunflower oil at two hour intervals. Controls received six portions of 2 ml sunflower oil at 2- hour intervals. Rats were monitored for 12 days for mortality, clinical and behavioural symptoms and any adverse reactions. Food consumption and body weight were measured every third day. The single-dose administration of lutein and lutein ester up to a concentration of 4 g/kg did not produce any mortality. The body weight of the animals did not differ much during the period of study. Food consumption was initially low, but was similar to controls from day 3 onwards. Also diarrhea was observed in all animals, including controls, for the first two days, and decreased from day three onwards; this latter effect may be related to the high amount of sunflower oil in the diet (Harikumar et al., 2008).

The Panel concluded that the acute oral toxicity of lutein is low.

3.2.2. Short-term and subchronic toxicity

Rats

EFSA (2006) described a 4-week pilot toxicity study and a 13-week toxicity study. Both studies are also reported by JECFA (2006).

A 4-week pilot toxicity study was conducted in Han Wistar rats to determine the oral toxicity following administration in the diet of a lutein product derived from marigold flowers (Kruger et al., 2002). A compositional analysis of the test product identified 97% of the components and indicated that 84% of the product consisted of the carotenoids lutein and zeaxanthin (79 and 5% respectively). The remaining content consisted of waxes, palmitic acid/palmitate, potassium and water (Kruger et al., 2002). Seven dose groups were used (0, 2.6, 7.7, 26.0, 77.3, 260 and 773.2 mg of lutein product/kg bw/day). The study was performed essentially according to OECD Test Guideline 407, with the exception that hematology parameters measured did not include blood clotting time/potential and that thymus and brain weights were not determined. Necropsies were performed after four weeks, after an overnight fast. Tissues were collected for gross examination. Organ weights were determined for adrenals, heart, kidney, liver, ovaries, spleen, testes and epididymides, and thyroids and parathyroids. Histopathology was performed on liver, spleen, skin and mesenteric lymph nodes. According to Kruger et al. (2002) the hematology and clinical chemistry analysis revealed sporadic, statistically

significant effects on several parameters compared with the control. However, there was no consistent dose-response, changes were small, and there was no correlation of the changes with any adverse treatment-related histopathology findings. These changes were therefore not considered to be treatment-related or biologically significant by the authors. Histopathological findings were generally infrequent and consistent with the expected pattern of background findings in rats of this strain and age. Histiocyte foci in the mesenteric lymph node of some animals from the high-dose group, particularly females, were noted. This was possibly related to the physical uptake of the test article and not to specific target organ toxicity. Mesenteric lymph nodes from other groups were comparable to the control. Necropsy findings indicate that most tissues were macroscopically unchanged compared to controls. The authors concluded that oral administration of this lutein product to rats at dose levels up to 773.2 mg/kg bw/day (highest dose level tested, corresponding to 611 mg lutein/kg bw/day since the lutein content of the product was 79%) for 4 weeks did not result in test article related toxicity and was well tolerated by the rats (Kruger et al., 2002).

Han Wistar rats (10/sex/dose) were exposed to 0, 2.6, 26 and 260 mg of a lutein product (similar specifications as described above)/kg bw/day for 13 weeks (Pfannkuch et al., 2000a; Pfannkuch et al., 2001a; Kruger et al., 2002). This study was performed according to OECD Test Guideline 408. Test article administration was conducted in the same manner as in the 4-week study described above. For the recovery study, five rats per sex from the control and high-dose group were fed basal diet for an additional four weeks following termination of the treatment and prior to necropsy. In addition to standard histopathological examinations, livers and kidneys from all animals in the low-dose group and middle-dose groups and spleens from selected animals in the low-dose group and middle-dose group and from animals identified by group examination in the control and high-dose groups (recovery period) were examined histopathologically.

No clinical signs or adverse effects that could be attributed to the test material were observed throughout the course of the study. One female in the low-dose group died in week 13, but the authors indicated that this was likely to be related to the blood sampling and not treatment-related. According to Kruger et al. (2002), body weight gains were slightly higher in females in the low-dose group, but this effect was not observed in the middle or high-dose group or in males from any dose group. There were no changes in the haematology, clinical chemistry and feed intake related to the administration of the test article. The activated partial thromboplastin time (APTT) was significantly higher in treated female groups compared with controls. This was however not dose-related and also not observed in males. According to the authors, in comparison to the control, microscopic examination of livers and kidneys from females in the high-dose group revealed an apparent increase in the incidence of hepatocyte vacuolation in the liver and tubular degeneration/regeneration in the kidney. These increases were reported to be not statistically significant and were not observed in any of the males from the control or treatment groups. Because there was no correlating evidence from clinical chemistry, indicative of toxicity to either organ in any of the dose groups, this effect was not considered toxicologically relevant by the authors. It can be concluded that a dose level of 260 mg lutein product/kg bw/day (highest dose level tested) for 13 weeks did not result in toxicity. This corresponds to a lutein dose of 200 mg/kg bw/day, since the lutein content of the product was 79% (Kruger et al., 2002). In 2006, JECFA established a group ADI of 0-2 mg/kg bw/day for lutein from *Tagetes erecta* and for zeaxanthin based on the NOAEL of 200 mg/kg bw/day (highest dose tested) from this 90-day study in rats and application of an uncertainty factor of 100.

The JECFA (2006) evaluation described several additional short-term toxicity studies.

Mice

Groups of 36 eight week old female BALB/c mice were given various doses of marigold extract (37% lutein esters and 0.5% zeaxanthin esters) by feeding a diet containing 0, 0.05, 0.1, 0.2 or 0.4% lutein for up to 28 days. Intakes of lutein were equivalent to approximately 0, 75, 300 or 600 mg/kg bw/day, intakes of zeaxanthin esters equivalent to approximately 0, 1, 2, 4 or 8 mg/kg bw/day. Six mice per group were killed on days 0, 3, 7, 14, 21 and 28. No differences in body, liver, or spleen weights

among treatment groups were reported, and there were no significant differences in feed intake throughout the experimental period (Park et al., 1998).

Groups of 10 females of apolipoprotein E (ApoE)-null mice and low-density lipoprotein (LDL) receptor-null mice received either a diet supplemented with lutein (no details on lutein source) (0.2% by weight in the diet, corresponding to approximately 500 mg/kg bw/day) or basal diet for eight weeks. The study was designed to investigate the possible protective effects of lutein on atherosclerotic lesion formation and cardiovascular effects. Supplementation with lutein was well tolerated, and there were no adverse effects on body weight gain (Dwyer et al., 2001).

Monkeys

Cynomologus monkeys aged 4–7 years (2/sex/group) were given lutein (extracted from marigold petals, no details on purity provided) at doses of 0, 0.2 or 20 mg/kg bw/day by gavage for 52 weeks. An additional male and female were included in the group receiving the highest dose, which were designated for examination at six months. The study was primarily designed to investigate the ocular effects of lutein and complied with GLP principles.

All animals survived the treatment period. No effects were observed on body weight gain, feed intake, haematology, blood chemistry, urine analysis measurements and organ weights. Histopathological examinations revealed no treatment-related findings. There were no treatment-related changes in data on electrocardiogram waveform or blood pressure. In the highest treatment group, all animals had orange/yellow discolouration of the faeces from day two onwards. Most of the animals showed dark yellow-coloured mesenteric fat at interim sacrifice and golden yellow mesenteric fat at terminal sacrifice; these latter two effects were attributed to the lutein treatment. Occasional retinal changes, such as inclusions in the macula, were observed in some groups of animals, including controls, and were considered to be unrelated to treatment. Overall, comprehensive ophthalmic examinations (ophthalmoscopy and biomicroscopy examinations, fundus photography, electroretinography (considered to be a very sensitive procedure to detect early signs of generalized retinal degeneration), and *post mortem* examinations of the right retina (including macroscopic inspection, microscopic pathology under polarized and bright light for peripheral retina and macula, confocal microscopy of the macula and histopathological examination of the peripheral retinal) showed no evidence of treatment-related adverse changes, including no evidence for the formation of crystals in the eyes during or after 52 weeks of treatment with lutein. Dose-dependent increases in lutein concentrations in the peripheral retina were reported. In the central retina and lens, lutein content was markedly increased in animals at the highest dose, but there was no evidence for crystalline deposits. Animals showed a dose-related increase in plasma and liver concentrations of lutein. Lutein was thus considered to be well tolerated. The NOAEL in this 52-week study in cynomologus monkeys was 20 mg/kg bw/day, the highest dose tested (Pfannkuch et al., 2000b,c; Pfannkuch, 2001b).

New studies

Since the previous evaluations by JECFA (2006) and EFSA (2006), new 4-week toxicity and 13-week toxicity studies in rats were available, as well as a tolerable dose finding 5-week study. Furthermore, a one-year toxicity study in female rhesus macaques, designed to investigate the plasma levels, ocular disposition levels and the ocular toxicity of lutein, was available.

Six group of Wistar rats (5/sex/group) were given either 4, 40 or 400 mg/kg bw/day lutein or lutein ester, both isolated from marigold flowers by hexane extraction, saponification and crystallization to 85% purity, dosed at 0.05%, 0.5% and 5% molar equivalents of lutein concentrations in 2 ml sunflower oil, once daily, by oral gavage, for 13 weeks (Harikumar et al., 2008). An additional control group received sunflower oil only. The animals were monitored for mortality, clinical and behavioural symptoms and any adverse reaction to lutein or lutein ester. Food consumption and body weight were recorded on every fifth day. At termination of the study, the animals were sacrificed under light ether anaesthesia. Blood was collected by direct heart puncture. Haematology (red blood cell, haemoglobin, platelet, total white blood cells, lymphocytes) and serum chemistry measurements (liver function

markers, kidney function markers, lipid profile) were conducted. Necropsy was performed and selected organs such as liver, lungs, thymus, spleen, kidney, brain and eyes were dissected out and weights were recorded (Harikumar et al., 2008).

Body weight, body weight gain, food intake and mortality were not affected by the treatment with lutein or lutein ester. No clinical signs of any adverse or toxic symptoms were noticed. No changes in the various organs at necropsy and no changes in the weight of the lungs, spleen and kidneys were noted. In males, inconsistent incidental changes in liver, thymus and eye weights were observed. A slight decrease in AST was observed in males that received 400 mg/kg bw/day lutein or lutein ester and in females that received 40 mg/kg bw/day and 400 mg/kg bw/day lutein. No other changes in hepatic function parameters were observed. Blood urea was increased in several groups of treated males but decreased in several treated groups of females. Serum creatinine was significantly decreased in females fed 400 mg lutein. A small increase in chloride was seen in males treated with 400 mg/kg bw/day lutein and in males and females given 4, 40 and 400 mg/kg bw/day of lutein. Bicarbonate levels were significantly decreased in females of the 400 mg lutein ester group. No consistent changes were observed in the lipid profile. No changes were observed in haematological parameters. Histopathological analysis of the brain, spleen, kidney, liver and eyes did not reveal any pathological changes. Lutein and lutein ester did not produce any toxicity in Wistar rats when given in concentrations up to 400 mg/kg bw/day for 13 weeks (Harikumar et al., 2008).

The Panel noted that the study included only 5 animals/sex/group and that only a limited number of tissues were examined. Therefore, the Panel considers that, compared to the standard regulatory studies, the study is too limited to identify a NOAEL for the safety evaluation of lutein.

F344 rats (8/group) were exposed to 0, 500, 1000, 1500, 2000 or 2500 mg/kg diet of lutein for 5 weeks to determine the tolerable dose for the aberrant crypt foci assay described under 'carcinogenicity studies' (lutein was obtained from the National Cancer Institute Repository, no further details on the lutein source were provided). Body weights were recorded biweekly. All animals were examined daily for any symptoms of toxicity. After 5 weeks, the rats were sacrificed, and colon, small intestine, stomach, liver and kidney were examined grossly for abnormalities. Lutein was well tolerated at doses up to 2500 mg/kg in the diet (equivalent to 125 mg/kg bw/day), based on body weight gain and toxicity observations (Raju et al., 2005).

Female rhesus macaques (5/group) were exposed to 10 mg/kg bw/day of lutein supplements providing 9.34 mg lutein and 0.66 mg zeaxanthin, 10 mg/kg bw/day of zeaxanthin supplements, or supplements of a combination of lutein and zeaxanthin (each at 0.5 mg/kg bw/day) for 12 months. The latter dosing was chosen to investigate the possible interaction between these two carotenoids at a dose that would be three- to six-fold higher than that to be used in future human clinical trials. A control group of three non-treated macaques was also included. After 12 months, one control animal, two lutein-treated animals, two zeaxanthin-treated animals and all lutein and zeaxanthin combined-treated animals were killed. The other animals were kept under observation for six additional months without receiving further supplementation and were then killed. All animals were kept on the same standardised monkey diet. Carotenoid levels in this diet were analysed. Plasma and ocular carotenoid analyses, fundus photography and retina histopathology were performed on the animals.

Supplementation of female rhesus macaques with 9.34 mg lutein/kg bw/day or 10 mg zeaxanthin/kg bw/day for 12 months resulted in 3.2-fold and 3.7-fold increases in the mean concentrations of lutein and 4.0-fold and 4.3-fold increases in the mean concentrations of zeaxanthin, in plasma and retina, respectively. Supplementation with lutein and zeaxanthin, both at 0.5 mg/kg bw/day, for 12 months increased the mean plasma concentrations of these carotenoids during the first six months of the study from 0.26 to 0.61 µmol/l (lutein) and from 0.11 to 0.33 µmol/l (zeaxanthin) but thereafter the levels returned to baseline. Supplementation of monkeys with lutein or zeaxanthin for one year at a dose of approximately 10 mg/kg bw/day did not cause ocular toxicity and had no effect on biomarkers associated with nephrotoxicity (Khachik et al., 2006).

An Aberrant Crypt Foci (ACF) study is described in the new literature. Aberrant Crypt Foci are putative precursors of colon cancer and therefore this study is included here although the study duration is only nine weeks. Male F344 rats (10/group; seven weeks of age) were given diets containing 0 (controls), 100, 200, 1000 or 2000 mg/kg diet of lutein (equivalent to 0, 5, 10, 50 and 100 mg lutein/kg bw/day). Lutein was obtained from the National Cancer Institute Repository, no further details on the lutein source were provided. At nine weeks of age, all rats received s.c. injections of azoxymethane at a dose of 15 mg/kg bw per week for two weeks. Lutein treatment continued until the animals were 16 weeks of age. All animals were sacrificed by CO_2 asphyxiation. The colons were removed and assessed for any macroscopic changes. Aberrant Crypt Foci were quantified. Rats fed 2000 mg/kg diet of lutein showed significantly (but only slightly) higher body weights than the controls. No significant differences in body weights were observed between the different treatment groups. Lutein at 10 mg/kg bw/day significantly inhibited the azoxymethane-induced ACF formation by about 30%. At the highest dose tested however, 100 mg/kg bw/day, lutein significantly increased the azoxymethane-induced ACF by about 40% (Raju et al., 2005).

3.2.3. Genotoxicity

EFSA (2006) describes a study in which the mutagenic potential of a lutein product (from marigold petals, containing 79% lutein and 5% zeaxanthin) was investigated in the Ames test according to OECD Test Guideline 471. *Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102 with and without metabolic activation (S9 fraction from rat liver), were used. Two formulations were tested: beadlets containing 10% lutein product (158-15 800 µg beadlets/plate (i.e. 12.8-1280 µg lutein/plate)) and the lutein product as such (15.8 – 1580 µg lutein product/plate (i.e. 12.8-1280 µg lutein/plate)). For both formulations, the plate incorporation method and the pre-incubation method were used. The results obtained were as follows: Beadlet formulation: the maximal dose level of 15 800 µg beadlets/plate was not evaluated due to precipitation. No increase in the number of mutant colonies was observed with the beadlets formulation. The mutant frequencies of the controls were within the range of historical control values and data published in the literature. Lutein product: no toxicity was apparent for any strain, except TA102, which showed reduced growth, most prominently in the absence of S9. No increase in the number of mutant colonies was observed with the lutein product. The mutation frequencies of the controls were within the range of the historical control values and the data published in the literature (Kruger et al., 2002).

JECFA (2006) summarised several *in vitro* and *in vivo* assays with lutein. In these studies there was no evidence of genotoxicity (Table 6).

Table 6: Overview of studies on the genotoxicity of lutein

End-point	Test system	Concentration/dose	Results	Reference
In vitro				
Bacterial reverse mutation (Ames) assay with metabolic activation from S9	*S. typhimurium* TA98	0.1 ml of diluted eggplant fruit juice extracts containing lutein in the methanol layer	Negative	Yoshikawa et al., 1996
Bacterial reverse mutation (Ames) assay with metabolic activation from S9	*S. typhimurium* TA98 and TA100	25, 50, 75, or 100 µl of solvent extracts from fruits and vegetables containing lutein; isolated lutein also tested	Negative	Rauscher et al., 1998

End-point	Test system	Concentration/dose	Results	Reference
Chromosome aberration	Human peripheral blood lymphocytes	10% lutein in a beadlet formulation: 3.9–125 µg/ml for 3 or 24 hours in the absence of metabolic activation, or for 3 and 5hours in the presence of microsomes from phenobarbital- and 5,6-benzoflavone-pretreated rats, respectively	Negative	Chételat et al., 2002a
In vivo				
Micronucleus formation	Male NMRI mice	180 mg/kg bw per os	Negative	Rauscher et al., 1998
Micronucleus formation	Rat	45, 89, or 178 mg 10% lutein beadlets/kg bw on 2 consecutive days	Negative	Chételat et al., 2002b
Comet assay	Human lymphocytes	Human subjects from whom lymphocytes were obtained had consumed 15 mg supplemental lutein/day for 12 weeks with measurement at 16 weeks	Negative	Collins et al., 1998

S9, 9000 x *g* supernatant from rat liver.

New results from an Ames test and a chromosomal aberration test are available for lutein since those included in the previous evaluations done by JECFA (2006) and EFSA (2006).

Lutein (80% purity, extracted from dried marigold flowers) was tested in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 in the absence and presence of S9 at three concentrations (334, 668 and 1335 µg lutein/plate (no adjustment for purity)) (Wang et al., 2006). No mutagenicity was observed.

A chromosomal aberration test was performed with Chinese Hamster Ovary (CHO) cells. Lutein (80% purity, extracted from dried marigold flowers) in concentrations up to 267 mg/l did not show significant clastogenicity both in the absence and presence of S9 at 24- and 48- hour incubations (Wang et al., 2006).

Rauscher et al. (1998) concluded that it could be demonstrated that the number of cyclophosphamide (180 mg/kg bw) or benz(*a*)pyrene (150 mg/kg bw) induced micronuclei in polychromatic erythrocytes (MNPCEs) in bone-marrow of male NMRI mice was reduced significantly from respectively 40.44 $\pm$ 2.30 to 26.43 $\pm$ 0.98 MNPCEs/1000 PCEs (34.6%)($p<0.01$) for cyclophosphamide and from 12.78 $\pm$ 0.97 to 8.33 $\pm$ 0.91 MNPCEs/1000 PCEs (34.8%)($p<0.01$) for benzo(a)pyrene upon dosing the animals with the mutagen together with 180 mg/kg bw lutein.

Collins et al. (1998) used the Comet assay to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes from volunteers supplemented with lutein or placebo. The dose level of lutein tested amounted to 15 mg/day (80% *trans*-lutein, 20% 13-15-*cis*-lutein). Lutein supplementation did not induce DNA damage.

Chételat et al. (2002b) reported results of a micronucleus test in rat bone marrow upon oral administration (gavage) of 45, 89, or 178 mg beadlets containing 10% lutein /kg bw/day (i.e. 4.5, 8.9 or 17.8 mg lutein/kg bw/day) on 2 consecutive days. This study was evaluated by JECFA which concluded that the results did not reveal a genotoxic potential, but the original unpublished study report was not available to the Panel for further evaluation.

All together a number *in vitro* (bacterial reverse mutation and chromosomal aberration) and *in vivo* (micronucleus formation, Comet assay) genotoxicity studies are available for lutein. The Panel concluded that based on these studies there is no concern with respect to genotoxicity for lutein.

3.2.4. Chronic toxicity and carcinogenicity

In previous evaluations no chronic toxicity/carcinogenicity studies have been described for lutein. JECFA (2006) described several studies that investigated potential chemopreventive effects of lutein in mice but these studies have not been included in this opinion because they are not considered relevant for the evaluation of the safety of lutein.

No long term or carcinogenicity studies on lutein were described since the previous evaluations.

3.2.5. Reproductive and developmental toxicity

No multigeneration studies are available. JECFA (2006) describes one developmental toxicity study.

Female Sprague-Dawley rats (mated when they were aged 10–13 weeks) were given diets mixed with beadlets containing 10% lutein (from marigold extract; 79% lutein, 5% zeaxanthin), corresponding to dietary intakes of 0, 252, 535 and 1118 mg/kg bw/day, from day 6 to day 20 of gestation. Placebo beadlets were used to ensure similarity in the total concentration of beadlets received by all treatment groups. The study complied with GLP guidelines.

There was no evidence of an adverse effect of lutein in the dams. However, there was an inverse dose-related reduction in food consumption and in both maternal and fetal body weights at the lowest and intermediate doses (i.e. reduction was highest for the lowest dose). This was attributed to the decreased palatability of the diet due to the lower palatability of empty beadlets compared with lutein beadlets. There were no effects of lutein on pre- or post-implantation loss, embryo-fetal survival, or sex ratios. Fetuses were examined for visceral and skeletal abnormalities and soft tissue changes. There was an inverse dose-related increase in the forms of reduced ossification, but the degree of ossification in the fetuses at the highest dose was similar to that for historical controls. These findings were considered to be in line with the maternal findings of decreased food consumption in the control group and at the lowest dose.

There were no adverse effects of treatment with lutein on the incidence of external or skeletal abnormalities in any treatment group. Minor visceral abnormalities were observed in one or two fetuses in each of the treated groups, but the incidence of these changes was similar to that for historical controls and was therefore not considered to be treatment-related. There was a slight, dose-related increase in the incidence of rudimentary extra lumbar ribs in the groups receiving the intermediate and highest doses. However, these findings were not considered to be of toxicological significance owing to the known reversibility of this minor skeletal finding. Analyses of blood samples showed dose-dependent increases in mean total plasma concentrations of lutein on days 7 and 16 of gestation. Mean total plasma lutein concentrations were approximately 80% higher on day 16 of gestation than on day 7. These data indicate that animals were adequately exposed to lutein throughout the experimental period. The NOAEL in this study of embryotoxicity/teratogenicity in rats was 1000 mg/kg bw/day, the highest dose tested (Edwards et al., 2002).

No new literature is available since the JECFA evaluation (2006).

3.2.6. Other studies

JECFA describes some studies investigating possible beneficial effects of lutein. However, beneficial effects are not relevant for the risk evaluation for the use of lutein as a food colour, and therefore, these studies are not included in this evaluation.

JECFA (2006) evaluated several studies on the effect of lutein on immune responses.

Mice

The effects of lutein on mitogen-induced lymphoproliferation, cytotoxicity, and interleukin-2 (IL-2) production were investigated in BALB/c mice given diets containing 0.1 or 0.4% lutein esters from marigold extracts (37% lutein esters and 0.5% zeaxanthin esters; corresponding to approximately 200

and 803 mg/kg bw/day for lutein, and 2.7 and 10.9 mg/kg bw/day for zeaxanthin) for two or four weeks. No significant treatment-related differences in body weight gain or food intake were reported. Dietary lutein enhanced phytohaemagglutinin (PHA)-induced lymphocyte proliferation, but had no effect on IL-2 production or lymphocyte cytotoxicity (Chew et al., 1996).

The possible effects of lutein on the expression of the *pim*-1 gene, which is involved in early activation of T cells and cells of other lineages, were investigated in BALB/c mice fed diets containing 0, 0.02 or 0.4% lutein (source not specified) for 14 days, corresponding to approximately 0, 40 or 780 mg/kg bw per day, respectively. No external signs of toxicity were noted in any of the treated mice. Splenocytes isolated from mice fed with lutein that were cultured in the presence of concanavalin A showed a dose-dependent increase in steady-state levels of *pim*-1 mRNA. This is a potential mechanism through which lutein may modulate immune function (Park et al., 1999).

Cats and dogs

The effects of diets containing lutein (crystalline, from marigold petals containing about 77% lutein and about 5% zeaxanthin) on humoral and cell-mediated immune responses were investigated in female tabby cats (Kim et al., 2000a) and female beagle dogs (Kim et al., 2000b). In each study, animals (56/species) received basal diets supplemented with lutein at 0, 1 (cats only), 5, 10 or 20 (dogs only) mg/day for 12 weeks. In cats, this corresponded to approximately 0, 0.7, 3.5 or 7.1 mg lutein/kg bw/day and approximately 0, 0.05, 0.25 and 0.5 mg zeaxanthin/kg bw/day. In dogs, this corresponded to 0, 0.4, 0.9 or 1.75 mg lutein/kg bw/day and 0, 0.03, 0.06 and 0.12 mg zeaxanthin/kg bw/day. In dogs only, blood was collected from weeks 13 to 17 to determine changes in plasma concentrations of immunoglobulin following second and third challenges. No significant changes in body weight were observed. In cats, there was a significant dose-related increase in delayed-type hypersensitivity response to vaccine, but not to concanavalin A, and significantly enhanced concanavalin A and pokeweed mitogen (PWM)-stimulated proliferation of peripheral blood mononuclear cells. In cats at the highest dose (7.1 mg lutein/kg bw/day), the percentages of CD4+ and CD21+ cells were significantly elevated at week 12, and in the groups fed lutein at 0.7 and 7.1 mg/kg bw/day, concentrations of IgG were significantly higher from weeks 8 to 12. In dogs, supplementation with lutein significantly increased the delayed-type hypersensitivity response to vaccine and PHA, and significantly increased mitogen (PHA, Con A, and PWM)-stimulated proliferation of peripheral blood mononuclear cells. The percentages of cells expressing CD5, CD4, CD8 and major histocompatibility complex class II molecules were significantly increased, and the production of IgG significantly increased after the second antigenic challenge. There were no differences in IL-2 production in cats or dogs throughout the experimental periods. These results suggest that dietary lutein stimulated both cell-mediated and humoral immune responses in cats and dogs.

Studies in mice, cats and dogs have shown that lutein may stimulate both cell-mediated and humoral immune responses. In the cat study of Kim et al. (2000a) immune stimulating effects were observed at dose levels from 0.7 mg lutein/kg bw per day onwards.

The mechanism of immunological benefits induced by carotenoids has not been fully elucidated. Katsuura et al. (2009) investigated some of the immunity-related properties of beta-carotene and two other carotenoids, beta-cryptoxanthin, and lutein, on the murine macrophages cell line RAW264. beta-Carotene added to the culture medium accumulated in the cells in a time- and dose-dependent manner. The accumulation was positively correlated with cellular lipid peroxidation, demonstrating the pro-oxidative activity of beta-carotene, and also with the synthesis of glutathione, an intracellular antioxidant. Conversely, accumulation of beta-carotene was negatively correlated with the transcription of immune-active molecules, such as IL-1beta, IL-6, and IL-12 p40, in cells stimulated by LPS and INF-gamma. The transcription of the pro-inflammatory cytokines IL-1beta and IL-6 was more sensitive to the accumulation of beta-carotene than was IL-12 p40. The accumulation of beta-cryptoxanthin in cells resulted in effects similar to those of beta-carotene. However, lutein accumulated minimally and did not significantly affect the cells. The authors of the study concluded that these results demonstrate that beta-carotene, and beta-cryptoxanthin as well, can accumulate in

RAW264 cells and induce changes in intracellular redox status, which in turn regulate the immune function of macrophages.

The Panel considered that immunostimulating and immunomodulating effects of lutein have not been demonstrated in a robust and reproducible way, which could enable them to be used as pivotal studies for risk assessment.

In humans, plasma concentrations of lutein have been negatively associated with the activity of Cytochrome P450 1A2 (CYP1A2) (caffeine test), a liver enzyme involved in the metabolic activation of a number of human carcinogens (Le Marchand et al., 1997).

Male Wistar rats (8/group) received for 16 days, either (i) a basal diet that had not been supplemented with lutein (negative controls), (ii) a basal diet supplemented with lutein (oleoresin extracted from marigold petals) to provide a dose of approximately 45 mg/kg bw/day or (iii) a basal diet supplemented with 3-methylcholanthrene (3-MC) (positive controls). There were no changes in feed intake, body weight gains, or organ weights after dietary supplementation with lutein. Lutein content was increased in the tissues examined. No effect on glutathione-S-transferase (GST) activity or glutathione levels (GSH) were detected in any of the tissues examined. Cytochrome P450 activities (ethoxyresorufin-O-deethylation (EROD), methoxyresorufin-O-demethylation (MROD), and pentoxyresorufin-O-dependentylation (PROD)) were not induced in the liver, kidneys, or lungs after supplementation with lutein. Benzyloxyresorufin-*O*-dearylation (BROD) activity was not changed in the liver and kidney, but significantly decreased in the lung. CYP450 enzyme activities were undetectable in the small intestine (Jewell and O'Brien, 1999).

Male SPF Wistar rats (6/group) received diets containing corn oil (control) or 10% lutein oleoresin (extracted from marigold petals, mixed with corn oil) at 300 mg/kg of diet for 15 days, corresponding to approximately 2.8 mg/kg bw/day. No changes were observed in food intake, body weights, or organ weights. Lutein was present in liver microsomes at approximately 0.3 nmol/mg of protein, but did not induce any significant changes in total microsomal P450 and associated cytochrome c reductase activities, as well as EROD, MROD, PROD, BROD, erythromycin N-demethylase (ERDM), nitrosodimethylamine N-demethylase (NDMAD), *p*-nitrophenol- and 4-hydroxybiphenyl UDP glucuronosyl transferases (4NP-UGT and 4-HBT-UGT), and total cytosolic GST activity (Gradelet et al., 1996).

3.2.7. Human data

EFSA (2006) summarises several dietary intervention studies.

Many studies have been conducted to study the effects of dietary intervention with foods high in lutein + zeaxanthin or lutein supplements. In these studies effects on plasma levels were evaluated after ingestion of lutein at concentrations which ranged from 0.4 to 30 mg/day for a period up to 12 months (Broekmans et al., 2000; Landrum et al., 1997b; Olmedilla et al., 2002; Hininger et al., 2001; Roodenburg et al., 2000; Micozzi, 1992; Rock et al., 1997; Rock et al., 2002, Castenmiller et al., 1999; Handelman et al., 1999; McEligot et al., 1999; Muller et al., 1999, Van het Hof et al., 1999a; Olmedilla et al., 1997). With the exception of the multi-centre Olmedilla study (Olmedilla et al., 2002) no side-effects were reported in these studies with respect to increased carotenoid consumption. However, these studies were not designed to detect adverse effects. In the Olmedilla study, 40% of the subjects in the Spanish cohort only, supplemented for 20 weeks with 15 mg/day lutein from a lutein-rich marigold extract, showed carotenodermia, but no changes in biochemical or hematological indices were noted.

JECFA (2006) indicates that several studies have investigated correlations between dietary or supplemental intake of lutein or zeaxanthin or serum concentrations of lutein or zeaxanthin and the incidence of age-related macular degeneration, macular pigment density or cataractogenesis, with varying results. The studies were not described in the JECFA evaluation, but it was indicated that none of these studies reported adverse effects of lutein/zeaxanthin, including ocular toxicity.

JECFA (2006) summarized a few other intervention studies with lutein.

Daily ingestion of 11.3 mg of lutein in a liquid spinach powder preparation administered daily with meals was well tolerated by all subjects (23 healthy volunteers). There were no significant changes in blood concentration of haemoglobin, leukocytes, or serum electrolytes (sodium, potassium, chloride) (Müller et al., 1999).

After administration of diets containing crystalline lutein from marigold petal extracts (suspension in vegetable oil with β-carotene) at 6.6 mg/day for three weeks to non-obese, non-smoking, normolipidaemic men and women (aged 18–58 years), no significant differences in serum concentrations of cholesterol or triacylglycerol were reported compared with the control group that received a control diet (Castenmiller et al., 1999).

In a double-blind, parallel, placebo-controlled intervention study to investigate the effects of significant elevations in plasma concentrations of lutein on fasting plasma fatty acid profiles, healthy non-smoking males received daily supplementation of lutein (lutein-rich marigold extract, encapsulated) at 15 mg/day (10 subjects) or placebo (encapsulated corn oil) (11 subjects) for 26 days. Blood samples were taken before treatment (baseline) and on day 28 for analysis of concentrations of long-chain fatty acid. Lutein supplementation had no effect on individual fatty acids, total fatty acids, total saturated, unsaturated, mono-unsaturated, or poly-unsaturated fatty acids, or on ratios of unsaturated versus saturated fatty acids (Wright et al., 1999).

JECFA (2006) also summarized several epidemiological studies.

A large prospective study examined the relationship between serum concentrations of carotenoids and the subsequent risk of developing cancers of the stomach and upper digestive tract in a region of China with epidemic rates of oesophageal and gastric cancer (Abnet et al., 2003). There was an association between the incidence of gastric non-cardia cancer and the serum concentrations of lutein/zeaxanthin derived from normal dietary sources. However, a Dutch cohort study has suggested that dietary intake of lutein/zeaxanthin is not associated with the risk of gastric cancer, although intakes of retinol and β-carotene were positively associated with the risk of this cancer (Botterweck et al., 2000).

No adverse outcomes have been reported between increased serum levels of lutein and zeaxanthin and the risk of subsequent myocardial infarction (Street et al., 1994).

Three new studies are available.

An epidemiological case-control study was conducted in an occupational cohort (miners) from the Yunnan Tin Corporation in China. During six years of follow-up, 339 cases of confirmed lung cancer were diagnosed and those cases that donated pre-diagnostic blood (n = 108) were included in the study. For each case, two individuals alive and free of cancer at the time of the case diagnosis, matched for age, sex and date of blood collection, were selected as controls. Risk estimates were adjusted for tobacco use (g/day) and radon exposure. No significant association was observed between lutein and zeaxanthin levels and lung cancer in the whole study population. After stratification by alcohol drinking status (based on one 24-hour recall), higher lutein/zeaxanthin serum levels (third tertile, > 61 µg/dl) were significantly associated with increased lung cancer risk (Odds Ratio (OR) 2.3, 95% Confidence Interval (CI) 1.2-6.6) among alcohol drinkers (based on one 24-hour recall). Conversely, among non-drinkers an OR of 0.4 (95% CI 0.2-1.1) was found at the third tertile of lutein/zeaxanthin intake, suggesting a possible protective association for higher carotenoid levels (Ratnasinghe et al., 2000). It is noted that the use of one 24-hour recall may not be a very reliable method to make a distinction between drinkers and non-drinkers.

A prospective observational cohort study was performed with 812 white women who were expecting their first child. Only women with singleton pregnancies were included in the study. The relationships of dietary intakes and serum levels of antioxidant nutrients and the risk of prelabour rupture of the membranes [amniotic sac] preterm were studied. Women with high serum lutein concentrations (top

third) in early pregnancy were at four times greater risk for prelabour rupture of the membranes than woman in the lowest third (95% CI 1.3-11.9; P = 0.009), after adjusting for the adverse effects of smoking. Similar results were obtained using lutein levels from later pregnancy. The authors indicate that this observation does not necessarily imply a causal relationship (Mathews and Niel, 2005).

In the Melbourne Visual Impairment Project, 3040 human volunteers were recruited in 1992-1994. Follow-up investigations were performed with 85% (2594) of volunteers in 1997-1999. At both time points, participants underwent a standard procedure including an ophthalmic examination and an interview regarding socioeconomic and demographic characteristic, historic and current symptoms of eye diseases, medical history and medication use. The follow-up investigations also included a food frequency questionnaire. Age-related macular degeneration (AMD) was diagnosed by either clinical or photographic examinations. The authors indicate that a possible protection of high lutein/zeaxanthin intake on AMD was observed among those with low level (< 7.17 mg) linoleic acid intake. On the other hand, among those with daily linoleic acid intake ≥ 7.17 mg, the risk of having AMD increased up to five-fold for 1 mg increase in adjusted daily lutein/zeaxanthin intake and up to threefold for 1 mg increase in crude daily lutein/zeaxanthin intake. However, it is not completely clear from the results presented how these conclusions are derived (Vu et al., 2006).

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

Lutein (E 161b) is a natural carotenoid dye authorised as a food additive in the EU (E 161b) and previously evaluated by the SCF in 1975 and JECFA in 2006. JECFA established a group ADI of 0-2 mg/kg bw/day for lutein from *Tagetes erecta* and for zeaxanthin. The SCF could not establish an ADI but concluded that xanthophylls prepared from natural foods by physical processes are acceptable for use in food (SCF, 1975).

Specifications have been defined in the EU legislation Directive 2008/128/EC and JECFA (JECFA, 2006b). EU specifications for lutein only describe 4% of the commercial product. It is not clear what makes up the other 96%. The Panel concluded that the existing specifications need to be extended to include the material not accounted for.

The Panel noted that separate specifications are available for lutein from *Tagetes erecta* in the JECFA but not in the EU specifications and that the JECFA specifications on lutein from *Tagetes erecta* are higher with respect to lutein content (> 70%) than the EU specifications (> 4%).

Furthermore, the EU specifications and the JECFA specifications (both for mixed carotenoids and for lutein from *Tagetes erecta*) on the purity, differ with respect to solvent residues, metals, moisture, ash, zeaxanthin and waxes.

The Panel noted that lutein as defined by the SCF and JECFA specifications may be obtained from sources that could not be regarded as edible plant materials or foods (Lucerne and *Tagetes erecta*).

The Panel noted that the term “marigold” may be used to denote *Tagetes erecta*, but that this common name may be used for other species including for example *Calendula officinalis*, and that for this reason the term “marigold” should not be used in the specifications.

Finally, the Panel also noted that the current JECFA ADI for lutein is based on a study using a lutein product derived from *Tagetes erecta* with a lutein content of 79%. Since in the EU specifications a lutein content of only 4% is required in food colour preparations, the material tested may differ substantially from the food colour preparations that are on the market.

The JECFA ADI was based on a NOAEL from a 90-day study in rats (Pfannkuch et al., 2000a; Pfannkuch et al., 2001a; Kruger et al., 2002). The lutein tested consisted of a lutein product derived from marigold flowers (Kruger et al., 2002). A compositional analysis of the test product identified 97% of the components and indicated that 84% of the product consisted of the carotenoids lutein and zeaxanthin (79 and 5% respectively). The remaining content consisted of waxes, palmitic acid/palmitate, potassium and water (Kruger et al., 2002). The NOAEL of this study was 200 mg/kg bw/day (the highest dose tested) and an uncertainty factor of 100 was applied. Although the ADI was based on the results of a short-term study, the JECFA concluded on the basis of the supporting data and lack of effects at much higher lutein doses in some studies (e.g. a study of developmental toxicity) that an uncertainty factor of 100 was appropriate. Zeaxanthin was included in the ADI in view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin. The group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications. According to the JECFA specifications, lutein from *Tagetes erecta* should contain 'not less than 80% total carotenoids, not less than 70% lutein'. However, according to the 'mixed carotenoids' JECFA specifications, total colouring matter (as lutein) should be 'not less than declared'. The Panel noted that it is not completely clear how the JECFA group ADI relates to these 'mixed carotenoids' specifications.

The Panel noted that in a more recent 90-day study in rats (Harikumar et al., 2008) no adverse effects were reported up to dose levels of 400 mg/kg bw/day. However, the Panel noted that the study included only 5 animals/sex/group and that only a limited number of tissues were examined. Therefore, the Panel considers that, compared to the standard regulatory studies, the study is too limited to identify a NOAEL for the safety evaluation of lutein.

From the animal studies it can be concluded that after a single oral dose of [^{14}C]-lutein, absorption of lutein results in peak concentrations at about 3-4 hours in rats, and after 12 hours in cows. Low tissue concentrations of radioactivity indicate that lutein and/or its metabolites do not accumulate. Highest concentrations were found in liver and gastrointestinal mucosa. In most studies, lutein is reported to be mainly excreted via the faeces and low urinary and biliary excretion indicated that there was poor absorption from the intestinal tract (about 10%). In one rat study, however, absorption up to 43% was reported when the substance was administered in the diet as beadlets. After oral daily supplementation with lutein, steady-state plasma concentrations were reached after about three days in rats.

From the human kinetic data it can be derived that lutein has to be solubilised in micelles to allow absorption by mucosal cells. The absorption of lutein may therefore be influenced by the food matrix. Fibre and β-carotene may decrease the rate of absorption, whereas dietary fat may increase the absorption. The relative absorption of lutein from a mixed vegetable diet was lower than from a diet supplemented with pure lutein, which may at least be partly due to the fibre content of the diet. Lutein occurs in nature predominantly in the all-*trans* configuration, but in human (and non-human primate) serum small amounts of *cis* isomers can also be detected. Serum concentrations of lutein are quite variable. After administration of a single dose, peak serum concentrations were observed after 2-16 hours. Steady-state plasma concentrations were reached after 28-40 days of supplementation. It is not clear from the available studies whether the elimination of lutein follows first order kinetics with an elimination half-life ranging from 33-78 days, or whether two pools of lutein will be formed, one with a fast excretion half-life of about five days and one with a longer half-life (Rock et al., 1992; Burri et al., 2001; Cohn et al., 2001). The main sites of lutein storage are the adipose tissue and the liver. The eye in general and the retina (fovea) in particular, contain high concentrations of lutein and zeaxanthin.

Little is known about the metabolism or degradation of lutein, but several metabolites have been detected in human serum. It can exist in equilibrium with zeaxanthin. Lutein has only a minimal, if any, provitamin A effect. Lutein does not have a pronounced effect on phase I and phase II biotransformation enzymes.

In mice given lutein from marigold extract (37% lutein esters and 0.5% zeaxanthin esters) in doses up to approximately 600 mg/kg bw/day for up to 28 days, no differences in body, liver, or spleen weights among treatment groups were reported, and there were no significant differences in feed intake throughout the experimental period.

In rats given a lutein product at dose levels up to 773.2 mg/kg bw/day (highest dose level used) for four weeks, no test article related toxicity was observed. Based on two 13-week toxicity studies in rats, no toxicity was observed with either lutein or lutein ester at dose levels up to 400 mg/kg bw/day (highest dose level used). The Panel noted that the 13-week toxicity study testing lutein or lutein ester at dose levels up to 400 mg/kg bw/day included only 5 animals/sex/group and examined only a limited number of tissues. Therefore, the Panel considered that, compared to the standard regulatory studies, the study is too limited to identify a NOAEL for the safety evaluation of lutein.

In monkeys exposed to lutein for 52 weeks at concentrations of up to 20 mg/kg bw/day, the only effects observed were yellow discoloration of faeces and fat. The study was designed to investigate ocular toxicity, and no ocular toxicity or other overt toxicity was observed.

No multigeneration reproductive toxicity studies with lutein are available. In a developmental toxicity study no adverse effects were observed and therefore the NOAEL was 1000 mg lutein/kg bw/day, the highest dose tested. The Panel noted that no effects on reproductive organs were observed in any of the available oral 90-day studies.

A number of *in vitro* (bacterial reverse mutation and chromosomal aberration) and *in vivo* (micronucleus formation, Comet assay) genotoxicity studies are available for lutein. The Panel concluded that based on these studies there is no concern with respect to genotoxicity for lutein.

No chronic toxicity/carcinogenicity studies are available for lutein.

Studies in mice, cats and dogs have shown that lutein may stimulate both cell-mediated and humoral immune responses. In the cat study of Kim et al. (2000a) immune stimulating effects were observed at dose levels from 0.7 mg lutein/kg bw/day and higher. The Panel considered that immunostimulating and immunomodulating effects of lutein have not been demonstrated in a robust and reproducible way, which could enable them to be used as pivotal studies for risk assessment.

The Panel also noted that 90-days studies did not indicate any effect pointing at immunotoxicity such as effects on haematology or histopathological effects on relevant tissues.

The Panel concluded, based on the NOAEL of 200 mg/kg bw/day (the highest dose level tested) in a 90-day rat study, the absence of developmental toxicity at dose levels up to 1000 mg/kg bw/day (the highest dose level tested), the fact that lutein is not genotoxic, the fact that in 90-day studies no effects on reproductive organs were observed, and the fact that lutein is a normal constituent of the diet, that an ADI can be derived. Given the absence of a multigeneration reproductive toxicity study and of chronic toxicity/carcinogenicity studies the Panel applies an uncertainty factor of 200 and establishes an ADI of 1 mg/kg bw/day.

The Panel noted that this ADI refers to lutein derived from *Tagetes erecta* containing at least 80% carotenoids consisting of lutein and zeaxanthin (79 and 5% respectively). According to specifications provided by NATCOL this may refer to the lutein with high concentrations of total saponified carotenoids at levels of at least 80% (cf. JECFA specifications for lutein from *Tagetes erecta*). The ADI does not refer to lutein preparations of lower purity or from other sources.

The Panel noted that other preparations of lutein are also on the market, i.e. lutein with low concentrations of total carotenoids at levels of ~5-12%, and lutein with high concentrations of total carotenoids extracted and present as esters at levels of at least 60%. The Panel concluded that the toxicological data base available on these preparations is too limited to conclude that the ADI also applies to these preparations.

The Panel noted that the JECFA specifications for lead are ≦ 5 or ≤ 3 mg/kg, whereas the EC specification is ≦ 10 mg/kg.

The Panel noted that, if available, the aluminium lake of the colour could add to the daily intake of aluminium for which a TWI of 1 mg aluminium/kg bw/week has been established (EFSA, 2008) and that therefore specifications for the maximum level of aluminium in the lakes may be required.

Tier 3 intake estimates, based on the maximum use levels from the NATCOL usage survey, ranged from 0.6-2.2 mg/kg bw/day. High level intakes ranged from 0.7-5.7 mg/kg bw/day (Table 4). Therefore, at the current use levels, the ADI of 1 mg/kg bw/day will be exceeded due to the use of lutein as a food colour at the upper end of the range. Furthermore, EFSA (2006) indicated that overall the dietary intake of lutein as such is estimated to be between 0.8 and 2.5 mg/day, equivalent to 0.01 – 0.04 mg/kg bw/day for a 60 kg person, indicating that the worst case scenario for intake of lutein used as a food colour in combination with its average intake from other dietary sources does exceed the ADI of 1 mg/kg bw/day.

CONCLUSIONS

Lutein (E 161b) is a natural carotenoid dye authorised as a food additive in the EU (E 161b) and previously evaluated by the EU SCF in 1975 and JECFA in 2006. JECFA established a group ADI of 0-2 mg/kg bw/day for lutein from *Tagetes erecta* and for zeaxanthin. The SCF could not establish an ADI, but concluded that xanthophylls prepared from natural foods by physical processes are acceptable for use in food (SCF, 1975).

The Panel concluded, based on the NOAEL of 200 mg/kg bw/day (the highest dose level tested) in a 90-day rat study, the absence of developmental toxicity at dose levels up to 1000 mg/kg bw/day (the highest dose level tested), the fact that lutein is not genotoxic, the fact that in 90-day studies no effects on reproductive organs were observed, and the fact that lutein is a normal constituent of the diet, that an ADI can be derived. Given the absence of a multigeneration reproductive toxicity study and of chronic toxicity/carcinogenicity studies the Panel applies an uncertainty factor of 200 and establishes an ADI of 1 mg/kg bw/day.

The Panel noted that this ADI refers to lutein derived from *Tagetes erecta* containing at least 80% carotenoids consisting of lutein and zeaxanthin (79 and 5% respectively). The ADI does not refer to lutein preparations of lower purity or from other sources.

The Panel concluded that at the current levels of use Tier 3 intake estimates are above the ADI of 1 mg/kg bw/day at the upper end of the range.

The Panel concluded that the average intake for adults from the regular diet amounts to 1-4 % of the ADI of 1 mg/kg bw/day. High level intakes from the regular diet would amount to 28% of this ADI for chidren (assuming an intake of lutein present in food of 7 mg/day and a body weight of 25 kg, equal to 0.28 mg/kg bw/day).

The Panel concluded that the existing specifications need to be extended to include the material not accounted for and to match the material tested in the toxicological studies.

The Panel noted that the JECFA specifications for lead are ≦ 5 or ≤ 3 mg/kg whereas the EC specification is ≦10 mg/kg.

The Panel noted that, if available, the aluminium lake of the colour could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established and that therefore specifications for the maximum level of aluminium in the lakes may be required.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

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APPENDIX A

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation, usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

1. **Decision rules taken to deal with QS authorisations for MPL: (see the decision tree in Figure 1)**

    a. If the category 'All other foodstuff' is QS, the highest observed MPL value should be used, which is 500 mg/kg

    b. At the food category level, if a colour is authorised QS in a food category for one or more colours

        i. If a value is available for only one colour, this value is used for all the colours

        ii. If many values are available for more than one colour, the highest value is used

        iii. If there is no available value, the available value for a similar food group for the same colour is used. If there is no similar food group, the highest MPL of 500 mg/kg is used.

Particular cases:

- **Edible casings QS**: If available use the pork-based products use level; if there is no value available, the highest MPL of 500 mg/kg is used.
- **Edible cheese rinds**: The MPL of 100 mg/kg (from the flavoured processed cheese category) is used, except for E 120 (Cochineal) whose level is 125 mg/kg for red marbled cheese.

2. **Rules to identify the maximum reported use levels to be used for the refined exposure assessment:**

    A maximum reported use level is the maximum value selected from reported usage by industry and analytical data provided to the Panel:

    a. If the identified maximum reported use level is greater than or equal to the actual MPL, then the actual MPL is used by default.

    b. If both maximum analytical and maximum current use level data are available, priority is given to the use level data, even if analytical values are lower or higher; the selected value is rounded to the nearest whole number.

    c. If no use level data are available, because either no uses were reported or industry was not asked to provide them, the choice is made between the highest analytical value or the MPL:

        i. if more than10 analytical data are available, the highest quantified reported value is used;

        ii. if less than10 analytical data are available, the MPL is used.

d. If the highest use level or the highest analytical data are higher than the proposed adjusted QS values for MPL, priority is given to the highest use level/analytical data.

3. Tiered approach to intake estimation

The basic principles of the stepwise approach for the estimation of additives' intakes involve, for each successive Tier, a further refinement of intakes from the conservative estimates for screening (Tier 1) to more realistic estimates (Tier 2 and 3) (EC, 2001). Depending on the information on use levels data available, the three screening tiers approach must be adapted (see Figure 2 for the decision rules).

The three screening tiers performed both for children and adult population are:

Tier 1: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours and the Budget method.

Tier 2: Estimates are based on the MPLs, as specified in the Directive 94/36/EC on food colours with adjustment for quantum satis usages, and national individual food consumption data.

Tier 3: Estimates are based on maximum reported use levels and national individual food consumption data.

In Tier 2 and 3, the following approach is used to calculate the high percentile consumption: The high consumption should be calculated by examining the 97.5th percentile of food additive intake per food group, and selecting the highest intake* and then adding this value to the sum of the mean intakes for the remaining food groups. This approach is slightly different to the usual approach, in which the two highest food group intakes at the 97.5th percentile of additive intakes are added to the mean consumption of the other food groups. The approach was modified based on evaluation of the Expochi study, as it provides a more realistic estimate of exposure.

*High consumption value of fruit wines (still or sparkling), cider (except cidre bouche) and perry, aromatized fruit wines, cider and perry from UK adult data is not taken into account for the calculation of high percentile exposure when this food category appeared to be the highest P95 exposure. In this case the second highest contributor is taken in the calculation.

GLOSSARY/ABBREVIATIONS

ACF	Aberrant Crypt Foci
ADI	Acceptable Daily Intake
AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
AMD	Age-related Macular Degeneration
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
APTT	Activated Partial Thromboplastin Time
AUC	Area Under the Curve
BROD	Benzyloxyresorufin-*O*-dearylation
CAS	Chemical Abstracts Service
CEN	European Committee for Standardization
CHO	Chinese Hamster Ovary cells
CRL	Community Reference Laboratory
DMNQ	2,3-dimethoxy-1,4-naphtoquinone)
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
ERDM	Erythromycin N-demethylase
EU	European Union
EXPOCHI	Refers to EFSA Article 36 2008 call for Proposals Focused on Children and Food Consumption
EROD	Ethoxyresorufin-*O*-deethylation
FAO/WHO	Food and Agriculture Organization/World Health Organization
FEEDAP	Scientific Panel on Additives and Products or Substances use in Animal Feed
FFQ	Food Frequency Questionnaire
FSMPs	Foods for Special Medical Purposes
GLP	Good Laboratory Practise
GSH	Glutathione levels
GST	Glutathione-*S*-transferase
HDL	High-Density Lipoprotein
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
IU	International Units

JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
LDL	Low-Density Lipoprotein
LOD	Limit of Detection
LQD	Limit of Quantitation
NATCOL	Natural Food Colours Association
NDMAD	Nitrosodimethylamine *N*-demethylase
3-MC	3-methylcholanthrene
MPL	Maximum Permitted Limit
MROD	Methoxyresorufin-*O*-demethylation
NDNS	UK National Dietary and Nutrition Survey
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
PARNUTs	Foods for Particular Nutritional Uses
PHA	Phytohaemagglutinin
PROD	Pentoxyresorufin-O-depentylation
SCF	Scientific Committee on Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
TRL	Triglyceride-rich lipoprotein
TWI	Tolerable Weekly Intake
UNESDA	Union of European Beverage Associations
US EPA	US Environmental Protection Agency
VLDL	Very Low-Density Lipoprotein