European Food Safety Authority

The EFSA Journal (2008) 893, 1-15

SCIENTIFIC OPINION

Safety of 'Lycopene Cold Water Dispersible Products from *Blakeslea trispora*' [1]

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

(Question No EFSA-Q-2008-697)

Adopted on 04 December 2008

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of Lycopene Cold Water Dispersible Products (CWD) from *Blakeslea trispora* as novel food ingredient.

Lycopene from *B. trispora* is biosynthesised by the fungus *B. trispora* through the same pathway as lycopene produced in the tomato. Following a fermentation process, lycopene is extracted from the biomass, purified and then crystallised. The lycopene CWD product is a dark red powder, which is formed when the lycopene crystals are mixed with tocopherol in methylene chloride, which is then mixed with an octenyl succinic anhydride (OSA)-starch solution to form a homogeneous emulsion and afterwards dried. Methylene chloride, meeting food grade specifications, is permitted for use as an extraction solvent for lycopene extracted from tomatoes under EU Directive 95/45/EC (Commission of the European Communities, 1995). OSA starch, meeting food grade specifications, is used in accordance with EU Directive 95/2/EC.

No information on the bio-availability of lycopene from *B. trispora* lycopene 10 and 20 % CWD products has been provided. However it is expected that the bio-availability of lycopene from the 10 and 20 % CWD forms will not differ from other lycopene formulations in a way that would cause safety concerns.

Toxicological information on lycopene from various sources including lycopene from *B. trispora* has been reviewed in previous opinions by the European Food Safety Authority (EFSA) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission on the safety of 'Lycopene Cold Water Dispersible Products (CWD) from *Blakeslea trispora*'. *The EFSA Journal* (2008) 893, 1-157.

The applicant proposes to use lycopene from *B. trispora* as a food ingredient in beverages, cereal products, fruit products, nut products; milk products; sugar, and confectionary products and preserves at use levels of 5 mg/kg.

The applicant provides an intake estimate based on these food uses. The Panel considered also 1) normal dietary intake of lycopene from food, 2) intake of lycopene from dietary supplements and 3) intake from use of lycopene as a food colour.

On an all-user basis, the highest mean and 95th percentile intakes of lycopene (as CWD) by the U.K. population, as observed in male teenagers, were estimated to be 3.54 mg/person/day (0.07 mg/kg bw/day) and 8.16 mg/person/day (0.16 mg/kg bw/day). On a body weight basis, children consumed the greatest amount of lycopene (as CWD), with mean and 95th percentile all-user intakes of 0.15 and 0.37 mg/kg bw/day, respectively.

In a recent opinion the 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 (AFC, 2008a). 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 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 total daily intakes up to 9.8 to 14.5 mg/day at the mean and 23 to 51 mg/day as high intakes. These values amount to 0.16 to 0.24 mg/kg bw/day and 0.38 to 0.85 mg/kg bw/day for a 60 kg person and can be, especially for the high intake estimates, higher than the ADI recently established by EFSA as a group ADI for lycopene considered to be 0.5 mg/kg bw/day from all sources, including lycopene occurring naturally in foods.

The Panel concludes that lycopene 10 and 20 % CWD formulations from *B. trispora* are as safe as lycopene from other sources.

The Panel also concludes that for the average user consumption of lycopene 10 and 20 % CWD formulations from *B. trispora* and from all other sources will be below the ADI. However, some users of lycopene products might exceed the ADI of 0.5 mg/kg bw/day.

Key words:

Lycopene, *Blakeslea trispora*, cold water dispersible, CWD, novel food ingredient, CAS Registry Number 502-65-8

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BACKGROUND

On 30 August 2007, Vitatene S.A.U submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market 'Lycopene Cold Water Dispersible Products (CWD) from *Blakeslea trispora*' as novel food ingredients.

On 17 October 2007, the competent authority of the United Kingdom (UK) forwarded to the Commission its initial opinion, which came to the conclusion that an additional assessment was required. The UK Advisory Committee on Novel Foods and Processes (ACNFP) concluded that this novel food ingredient was essentially an extension of use and differed only in its formulation when compared with the ingredient which has been granted marketing authorisation by the Commission Decision of 23 October 2006 (2006/721/EC) "authorising the placing on the market of lycopene from *B. trispora* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council". The UK Competent Authority was aware of the then current evaluation[2] of lycopene from different sources by the European Food Safety Authority and requested that an additional assessment is carried out in order to determine whether this application for additional food uses of lycopene from the fungus *B. trispora* meets the criteria for acceptance of a novel food ingredient defined in Article 3(1) of regulation (EC) 258/97, taking into account the conclusions of this ongoing EFSA review for any subsequent authorisation.

On 11 February 2008, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States agreed with the initial opinion of UK.

Additionally, one Member State submitted a comment of a scientific nature requesting that an additional assessment should in particular evaluate whether the bio-availability of the water-dispersible formulation in the application corresponds to the oil suspension formulation granted marketing authorisation by the Commission Decision of 23 October 2006 (2006/721/EC) "authorising the placing on the market of lycopene from *Blakeslea trispora* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council".

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

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Lycopene Cold Water Dispersible Products (CWD) from *Blakeslea trispora*' as a food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: 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.

[2] Use of lycopene as a food colour (EFSA Q-2007-001, Q-2007-081, 2008-076); Safety of lycopene oleoresin from tomatoes (EFSA-Q-2006-186); Safety of synthetic lycopene (EFSA-Q-2007-119).

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC, the ingredient concerned by the application belongs to Class 2.2 "Complex NF from non-GM sources; the source of the NF has no history of food use in the Community". For this reason this Opinion will be an assessment of the safety data provided by the applicant to comply with the information required for novel foods of Class 2.2, i.e. information requirements I, II, III, IX, XI, XII and XIII as detailed in the following text and does not include an assessment of the possible nutritional benefits of lycopene.

This present opinion refers to the following previous opinions:

- Opinion of the Scientific Panel on Dietetic products, nutrition and allergies (NDA) related to an application on the use of α-tocopherol-containing oil suspension of lycopene from *B. trispora* as a novel food ingredient (EFSA, 2005a),
- Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to an application on the use of α-tocopherol containing oil suspensions and cold water dispersible forms of lycopene from *B. trispora* as a food colour (EFSA 2005b),
- Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, on a request from the European Commission on the use of lycopene as a food additive (EFSA, 2008a),
- Opinion of the Scientific Panel on Dietetic products, Nutrition and Allergies on a request from the Commission related to an application on the safety of lycopene oleoresin from tomatoes (EFSA 2008 b),
- Opinion of the Scientific Panel on Dietetic products, Nutrition and Allergies on a request from the Commission related to an application on the Safety of Synthetic Lycopene (EFSA 2008c).

I. Specification of the novel food (NF)

Lycopene CWD products are derived from the fungus *B. trispora*. The predominant occurring lycopene isomer in the final manufactured material is all-*trans*-lycopene, which has CAS Registry number 502-65-8.

Lycopene is a carotenoid with the formula $C_{40}H_{56}$. It has a molecular weight of 536.85 g/mol. Its structural formula is:

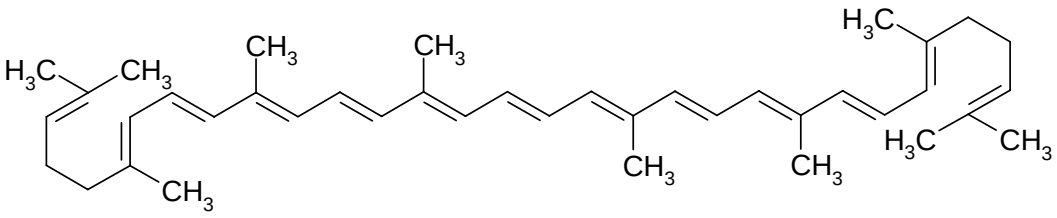

Figure 1. **Structural Formula**

Lycopene occurs in food predominantly in an all-trans form (Cronin 2000; Boileau et al., 2002) and has various cis isomers (common in human blood and tissue) (Cronin, 2000). All-trans lycopene is a red crystalline powder with a melting point of 173° C that is soluble in fats and certain organic solvents but virtually insoluble in water, methanol and ethanol (Cronin, 2000; Merck, 2001).

Lycopene from *B. trispora* is biosynthesised by the fungus *B. trispora* through the same pathway as lycopene produced in the tomato. The predominant isomer is all-trans lycopene.

Specifications for lycopene obtained from *B. trispora* have been described in the opinion of the NDA Panel on the use of α-tocopherol-containing oil suspension of lycopene from *B. trispora* as a novel food ingredient (EFSA, 2005a) and in the previous opinions on the use of α-tocopherol-containing oil suspensions and cold water dispersible forms of lycopene from *B. trispora* as a food colour (EFSA, 2005b, EFSA 2008a).

Table 1 presents the product specifications of lycopene crystals, the 10 % CWD product and the 20 % CWD product as provided by the applicant. The applicant indicates that the product specifications for lycopene crystals are similar to those established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2006b), with the exception of the following parameters: identification test for carotenoids and loss on drying, which are not included in the product specifications outlined below.

The major difference between the lycopene 10 % and 20 % CWD products and lycopene crystals is the % of lycopene and the residual amount of methylene chloride and octenyl succinic anhydride (OSA)-starch present in the CWD products. Methylene chloride is used as a solvent in the preparation of lycopene 10 and 20 % CWD products. Methylene chloride, meeting food grade specifications, is permitted for use as an extraction solvent for lycopene extracted from tomatoes under EU Directive 95/45/EC (Commission of the European Communities, 1995). OSA starch, meeting food grade specifications, is used in accordance with EU Directive 95/2/EC (European Parliament and the Council of the European Union, 1995).

Furthermore the lycopene formulated into the 10 % and 20 % CWD products is prepared in a way identical to lycopene form *B. trispora* formulated into the α-tocopherol-containing oil suspension previously evaluated (EFSA 2005a; EFSA 2005b).

Table 1. **Product specifications of lycopene crystals, the 10 % CWD product and the 20 % CWD product as provided by the applicant**

Parameter	Lycopene crystals	Lycopene 10 % CWD product	Lycopene 20 % CWD product
Solubility (1 % in chloroform)	clear	clear	clear
Identification (spectrometry: max in hexane)	ca 472	ca 472	ca 472
Assay (%)(472 nm)	≥ 95	≥ 10	≥ 20
Total lycopene (%)	≥ 95		
Trans-lycopene (%)	≥ 90		
Subsidiary colouring matters (%)	≤ 5		
Sulphated ash (%)	≤ 1		
Loss on drying (%)		≤ 8	≤ 8
Imidazole (mg/kg)	≤ 1		
Isopropanol (%)	≤ 1		

Isobutyl acetate (%)	≤ 1		
Methylene chloride (mg/kg)		≤ 10	≤ 10
Arsenic (mg/kg)	≤ 1	≤ 1	≤ 1
Lead (mg/kg)	≤ 1	≤ 1	≤ 1
Mercury (mg/kg)	≤ 1	≤ 1	≤ 1
Cadmium (mg/kg)	≤ 1	≤ 1	≤ 1

Analysis of representative batches of the lycopene crystals and the resultant lycopene CWD products as provided by the applicant demonstrate that the manufacturing process and final product formulation are both reproducible and capable of producing materials that meet the specifications.

II. Effect of the production process applied to the NF

The manufacturing process is carried out in two phases: the fermentation step and the isolation of the biosynthesised product via extraction followed by crystallization. Details of these steps are described in the Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to an application on the use of α-tocopherol-containing oil suspension of lycopene from *B. trispora* as a novel food ingredient (EFSA, 2005a).

The lycopene CWD product is a dark red powder, which is formed when the crystals are mixed under nitrogen and at controlled temperature, with α-tocopherol in methylene chloride, which is then mixed with an octenyl succinic anhydride (OSA)-starch–solution to form a homogeneous emulsion and then dried. A subsequent evaporation and a washing step reduce the content of methylene chloride to trace levels.

Due to its chemical structure (*i.e.*, long chain of conjugated carbon-carbon double bonds), lycopene is susceptible to chemical changes such as isomerisation and degradation when exposed to light and heat (Lee and Chen, 2002). To overcome the stability issues, lycopene production, including the recovery, formulation, and packaging processes of lycopene from *B. trispora* are carried out in the dark, at controlled temperature and under nitrogen atmosphere conditions. Manufacturing and further processing of lycopene is carried out in a continuous process in which lycopene crystals are not accumulated but immediately α-tocopherol is added as an antioxidant to the lycopene CWD product.

Stability trials with α-tocopherol-containing 10 % or 20 % CWD products of lycopene from *B. trispora* have been described in detail by the applicant. Stability was evaluated at 25°C ± 2°C and 60 % ± 5 % relative humidity (RH), at 40°C ± 2°C and 75 % ± 5 % RH, and under conditions of intended use at 5°C ± 3°C. Testing was conducted for periods of up to 6 months. The results of the stability trials indicate the stability of lycopene in a CWD formulation under all conditions tested.

The applicant indicates that since lycopene produced from *B. trispora* is similar to that which occurs naturally in food, it is expected that any breakdown products derived from the lycopene from *B. trispora* in CWD formulations are similar to those that would occur naturally. However, the presence of macromolecules in a food system have been suggested to offer additional protection for lycopene (Lee and Chen, 2002); therefore, the lycopene stability data presented above are expected to be a conservative representation of the stability of lycopene under the proposed conditions of intended use.

The stability of the lycopene in food is supported by a stability experiment conducted with rat feed containing lycopene at 0, 0.25, 0.50, and 1.0 % (Jonker *et al.*, 2003). For each

concentration level, diets were sampled immediately after preparation, following storage for 1 and 4 days at room temperature, and following storage for 7, 14, and 29 days at < -18°C. Based on measured lycopene concentrations following storage under the aforementioned conditions (1 to 4 days at room temperature, and 7 to 29 days at <-18°C), lycopene was considered to be stable in the rat feed at levels of 0.25, 0.50, and 1.0 % in the prepared diets (Jonker *et al*., 2003). The levels of lycopene in the feed are 500 to 2,000 times higher than the proposed use levels for lycopene (5 mg/kg) as a CWD product.

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

Details on the history of the organism used as the source of the NF are described in the Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to an application on the use of α-tocopherol-containing oil suspension of lycopene from *B. trispora* as a novel food ingredient (EFSA, 2005a).

IX. Anticipated intake/extent of use of the NF

The petitioner indicated that lycopene CWD products are intended for use as a nutritional food ingredient. The individual proposed food-uses for lycopene CWD products from *B. trispora* in the EU are summarized in Table 2.

Table 2. **Summary of the individual food uses and use-levels for lycopene (as CWD) proposed by the applicant**

Food category	Food use	Use-Level (mg/kg)
Beverages	Fortified juice mixtures	5
	Soft drinks	5
Cereal products	Biscuits	5
	Buns, cakes, and pastries	5
	Cereal bars	5
	Fruit pies	5
	Pudding powder	5
Fruit products and nut products	Pie and pastry fruit filling	5
Milk products	Fruit preparations for fromage frais	5
	Fruit preparations for yoghurt	5
Sugar products, preserves ad confectionary products	Bakery fillings	5
	Chocolate confectionary	5
	Sugar confectionary	5

The applicant provided estimates for the intake of lycopene (as CWD) by the UK population generated and collated by computer, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of lycopene (as CWD) represent projected 7-day averages for each individual from days 1 to 7 of NDNS data. T distribution from which mean and percentile intake estimates were produced was comprised of these average amounts. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.* when using youth data to estimate intakes) in order to provide representative intakes for specific UK population groups. All-person intake refers to the estimated intake of lycopene (as CWD) averaged over all individuals surveyed regardless of whether they consumed food products in which lycopene (as CWD) is currently proposed for use, and therefore includes “zero” consumers [those who reported no intake of food

products containing lycopene (as CWD) during the 7 survey days]. All-user intake refers to the estimated intake of lycopene (as CWD) by those individuals consuming food products in which the use of lycopene (as CWD) is currently under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which lycopene (as CWD) are proposed for use on one of the 7 survey days.

Mean and 95th percentile intake estimates based on sample sizes of less than 30 and 160, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of estimates for the intake of lycopene (as CWD) based on the consumption of these foods may be questionable for certain individual population groups.

Table 3 summarizes the estimated total intake of lycopene (as CWD) (mg/person/day) from all proposed food-uses by UK population groups, while Table 4 presents the data on a per kilogram body weight basis (mg/kg bw/day). The percentage of users was high among all age groups evaluated in the current intake assessment as would be expected for a 7-day survey. Greater than 90.9 % of the population groups consist of users of food products in which lycopene (as CWD) is currently proposed for use (Table 2).

Table 3. **Summary of the estimated daily intake of lycopene (as CWD) from all proposed food categories in the UK by population group (NDNS data)**

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-Users Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ - 4½	98.7	1,626	2.13	4.08	5.11	2.14	4.08	5.11
Young People	4-10	99.5	833	3.41	6.00	7.23	3.42	6.00	7.23
Female Teenager	11-18	97.8	436	2.68	5.16	6.57	2.67	5.26	6.73
Male Teenager	11-18	99.3	413	3.54	6.89	8.16	3.54	7.00	8.16
Female Adults	16-64	91.0	872	1.31	3.20	4.17	1.35	3.25	4.19
Male Adults	16-64	90.9	696	1.55	3.90	5.33	1.62	3.97	5.39

Table 4. **Summary of the estimated daily intake (in mg per kg bw) of lycopene (as CWD) from all proposed food categories in the U.K. by population group (NDNS data)**

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption (mg per kg bw)			All-Users Consumption (mg per kg bw)		
				Mean	Percentile		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ - 4½	98.7	1,626	0.15	0.28	0.36	0.15	0.29	0.37
Young People	4-10	99.5	833	0.13	0.25	0.31	0.14	0.25	0.31
Female Teenager	11-18	97.8	436	0.05	0.11	0.12	0.05	0.11	0.12
Male Teenager	11-18	99.3	413	0.07	0.13	0.16	0.07	0.13	0.16
Female Adults	16-64	91.0	872	0.02	0.05	0.06	0.02	0.05	0.06
Male Adults	16-64	90.9	696	0.02	0.05	0.06	0.02	0.05	0.06

The population group with the greatest percentage of users was that of young people at 99.5 %. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates, and as a consequence, only the all-user intake results will be discussed in detail.

Male teenagers were determined to have the greatest mean and 95th percentile all-user intakes of lycopene (as CWD) on an absolute basis of the individual population groups, with values of 3.54 and 8.16 mg/person/day, respectively (Table 3). Female adults had the lowest absolute intakes with mean and 95th percentile all-user intakes of 1.31 and 4.19 mg/person/day, respectively.

Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 95th percentile all-user lycopene (as CWD) intakes of 0.15 and 0.37 mg/kg body weight/day, respectively. Female and male adults had the lowest mean and 95th percentile intakes, at 0.02 and 0.06 mg/kg body weight/day (Table 4), respectively.

Consumption data and information pertaining to the individual proposed food-uses for lycopene (as CWD) were used to estimate the all-person and all-user lycopene (as CWD) intakes of specific demographic groups in the UK population. This type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the 4-day children's survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days (Gregory *et al*., 1995).

In summary, on an all-user basis, the highest mean and 95th percentile intakes of lycopene (as CWD) by the UK population, as observed in male teenagers, were estimated to be 3.54 mg/person/day (0.07 mg/kg body weight/day) and 8.16 mg/person/day (0.16 mg/kg body weight/day). On a body weight basis, children consumed the greatest amount of lycopene (as CWD), with mean and 95th percentile all-user intakes of 0.15 and 0.37 mg/kg body weight/day, respectively.

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 by the AFC Panel (EFSA, 2008a). 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. 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.

Previous opinions of the NDA Panel on the safety of lycopene oleoresin from tomatoes (EFSA 2008b) and the safety of synthetic lycopene (EFSA 2008c) evaluated intake from proposed novel food uses. An overview of all exposure estimates is presented in Table 5. It was concluded that intake from the proposed levels of novel food use would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and values that are for the high intake estimates substantially higher than the ADI recently established by EFSA (EFSA, 2008a) as a group ADI of 0.5 mg/kg bw/day from all sources.

Table 5. **Overview of lycopene exposure estimates from previous EFSA opinions (EFSA 2008a, b, c) and the present opinion**

Source of lycopene	Average (mg/day)	High (mg/day)	Reference
Naturally occurring	0.5 - 5	8 - 20	EFSA 2008a
Enriched foods	8 - 19	23 – 37	EFSA 2008c
Enriched foods	12	12	EFSA 2008b
Supplements	0 (no supplement use)	8-15	EFSA 2008b/2008c
Food Colour	2 - 6	11 – 23*	EFSA 2008
Enriched food CWD	1.3 - 3.5	4 - 8	Present opinion

* based on the 97.5th percentile intake estimates

Overall, the Panel concludes that intake from the proposed levels of use from CWD formulations with lycopene from *B. trispora* would lead to intake levels that will substantially increase the overall dietary intake of lycopene, and could lead to daily intakes from naturally occurring dietary lycopene and food colour use plus the uses proposed for CWD lycopene up to 9.8 to 14.5 mg/day at the mean and 23 to 51 mg/day as high intakes.

These values amount to 0.16 to 0.24 mg/kg bw/day and 0.38 to 0.85 mg/kg bw/day for a 60 kg person and can be, especially for the high intake estimates, higher than the ADI of 0.5 mg/kg bw/day recently established by EFSA (EFSA, 2008a) as a group ADI for lycopene from all sources, including lycopene occurring naturally in foods.

XI. Nutritional information on the NF

The lycopene from *B. trispora* has been considered by the Panel to be nutritionally equivalent to natural dietary lycopene (EFSA, 2005a). OSA-starch solution is used in the preparation of the 10 and 20 % CWD products. It is added to the lycopene crystal solution to form a homogeneous emulsion. OSA starch, meeting food grade specifications, is used in accordance with EU Directive 95/2/EC (European Parliament and the Council of the European Union, 1995).

It is expected that the lycopene 10 % and 20 % CWD products added to food are not nutritionally disadvantageous for the consumer compared to lycopene from other sources already on the market (EFSA, 2005a; EFSA, 2008b; EFSA, 2008c).

This Opinion does not include an assessment of the possible nutritional benefits of lycopene.

Bio-availability

There is no information on the bio-availability of lycopene from *B. trispora* lycopene 10 and 20 % CWD products. Taking into account that the lycopene from *B. tispora* in the lycopene 10 and 20 % CWD products is similar to lycopene from *B. trispora* when it is formulated in an α-tocopherol-containing oil lycopene suspension and considering the OSA-starch solution in which lycopene is suspended in the presented formulation would have effects similar to an average food matrix, it is expected that the bio-availability of lycopene from the 10 and 20 % CWD forms would not differ in a way that would cause safety concerns.

Previously the AFC Panel (EFSA 2008a) also concluded that it is expected that lycopene CWD from *B. trispora* when used in foodstuffs of comparable composition will be bio-available to a similar extent as lycopene from tomatoes.

XII. Microbiological information on the NF

The applicant provides data on batch testing for typical food-borne microbes. Moulds (≤ 100 fu/g), yeasts (≤ 100 fu/g), *Salmonella* (absent in 25 g), *Escherichia coli* (absent in 5 g) do not appear in the lycopene crystals or the 10 % and 20 % CWD products (in brackets the specifications for the analytical methods used).

XIII. Toxicological information on the NF

Toxicological studies on lycopene from various sources including *B. trispora* lycopene oil suspension and biomass, and CWD formulations of synthetic lycopene have been reviewed in previous EFSA opinions (EFSA 2005a, EFSA 2005b; EFSA 2008a, EFSA 2008b, EFSA 2008c). Based on these studies, JECFA established a group ADI for synthetic lycopene and lycopene from *B. trispora* of 0 - 0.5 mg/kg bw/day (JECFA, 2006a) and EFSA derived an ADI of 0.5 mg/kg bw/day for lycopene from all sources, including lycopene occurring naturally in foods (EFSA, 2008a).

Two studies, a bacterial mutation test and an *in vitro* chromosome aberration test, were conducted to evaluate the genotoxicity of lycopene 20 % CWD from *B. trispora* (CTBR, 2003a,b). Both studies were designed to meet the requirements of ICH (International Conference on Harmonisation Steering Committee, 1997) and other international regulatory agencies. The bacterial mutation test was conducted with the test article (maximum 5,000 µg/plate) and standard positive control agents in *Salmonella enterica* Typhimurium (strains TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* in either the absence or presence of S9 mix. The results indicated no significant increase in the revertant colony counts of any strain with or without S9. In addition, the effects of a range of lycopene concentrations and positive control agents on the incidence of chromosomal aberrations were evaluated in human lymphocytes with or without S9 mix. No statistically significant increases in the incidence of lymphocytes with chromosome damage were detected in cultures treated with lycopene 20 % CWD, whereas the positive control agents showed a highly significant increase in the numbers of chromosome aberrations, confirming the sensitivity of the system. Taking these two studies together it was concluded that lycopene 20 % CWD from *B. trispora* showed no evidence of genotoxic activity.

Based on the negative results reported in bacterial reverse mutation assays and a human lymphocyte chromosomal aberration test conducted on the lycopene CWD product from *B. trispora*, JEFCA had already concluded that there was no evidence of genotoxicity of lycopene from *B. trispora* (JECFA, 2006a).

The applicant indicates that it is unlikely that the micro-organism (*B. trispora*) would be present in the final crystals or CWD formulations given the purification process. Furthermore, no proteins were detected in samples of the high oleic sunflower oil, the 5 %, or the 20 % lycopene oil suspension, as determined by the Bradford assay (detection limit 1 µg protein/mL or 1 µg protein in 400 mg of lycopene oil suspension), which are made from the same lycopene crystals obtained from *B. trispora* as the 10 and 20 % CWD formulations, indicating a lack of allergenic potential

DISCUSSION

Lycopene from *B. trispora* is biosynthesised by the fungus *B. trispora* through the same pathway as lycopene produced in the tomato. The lycopene is formulated into α-tocopherol-containing 10 % and 20 % lycopene cold water dispersible (CWD) products, which are evaluated in the present opinion.

The lycopene CWD product is a dark red powder, which is formed when the crystals are mixed with tocopherol in methylene chloride, which is then mixed with an octenyl succinic anhydride (OSA)-starch solution to form a homogeneous emulsion and then dried.

There is no information on the bio-availability of lycopene from *B. trispora* lycopene 10 and 20 % CWD products, but it is expected that the bio-availability of lycopene from the 10 and 20 % CWD forms will not differ other lycopene formulations in a way that would cause safety concerns.

The Panel noted that the lycopene 10 and 20 % formulations are prepared in the presence of α-tocopherol as an antioxidant. Stability trials with α-tocopherol-containing 10 % or 20 % CWD products of lycopene from *B. trispora* have been described in detail by the applicant. The results of the stability trials indicate the stability of lycopene in a CWD formulation under all conditions tested. It is expected that any breakdown products derived from lycopene from *B. trispora* in the oil suspension or in the CWD product in the presence of α-tocopherol or in food would be identical to those that would occur with lycopene from other natural sources.

Overall, the Panel considers that the lycopene 10 % and 20 % CWD formulations are substantially equivalent to the lycopene from *B. trispora* evaluated in previous opinions (EFSA, 2005a, 2005b, 2008a).

The applicant proposes to use lycopene from *B. trispora* as a food ingredient in beverages, cereal products, fruit products, nut products, milk products, sugar and confectionary products and preserves at use levels of 5 mg/kg. The applicant provides an intake estimate based on these food uses.

On an all-user basis, the highest mean and 95th percentile intakes of lycopene (as CWD) by the U.K. population, as observed in male teenagers, were estimated to be 3.54 mg/person/day (0.07 mg/kg body weight/day) and 8.16 mg/person/day (0.16 mg/kg body weight/day). On a body weight basis, children consumed the greatest amount of lycopene (as CWD), with mean and 95th percentile all-user intakes of 0.15 and 0.37 mg/kg body weight/day, respectively.

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 go up to 23 mg at the high level (AFC, 2008). 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 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 daily intakes from naturally occurring dietary lycopene and food colour use plus the uses proposed for CWD lycopene up to 9.8 to 14.5 mg/day at the mean and 23 to 51 mg/day as high intakes. These values amount to 0.16 to 0.24 mg/kg bw/day and 0.38 to 0.85 mg/kg bw/day for a 60 kg person and can be, especially for the high intake estimates, higher than the ADI of 0.5 mg/kg bw/day recently established by EFSA (EFSA, 2008a) as a group ADI for lycopene from all sources, including lycopene occurring naturally in foods.

CONCLUSIONS AND RECOMMENDATIONS

The Panel considers that the submitted CWD formulations of lycopene from *B. trispora* are as safe as lycopene from other sources previously assessed by EFSA.

The Panel concludes that for the average user consumption of lycopene 10 and 20 % CWD and from all other sources will be below the ADI. However, some users of lycopene products might exceed the ADI of 0.5 mg/kg bw/day.

Lycopene preparations intended for use in foods and food supplements are formulated as suspensions in edible oils, direct compressible or water-dispersible powders. As lycopene may undergo oxidative changes in such formulations, sufficient anti-oxidative protection should be ascertained.

DOCUMENTATION PROVIDED TO EFSA

Lycopene Cold Water Dispersible Products (CWD) from *Blakeslea trispora*' as novel food ingredients (September 2008), submitted by Vitatene S.A., Spain.

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