European Food Safety Authority

The EFSA Journal (2008) 675, 1-22

Safety of Lycopene oleoresin from tomatoes [1]

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

(Question No EFSA-Q-2006-186)

Adopted on 24 April 2008

PANEL MEMBERS

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SUMMARY

Following a request from European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of 'lycopene oleoresin from tomatoes' for use as a novel food ingredient.

The novel food ingredient consists of a lycopene-rich oleoresin obtained as an ethyl acetate extract from the pulp of ripe tomatoes from a non-GM variety of tomatoes (*Lycopersicon lycopersicum* L. Karst. *ex* Farw) naturally selected for their high lycopene content.

Lycopene extracted from tomatoes is authorised within the EU as food colouring agent (E160d). The lycopene oleoresin from tomatoes to be used as a novel food ingredient contains 5-15 % lycopene and is prepared by a production process identical to that for the production of the additive E160d, although E160d is prepared using an additional concentration step to obtain an oleoresin that contains 60-70 % lycopene.

The identity and purity of the lycopene oleoresin from tomatoes is in compliance with the purity criteria for colouring agent E160d (lycopene) as food additive.
The applicant proposes to use the lycopene oleoresin as a food ingredient. Products will be formulated in such a way that they will provide about 2 mg lycopene per serving. Lycopene

[1] For citation purposes: Scientific Opinion of the Panel on Panel on Dietetic Products, Nutrition and Allergies on a request from the European Commission on the safety of 'lycopene oleoresin from tomatoes'. *The EFSA Journal* (2008) 675, 1-22.

oleoresin as a food supplement has been used in the UK since before 1997 to a significant degree and thus does not need further approval.
The Panel based its intake estimates for lycopene on four sources including 1) normal dietary intake of lycopene from food, 2) intake of lycopene from dietary supplements, 3) intake of lycopene from proposed food products and 4) use of lycopene as a food colour.

An overview of average dietary intakes of lycopene from foods in different populations was presented in previous EFSA evaluations. It was concluded that regular intakes of lycopene from natural dietary sources in different populations are, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional intakes of 20 mg lycopene/day or more.

The applicant indicates that the amount of lycopene used in dietary supplements is 5 to 15 mg/day.

The applicant estimates that the use of lycopene at the proposed use levels in the six food categories requested would lead to an overall additional lycopene intake of 12 mg per day.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food noted that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on average and up to 23 mg at the high level.

The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours of up to 43 mg of lycopene per day.

Overall, the NDA Panel concludes that intake of lycopene from the proposed levels of use as a novel food ingredient would lead to intake levels that would substantially increase the overall dietary intake of lycopene, and could lead to mean daily intakes from 14.5 - 23 mg/day and from 46 - 70 mg/day as high intakes. These values amount to 0.24 - 0.38 mg/kg bw/day and to 0.77 - 1.17 mg/kg bw/day for a 60 kg person and are for the high intake estimates substantially higher than the acceptable daily intake (ADI) recently established by the AFC Panel as a group ADI of 0.5 mg/kg bw/day for lycopene from all sources (EFSA, 2008). This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006).

The Panel considers that lycopene oleoresin from tomatoes is as safe as lycopene from other accepted sources.

The Panel concludes that for the average user consumption of lycopene from tomatoes oleoresin and from all other sources will be below the ADI. However, considering the various sources of lycopene (natural occurrence, fortified foods, supplements, food colour) some users of lycopene products may exceed the ADI of 0.5 mg/kg bw/day.

Key words:

Lycopene oleoresin from tomatoes, novel food ingredient, E160d, Registry Number 502-65-8

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 7 September 2004, Barry Ottaway & Associates Ltd. submitted a request under Article 4 of the Novel Food Regulation (EC) No 258/97 to the Competent Authorities of the United Kingdom for placing on the market 'lycopene oleoresin from tomatoes' Lyc-O-Mato® as a novel food ingredient.

On 30 June 2005, the Competent Authorities of the United Kingdom forwarded to the Commission their initial assessment report, which had reached the conclusion that 'lycopene oleoresin from tomatoes' for the proposed uses is acceptable, subject to adherence to the proposed specification and the production parameters.

On 9 August 2005, the Commission forwarded the initial assessment report to the other Member States. Several of these Member States submitted additional comments/objections. In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of scientific nature raised by the Competent Authorities of Member States can be summarised as follows:

- Details on the production process, the absence of harmful substances and the specifications of the final product and the tomato source should be clarified.
- Lycopene is also authorised as a food additive. The proposed levels for use as a novel food ingredient are higher and for more food categories than those for use as a food colour. It should be guaranteed that the authorisation as a novel food ingredient would not lead to its use as a colouring agent without permission.
- Toxicological data are insufficient to guarantee the safety of intake levels above the normal dietary intake. Due to the large range of products for which use levels are foreseen the exposure will increase. A complete risk assessment of the intake of lycopene from all sources should be made.
- In a 13-week oral toxicity study in rats, there was a significant increase in lung weight in females. Arguments to support that this effect is not of toxicological concern should be provided and/or, given the absence of adequate mutagenicity data, a long-term study should be required.
- There should be clarification regarding the nutritional benefit of adding lycopene to the diet
- It would be important to present studies regarding certain factors that could potentially affect the bio-availability of lycopene, as well as an evaluation of its interaction with other carotenoids present in the diet, in order to assess the possible differences between this product and foods naturally rich in lycopene.
- It is important to protect allergic individuals and prevent small children from over-consumption of tomato lycopene.

In addressing Member States' comments of a scientific nature, and considering the overall safety of lycopene oleoresin from tomatoes the Panel has used information from the original dossier provided by the applicant, the initial assessment carried out by the authorities of the

United Kingdom, the comments given by the Member States, the response from the applicant to the issues raised by the Member States and the recent opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on the use of lycopene as a food additive (EFSA, 2008).

Existing authorisations and evaluations

Lycopene, extracted from tomatoes, is authorised as a food colouring agent within the EU (E160d) (Directive 94/36/EC) and the US (CDR 21 73.295).
Lycopene was evaluated by the SCF in 1975 when it was unable to allocate an ADI but felt able to accept the use of lycopene prepared from natural foods by physical processes, without further investigations, as a colouring matter in food, provided that the amount consumed did not differ significantly from the amount consumed through the relevant foodstuffs (SCF, 1975). This Opinion was reiterated in 1989 (SCF, 1989). When JECFA evaluated lycopene from natural sources in 1977 they postponed a decision because of lack of data (JECFA, 1978).

In 1999 the SCF evaluated synthetic lycopene, but the available data were not sufficient to be accepted by the SCF. The SCF concluded (SCF, 1999): "The proposed specification 'not less than 96 %' lycopene is not acceptable because highly concentrated lycopene is sensitive to oxygen and light, forms degradation products with mutagenic activity, and is not identical with the beadlet formulation that has been tested toxicologically," and "The toxicological data provided on the beadlet formulation are insufficient. Therefore the Committee is not able to allocate an ADI and considers its use in food unacceptable at present."

Synthetic lycopene is currently not approved for colouring matters within the EU. It is considered generally recognised as safe (GRAS) for use as a food ingredient in the US (GRAS notice No GRN 000119).

Recently the Panel evaluated the use of an α-tocopherol-containing oil suspension of lycopene obtained from *Blakeslea trispora* for use as a novel food (EFSA, 2005). It was concluded that an α-tocopherol-containing oil suspension of lycopene obtained from *B. trispora* to an additional intake of up to about 2 mg/day is not of concern from the safety point of view. It was also concluded that this does not hold for the proposed levels of use of lycopene in foods that would give rise to an additional intake of 20 mg per day.

In July 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) derived an ADI of 0 - 0.5 mg/kg bw/day based on a 104-week study in rats using a safety factor of 100 for synthetic lycopene. This ADI was made into a group ADI to include lycopene from *B. trispora*. This level equates to 30 mg lycopene/day maximum for a 60 kg individual.

The Panel noted that the ADI set by JECFA (JECFA, 2006) does not include lycopene from tomatoes. This was due to the fact that JECFA was not evaluating lycopene from tomatoes.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA, 2008) evaluated the new toxicological data on lycopene and derived an ADI of 0.5 mg/kg bw/day. This ADI refers to lycopene from all sources.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 9 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'lycopene oleoresin from tomatoes' (Lyc-O-Mato®) in the context of Regulation (EC) No 258/97.

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

Furthermore, EFSA is asked to consider whether there are reasons to believe that 'lycopene oleoresin from tomatoes' as specified by the applicant is less safe than lycopene stabilised by other means.

ACKNOWLEDGEMENTS

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

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC, the ingredient concerned by the application belongs to Class 2.1. "Complex NF from non-GM sources: the source of the NF has a history of food use in the Community". For this reason the opinion will be an assessment of the safety data provided by the applicant to comply with the information required for novel foods of Class 2.1, i.e. information requirements I, II, III, IX, X, XI, XII and XIII as detailed in the following text. It does not include an assessment of the possible nutritional benefits of lycopene oleoresin.

I. Specification of the novel food (NF)

The novel food ingredient (NI) (Lyc-O-Mato®) consists of a lycopene-rich oleoresin obtained as an ethyl acetate extract from the pulp of ripe tomatoes from a non-GM hybrid variety (*Lycopersicon lycopersicum* L. Karst. *ex* Farw) that has been selected for its naturally high lycopene content.

Lycopene is a carotenoid with the formula $C_{40}H_{56}$. It has a molecular weight of 536.85 and the CAS Registry Number 502-65-8. Its structural formula is:

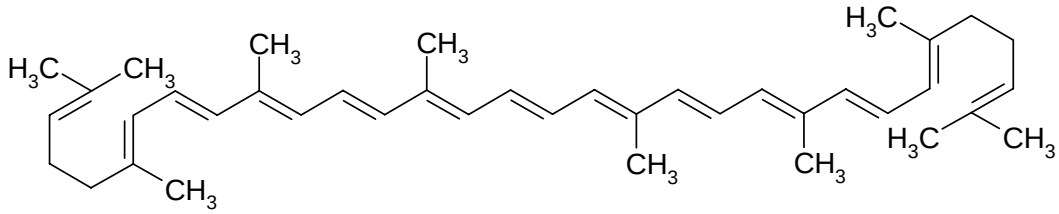

Fig 1. **Structural Formula**

Lycopene occurs in food predominantly in an all-*trans* form (Cronin, 2000; Boileau *et al.*, 2002). Regular tomatoes and tomato products mainly contain the all-E (*trans*-)isomers of lycopene (between 35-96 % of total lycopene content), but also some Z (*cis*-)isomers, mainly as 5Z, 9Z, 13Z and 15Z in percentages varying between 1-22 % (Schierle *et al*., 1997).

Carotenoids from tomato or tomato extract can be analysed using HPLC (Ishida *et al.*, 2001).

Based on the analytical information provided by the applicant, the novel food ingredient consists of 5-15 % lycopene together with a number of other constituents that occur naturally in tomato, including fatty acids and acylglycerols (69-74 %), unsaponifiable matter (14-19 %), water soluble matter (2.7-4.7 %), water (0.48-0.86 %), phosphorous compounds (0.35-0.52 %), phospholipids (8.9-14 %), nitrogen (0.16-0.31 %) and sulphated ash (upon drying)(0.7-0.8 %).

Lycopene in the proposed novel food ingredient consists of 90-95 % (all-*trans*)-lycopene. Different *cis* isomers are also likely to be present at small quantities. Other carotenoids and related substances including β-carotene, phytofluene, phytoene and tocopherols are, according to the applicant, present in small amounts (0.1-2.5 %).

The applicant notes that the percentage of lycopene in the oleoresin may vary between 5 and 15 % because the composition of the tomatoes is subject to natural fluctuations. The analysis of 25 commercial batches produced between 1995 and 2003 revealed a range of 5.8 to 15.6 %.

Specifications for the lycopene oleoresin from tomatoes have been provided by the applicant and are presented in Table 1. The applicant indicates that the levels of solvent residues, pesticides, microbiological contamination and heavy metals are continuously monitored.

Table 1. **Proposed Specifications of lycopene oleoresin from tomatoes as provided by the applicant**

Analysis	Method	Specification	
Physical State	Observation against standard	Red to dark brown viscous liquid	
Clarity	LAB/123/01	Clear solution	
Lycopene identity	LAB/109/01[a]	HPLC retention time	
Total lycopene[b]	LAB/109/01[a]	5.0 to 15.0 %	
% *trans*-Lycopene	LAB/109/01[a]	90 to 95 %	
Total carotenoids[c]	LAB/102/02[a]	6.5 to 16.5 %	
Other carotenoids	LAB/118/01[a]	Phytoene: 0.5 to 0.75 % Phytofluene: 0.4 to 0.65 % ß-carotene: 0.2 to 0.35 %	
Total tocopherols	LAB/118/01	1.5 to 3.0 %	
Unsaponifiable matter	Study 98/021[a]	13 to 20 %	
Total fatty acids[d]	Study 98/021	60 to 75 %	
Phytosterols	Study 98/021	0.5 to 2.5 %	
Lycopene crystal particle size	Microscopic	90 % < 5µ 99 % < 10µ	
Water	Karl Fisher	0.5 % max	
Sulphated ash	AOAC 34.104	0.5 to 1.5 %	
Residual solvent (ethyl acetate, ethanol)	LAB/114/01[a]	50 mg/kg max	
Pesticides	DFG-S19[e]	Below 3 ppm	
Heavy metals	I.C.P.[e]	Pb < 2 mg/kg Cd, Mo, Ni, Hg all <1 mg/kg	
Arsenic	I.C.P.[e]	As < 2 mg/kg	
Microbiology	USP 24 NF 19/<61>	Total viable count Moulds Yeasts Escherichia coli: Salmonella sp Staphylococcus aureus Pseudomonas aeruginosa Clostridium perfringens	 < 1000/g < 100/g < 100/g Not detected in 10g Not detected in 20g Not detected in 10g Not detected in 10g

[a]LycoRed Method, SOP available on request
[b]Combined *cis*- and *trans*-lycopenes
[c]Calculated as lycopene
[d]Myristic acid (14:0); palmitic acid (16:0); stearic acid (18:0); oleic acid (18:1); linoleic acid (18:2); linolenic acid (18:3); arachidic acid (20:0); behenic acid (22:0); mono-, di- and tri-glycerides; free fatty acids
[e]Analysis method SOP available on request

The applicant demonstrated the stability of the lycopene oleoresin from tomatoes under storage at 4°C and room temperature for up to 37 months. Stability was assessed by spectrophotometry and HPLC.

For the production of lycopene as a colour additive, the lycopene oleoresin from tomatoes which is the subject of the present opinion is further processed by removal of part of the tomato lipids to form the concentrated product, of which the final colour additive formulations are prepared.

Attempts carried out including the Bradford assay for protein detection did not reveal residual protein. The detection limit of the analysis for residual proteins was estimated to be about 1 μg/g.

The identity and purity of lycopene oleoresin from tomatoes is in compliance with the purity criteria for colouring agent E160d (lycopene) a food additive (EC, 1995).

II. Effect of the production process applied to the NF

The manufacturing process has been described by the applicant and is carried out in two steps: the tomato pulp production and the extraction of the lycopene from the tomato pulp.

The production process for lycopene oleoresin from tomatoes is identical to that for the production of the additive E160d, which has been examined and approved by the SCF (SCF, 1975, 1978), although E160d is prepared using an additional concentration step to obtain an oleoresin that contains 60-70 % lycopene.

Lycopene oleoresin from tomatoes is produced by crushing lycopene-rich tomatoes to pulp. The applicant states that the production process introduces no exogenous substances to the tomato pulp, protects the tomato phytonutrients from oxidation and assures that the extraction is conducted on unchanged and non-deteriorated raw material. The tomato pulp is then separated from the serum and extracted with ethyl acetate. The extract is separated from the tomato pulp, and lycopene oleoresin is obtained after solvent removal by evaporation under vacuum at 40-60° C.

Analytical methods to quantify the components of lycopene oleoresin and possible impurities (organic volatiles, microbes, acid-soluble metals) were provided by the applicant.

Due to the chemical nature of lycopene (i.e. long chain with conjugated carbon-carbon double bonds) it is susceptible to chemical changes such as isomerisation and degradation when exposed to light, heat and oxygen (Lee and Chen, 2002). The applicant indicates that the production process is designed such that it protects lycopene from oxidation.

The lycopene oleoresin obtained is packed under nitrogen and stored at 4 ºC.

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

High lycopene non-GMO tomatoes are used to produce the novel food. The applicant indicates that conventional breeding methods utilising the natural gene pool of the genus *Lycopersicon* have been applied in order to create a tomato plant with a high content of lycopene. This particular variety is not consumed directly but is used in the production of tomato products.

IX. Anticipated intake/extent of use of the NF

The Panel notes that intake of lycopene may result from four sources including 1) normal dietary intake from food, 2) intake from dietary supplements, 3) intake from proposed use in food products and 4) use as a food colour.

Intake of lycopene from normal dietary sources

The applicant indicated that intakes of lycopene from natural sources may amount to a maximum lycopene intake in the Netherlands (for the age group 55-69 years) from a normal diet by heavy users of tomato products of 26.1 and 18.6 mg/day for men and women respectively, corresponding to 0.44 mg/kg bw/day (assuming 60 kg bw for men) and 0.37 mg/kg bw/day (assuming 50 kg bw for women).

An overview of average dietary exposure to lycopene from foods in different populations was presented in previous EFSA evaluations (EFSA, 2005a and b; EFSA, 2008). It was concluded that regular exposure to lycopene from natural dietary sources in different populations are, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional exposure to 20 mg lycopene/day or more.

Intake of lycopene from proposed supplement use

The amount of lycopene used in dietary supplements is 5 to 15 mg/day, equivalent to 83-250 mg lycopene oleoresin containing 6 % lycopene and smaller amounts when oleoresin with higher lycopene content is used.

Intake of lycopene from use in food products at the proposed uses and use levels.

The applicant indicates that in case of addition to food, products will be formulated in such a way that they will provide ca. 2 mg lycopene per serving. Table 2 presents an overview of the intended use levels in the different food categories as provided by the applicant.

Table 2. **Summary of recommended food uses and use levels of lycopene oleoresin in different food categories proposed by the applicant.**

Food category	Food Product (examples)	Added lycopene (mg/kg)	Portion Size (g)	Added lycopene Per Serving (mg)
Dairy products	Yoghurt	16	125	2.0
	Ice cream	25	80	2.0
Non-alcoholic flavoured drinks	Fruit and vegetable based beverages (not as colorant)	8	250	2.0
Cereal and cereal products	Breakfast cereals	67	30	2.0
	Cereal bar	50	40	2.0
Canned products	Soups (other than tomato)	10	200	2.0

Bread and baked goods	Bread Crisp breads	20 40	100 50	2.0 2.0
Spreads	Fat-based spreads	201	10	2.0

The applicant notes that the levels of incorporation are significantly lower than those permitted for use of lycopene as a food colour (E160d).

The applicant estimates that the use of lycopene at the proposed use levels in the six food categories would lead to an additional lycopene intake of 12 mg per day.

Intake of lycopene resulting from use of lycopene as a food colour

An overview of average dietary exposure to lycopene from its use as a food colour in different populations was presented in a previous EFSA evaluation (EFSA, 2008). It was concluded that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on average and up to 23 mg at the high level.

Intake estimate from all sources

The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours of up to 43 mg of lycopene per day.

Table 3. **Summary of typical lycopene exposure estimates**

Source of lycopene	**Average** (mg/day)	**High** (mg/day)	**reference**
Natural occurrence	0.5 - 5	8 - 20	AFC 2008
Fortified foods*	12	12	Present opinion
Supplements	0 (no supplement use)	15	Present opinion
Food Colour	2 - 6	11 - 23**	AFC 2008

* fortified food: lycopene added to foods

** based on the 97.5th percentile intake estimates

Overall, the Panel concludes that intake from the proposed levels of use would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to mean daily intakes from 14.5 - 23 mg/day and from 46 - 70 mg/day as high intakes. The estimates for the high intakes are based on conservative assumptions. These values amount to 0.24 - 0.38 mg/kg bw/day and to 0.77 to 1.17 mg/kg bw/day for a 60 kg person and are for the high intake estimates substantially higher than the ADI recently established by JECFA (JECFA, 2006) and EFSA (EFSA, 2008) as a group ADI of 0.5 mg/kg bw/day.

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

The source for production of the novel food ingredient are high lycopene content non-GMO tomatoes created by using conventional breeding methods utilising the natural gene pool of the genus *Lycopersicon*. This particular variety is not consumed directly but is used in the

manufacture of tomato products. Thus there is no information from previous human exposure to the novel food or its source.

In response to a query from a Member State the applicant reported that over 400 tonnes of the novel food ingredient were used in Europe, the US and the Far East between 1995 and 2004. It was also stated that during this period no adverse events related to the consumption of these food supplements were reported to the applicant.

Case studies reported pigment deposition in liver and skin on the hands, forearms, face and soles of feet, abdominal pain, nausea, vomiting and diarrhoea and an elevated concentration of lycopene in serum and liver in individuals consuming diets rich in tomatoes or tomato products (Reich, 1960, La Placa *et al*. 2000; Bonnetblanc *et al.,* 1987; Gandhi *et al.,* 1988). According to Clinton (1998) the amount of lycopene consumed in one of these case reports may correspond to 100-232 mg lycopene/day.

A recent multi-centre, placebo-controlled supplementation study investigated the effect of supplementation with 13.3 mg lycopene (from tomato extracts)/day during 20 weeks in 400 healthy male and female volunteers (Olmedilla *et al.,* 2002). Neither significant side effects except for carotenodermia in 25 % of the subjects in the Spanish cohort nor changes in biochemical or haematological indices, were observed throughout the study. In a study by Hininger *et al.* (2001) 175 healthy male volunteers received 15 mg lycopene (from tomato extracts)/day or placebo for 3 months. No side effects were reported.

Several clinical studies evaluating various endpoints upon lycopene supplementation through protocols using tomato-based products or tomato based capsules, did not reveal any abnormalities in body weights, full blood counts, liver function tests or immune function tests in subjects supplemented with lycopene at levels ranging from 0.5 mg/day for 4 weeks, 15 or 30 mg for 3 weeks, 47.1 mg/day for 8 weeks to 75 mg/day for 1 week, and these doses were generally well tolerated with no reports of any illness or adverse biological effects (Micozzi *et al.,* 1992; Carughi and Hooper, 1994; Olmedilla *et al.,* 2002; Agarwal and Rao, 1998; Müller *et al.,* 1999; Kucuk *et al.,* 2001; Chen *et al.,* 2001; Hininger *et al.,* 2001; Chopra *et al.,* 2000; Watzl *et al.,* 2000). However, these studies were not designed to evaluate the safety of lycopene and were all of short duration.

XI. Nutritional information on the NF

The applicant states that, whilst the lycopene component of the oleoresin can be considered to be nutritionally equivalent to lycopene from conventional tomatoes, tomato products and the food additive E160d, small variations in the levels of other carotenoids and plant ingredients may occur due to the differences in tomato varieties used and/or the effects of the production process.

The panel considers that lycopene from tomato oleoresin does not have a nutritional impact. This Opinion does not include an assessment of the possible benefits of lycopene.

Bio-availability

No information on the toxicokinetics of the lycopene oleoresin was provided by the applicant.

Plasma responses in man and experimental animals upon supplementation of synthetic lycopene or lycopene from tomatoes have been investigated frequently and some data on the bio-availability of these forms of lycopene is presented here taken from a previous EFSA opinion (EFSA, 2005).

In toxicokinetic studies in rats and monkeys, less than 10 % of an orally administered dose of radio-labelled lycopene was absorbed. Most of the absorbed material was rapidly eliminated in the faeces, and only small amounts of residual radioactivity were found in several organs and tissues, most of it in the liver (McClain and Bausch, 2003; SCF, 1999). Accumulation of lycopene in the liver was higher in rats fed beadlet (synthetic) lycopene compared to those fed tomato preparations which could be due to the higher bio-availability of synthetic lycopene compared to natural lycopene (SCF, 1999).

Besides single dose studies, the effect of lycopene supplementation for a longer period on plasma lycopene concentrations has been investigated in several intervention studies. Several studies used tomato extracts as the lycopene source. A recent multi-centre, placebo-controlled intervention study investigated the effect on plasma lycopene response of daily supplementation with 13.3 mg lycopene (from tomato extracts) during 20 weeks in 400 healthy male and female volunteers (Olmedilla *et al.*, 2002). After 4 weeks of supplementation plasma levels of lycopene reached a plateau of 2-fold (approximately 0.55 µmol/L) over background levels. In a study by Hininger *et al.* (2001) 175 healthy male volunteers received 15 mg lycopene (from tomato extracts) or placebo for 3 months. Plasma lycopene concentrations increased by 0.54 µmol/L and LDL lycopene concentrations increased by 65 ng per mg LDL cholesterol in the lycopene-supplemented group. Hoppe *et al.* (2003) described a study aimed at determining the relative bio-availabilities of synthetic and tomato-based lycopene in a single-blind, randomized placebo-controlled parallel trial. Synthetic and tomato-based lycopene, dosed at 15 mg/day for 28 days, resulted in similar significant increases above baseline of serum total lycopene by 0.58 and 0.57 µmol/L respectively.

Diwadlar-Navsariwala *et al.* (2003) presented a physiological pharmacokinetic model, validated by a phase I study in healthy male subjects (five per dose), describing the disposition of lycopene delivered as a tomato beverage formulation at lycopene doses of 10, 30, 60, 90 and 120 mg. The amount of lycopene absorbed was not statistically different between the doses and amounted to a mean value of 4.69 ± 0.55 mg. Independent of the dose, 80 % of the subjects absorbed less than 6 mg of lycopene.

Overall systemic exposure and bioavailability of lycopene from tomato-based products and dietary supplements seems to be similar, since lycopene absorption from purified or synthetic sources (i.e. supplemental lycopene form soft gel capsules containing tomato oleoresin) has been demonstrated by Böhm and Bitsch (1999) to be comparable to that from processed tomatoes, i.e. tomato juice.

In addition, interactions, both competitive and synergistic, between carotenoids have been shown to occur during the various stages of absorption (e.g., incorporation into mixed micelles, intracellular transport within enterocytes, and chylomicron assemblage), as well as during post-absorptive distribution (Furr and Clark, 1997; Van den Berg, 1999); however, the mechanisms *via* which this occurs are not clear, and definite relationships between specific carotenoids have not been established. However, as long as intake levels are within normal dietary intake, such interactions are unlikely to have a significant impact on the systemic bioavailability of an individual component.

Metabolism

Very little is known about the metabolism or degradation of lycopene in mammals (Clinton, 1998; Parker, 1996). It has been shown that lycopene does not exhibit provitamin A activity (Van Vliet *et al.,* 1996; Agarwal and Rao, 2000). Furthermore, few metabolites of lycopene have been documented in human plasma or tissues. For example, two oxydised lycopene metabolites, identified as epimeric 2,6-cyclolycopene-1,5-diols, have been detected in breast milk and serum of three lactating mothers (Khachik *et al.,* 1997). It is postulated by the authors that these compounds may result via an *in vivo* metabolic oxidation of lycopene to lycopene epoxide. Upon oral administration of ^{14}C labelled lycopene to rats and monkeys no evidence for any metabolic products of lycopene was observed (McClain and Bausch, 2003).

XII. Microbiological information on the NF

The lycopene oleoresin is produced without the aid of microbiological processes. The applicant indicates that great care is taken throughout the production process to prevent microbial contamination and that therefore the production process is not expected to result in the presence of micro-organisms or their metabolites other than would be present with current uses or mentioned in the specifications.

XIII. Toxicological information on the NF

The lycopene oleoresin is a pure tomato product without any additives. There are no chemical reactions involved in its production. The specifications do not suggest the presence of new toxicants or changed levels of existing toxicants compared to the traditional counterpart. No information was provided on the tomatine levels in the lycopene oleoresin. On the other hand, the applicant argues that only ripe tomatoes are used for the production of the lycopene oleoresin and that the concentration of tomatine diminishes with increasing maturity. Practically ripe tomatoes do not contain tomatine. Furthermore, since tomatine is a polar molecule it may not be extracted with the ethyl acetate extraction procedure. The applicant indicated that two batches of the lycopene oleoresin (containing 6 and 7 % lycopene) were tested and tomatine was not detected at a limit of detection of 1 mg/kg.

The applicant provided results from toxicological studies, including acute toxicity data, data on skin and eye irritation and skin sensitisation, subchronic toxicity and mutagenicity data from studies performed with the lycopene oleoresin containing 5 % or 6 % lycopene.

Acute oral toxicity

Studies provided by the applicant revealed that the lycopene oleoresin from tomatoes containing 5 % lycopene exhibits low acute oral toxicity (LD50 levels > 5000 mg/kg bw) in Sprague Dawley rats.

The oral LD_{50} value in mice of lycopene was assessed to be higher than 3000 mg/kg bw (Milani *et al.,* 1970). The source of lycopene used in this study is not specified by the authors.

Subacute and subchronic toxicity

The applicant provided results from a 13 week oral toxicity study in rats exposed to daily doses by gavage of 0, 45, 450 or 4500 mg of the lycopene oleoresin (containing 5 % lycopene) per kg bw, amounting to doses of 0, 2.25, 22.5 or 225 mg lycopene/kg bw/day, and performed in compliance with GLP and according to OECD guidelines.

Some animals showed staining of the tail but this was attributed to accidental transfer of the lycopene oleoresin during dosing. An increase in absolute lung weight in female rats in the two highest dose groups was observed. These were not accompanied by histopathological changes and were therefore considered by the applicant and by the UK Committee to be not toxicologically relevant.

The UK Committee asked the applicant for further histopathological data to clarify the significance of the increase in lung weights that was observed in the upper dose groups. The applicant provided some additional information and the UK Committee requested that a toxicologist with expertise in animal pathology be contacted in order to assess the significance of the findings. The nominated expert confirmed that the observed increased absolute lung weights were not indicative of a target organ toxic effect and related to the body weight increases for female rats, caused by the treatment.

The Panel noted that the increase in absolute lung weight was not observed in male rats, that the increase amounted to +13.2 % ($p<0.01$) in the 450 mg dose group and to +10.4 % ($p<0.05$) in the 4500 mg dose group and thus did not reveal a clear dose response, and that the effect was smaller and no longer statistically significant when expressed on a body weight basis (+6.7 % and +6.7 % for the two dose groups respectively), supporting the conclusion that the effect was not indicative of a target organ toxicity and related to the body weight increase of the female rats.

It can therefore be concluded that the NOAEL for this study was 4500 mg 5 % lycopene oleoresin/kg bw/day amounting to 225 mg lycopene/kg bw/day.

An overview of other subchronic studies with oral administration of synthetic or natural lycopene to experimental animals can be found in previous evaluations on the safety of lycopene from *B. trispora*, tomatoes or synthetic lycopene by the SCF (1999), EFSA (2005; 2008) and JECFA (JECFA, 2006).

In July 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) derived an ADI of 0 - 0.5 mg/kg bw/day based on a 104 week study in rats using a safety factor of 100 for synthetic lycopene. This ADI was made into a group ADI to include lycopene from *B. trispora*. This level equates to 30 mg lycopene/day maximum for a 60 kg individual.

Chronic toxicity and carcinogenicity

To date, no long-term feeding studies conducted with the 5-15 % lycopene oleoresin from tomatoes have been performed.

In the most recent AFC Opinion two long term studies in rats with synthetic lycopene are described (51 and 104 weeks respectively) (EFSA, 2008).

Mutagenicity

The applicant provided results from genotoxicity studies performed with the 5 % lycopene oleoresin from tomatoes. The preparation was negative in bacterial tests with *Salmonella enterica var. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* WP2uvrA all with and without metabolic activation.

No genotoxicity studies in mammalian cells have been performed with the 5-15 % lycopene oleoresin from tomatoes.

Literature studies report lycopene from various sources including tomato paste but also synthetic lycopene, to be negative in the Ames test with various strains of *Salmonella typhimurium* with and without metabolic activation (He and Campbell 1990; Aizawa *et al.,* 2000; McClain and Bausch, 2003), in *E. coli* with and without metabolic activation (Aizawa *et al.,* 2000), in the test for mutations at the tk locus in mouse lymphoma cells (McClain and Bausch, 2003) and in the test for chromosome aberrations in human lymphocytes *in vitro* (McClain and Bausch, 2003).

In vivo mutagenicity tests were performed in mice, rats and humans. Lycopene administered at daily doses of around 15 mg/day through synthetic or tomato-based products in the diet, did not induce DNA damage, detected by the Comet assay, in human lymphocytes (Pool-Zobel *et al.,* 1997; Collins *et al.,* 1998; Riso *et al.,* 1999). McClain and Bausch (2003) reported the absence of clastogenicity in the micronucleus assay performed with mouse peripheral blood cells upon administration of a beadlet formulation or of tomato juice containing natural lycopene. They also reported the absence of genotoxicity in the UDS test with rat hepatocytes after *in vivo* exposure to a lycopene beadlet formulation.

Previously the SCF (1999), EFSA (2005; 2008) and JECFA (2006) have evaluated the genotoxicity of lycopene from various sources including natural lycopene from tomatoes, synthetic lycopene and lycopene from *B. trispora*, and concluded that genotoxicity data do not give reason for concern.

Altogether it is concluded that the present database on genotoxicity of lycopene from various sources indicates that there is no reason for concern with respect to genotoxicity.

Allergenicity and Irritancy

No eye irritating properties were observed of tomato oleoresin containing 5 and 6 % lycopene. One batch of tomato oleoresin containing 5 % lycopene was found to be positive in a sensitisation study (Guinea pig maximisation test) indicating the capacity to induce skin sensitisation. The applicant suggested that the problem was due to contamination of the pulp with lactic acid bacteria causing lactic fermentation of the pulp from which the particular lot of oleoresin was extracted. The applicant has since changed the production process to prevent this fermentation problem. Subsequent batches were tested on rabbits and guinea pigs and it was found that neither skin irritation nor contact hypersensitivity were induced by the lycopene oleoresin. The Panel accepts this additional confirmation and concluded that the lycopene oleoresin from tomatoes did not cause skin sensitisation.

Tomatoes are known to produce allergic reactions in some individuals. IgE cross-reactive profilins have been suggested to account for the symptoms in patients suffering from tomato allergy. Westphal *et al*. (2004) concluded that tomato profilin is a minor allergen in tomato fruit, which has the potential to account for clinical symptoms in tomato-allergic patients.

Given the fact that the extraction process does not enrich the protein fraction in this NF, the panel concludes that the potential allergenicity is, at most, similar to that observed for tomatoes.

DISCUSSION

High lycopene non-GMO tomatoes are used to produce the novel food. The applicant indicates that traditional and conventional breeding methods utilising the natural gene pool of the genus *Lycopersicon* have been applied in order to create a tomato plant with a high content of lycopene. This particular variety is not consumed directly but is used in the production of tomato products.

The Panel based its intake estimates for lycopene on four sources including 1) normal dietary intake of lycopene from food, 2) intake of lycopene from dietary supplements, 3) intake of lycopene from proposed fortified food products and 4) use of lycopene as a food colour.

An overview of average dietary intakes of lycopene from foods in different populations was presented in previous EFSA evaluations (EFSA, 2005a and b). It was concluded that regular intakes of lycopene from natural dietary sources in different populations are, according to dietary surveys, estimated to be on average between 0.5 and 5 mg/day, with high exposures up to about 8 mg/day. High consumption of fruits and vegetables, especially tomato products, may result in occasional intakes of 20 mg lycopene/day or more.

The applicant indicates that the amount of lycopene used in dietary supplements is 5 to 15 mg/day per day.

The applicant estimates that the use of lycopene at the proposed use levels in the six food categories would lead to an overall additional lycopene intake of 12 mg/day.

In a recent opinion the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) noted that total daily exposure to lycopene as a food colour could potentially range from 2 to 6 mg on average and up to 23 mg at the high level. The AFC Panel did not exclude an occasionally combined high exposure from both natural dietary sources and food colours up to 43 mg of lycopene per day.

Overall, the Panel concludes that intake of lycopene from the proposed levels of use as a novel food ingredient would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to mean daily intakes from 14.5 - 23 mg/day and from 46 - 70 mg/day as high intakes. These values amount to 0.24 - 0.38 mg/kg bw/day and to 0.77 - 1.17 mg/kg bw/day for a 60 kg person and are for the high intake estimates substantially higher than the ADI recently established by the AFC Panel as a group ADI of 0.5 mg/kg bw/day for lycopene from all sources (EFSA, 2008). This ADI is in line with the ADI of 0 - 0.5 mg/kg bw/day established by JECFA (JECFA, 2006).

The applicants provided results from a 13-week oral toxicity study in rats from which a NOAEL of 4500 mg 5 % lycopene oleoresin, amounting to 225 mg/kg bw/day lycopene could be derived.

Recently, the AFC Panel concluded that the safety of synthetic lycopene was demonstrated in subchronic and chronic toxicity studies in rats, a carcinogenicity study and a two-generation study in rats, and developmental toxicity studies in the rat and rabbit. Mutagenicity has been studied in an extensive program using formulated forms of lycopene and demonstrated no concern. The following NOAELs for formulated, synthetic lycopene, were established in guideline-conforming toxicity studies:

- 500 mg lycopene/kg bw/day (the highest dose level tested) in a 14-week rat study
- 500 mg lycopene/kg bw/day (the highest dose level tested) in a developmental toxicity study in the rat

- 500 mg lycopene/kg bw/day (the highest dose level tested) in a two-generation study in the rat
- 400 mg lycopene/kg bw/day (the highest dose level tested) in a developmental toxicity study in the rabbit
- 50 mg lycopene/kg bw/day in a one year rat study and
- 50 mg lycopene/kg bw/day (the highest dose level tested) in a two year rat carcinogenicity study.

The NOAEL from a 90-day oral toxicity study with lycopene extracted from *B. trispora* amounted to about 600 mg/kg bw/day.

From the lowest NOAEL of 50 mg/kg bw/day the AFC Panel derived an ADI of 0.5 mg/kg bw/day using a safety factor of 100 and indicated that this ADI refers to lycopene from all sources (EFSA, 2008).

CONCLUSIONS AND RECOMMENDATIONS

The Panel considers that lycopene oleoresin from tomatoes is as safe as lycopene from other accepted sources.

The Panel concludes that for the average user consumption of lycopene from tomato oleoresin and from all other sources will be below the ADI. However, considering the various sources of lycopene (natural occurrence, fortified foods, supplements, food colour) some users of lycopene products may exceed the ADI of 0.5 mg/kg bw/day.

DOCUMENTATION PROVIDED TO EFSA

Letter from the European Commission to the Chairman of the European Food safety Authority with the request for an opinion on the safety of 'lycopene oleoresin from tomatoes'. SANCO E4/Ak/ko D(2006) 540654

Initial assessment report on lycopene-rich oleoresin from tomato from the UK Food Standard Agency. Ref NFU 482

Letters from Member States with comments on the initial assessment report on lycopene-rich oleoresin from tomato from the UK Food Standard Agency.

Response to Member States comments on the United Kingdom Opinion for a lycopene-rich oleoresin derived from tomato as a novel food ingredient, and consideration of new data provided by the applicant.

Application under regulation No 258-97 for the use of lycopene-rich oleoresin derived from tomato as a novel food ingredient.

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