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 *Blakeslea trispora* as a novel food ingredient

(Request N° EFSA-Q-2004-169)

(adopted on 21 April 2005)

SUMMARY

EFSA has been asked to evaluate lycopene, obtained from *Blakeslea trispora*, for use as a novel food ingredient.

Lycopene is biosynthesised by the fungus *B. trispora* through the same pathway as lycopene produced in the tomato. The predominant lycopene isomer in the material is all-*trans* lycopene. The lycopene product is formulated into a 20% or 5% sunflower oil suspension with α-tocopherol at 1% of the lycopene level.

There is no information on the bioavailability of lycopene from the α-tocopherol-containing oil suspensions of lycopene from *B. trispora*. Results from human intervention studies with tomato extract as lycopene source indicate that lycopene from this source is bioavailable. It is expected that lycopene from *B. trispora* when used in foodstuffs of comparable composition is bioavailable to a similar extent.

The lycopene from *B. trispora* is considered by the Panel to be nutritionally equivalent to natural dietary lycopene. However, the Panel did not carry out an assessment of the possible nutritional benefits of lycopene.

Average dietary intakes of lycopene from foods in different populations are, according to dietary surveys, estimated to be between 0.5 and 5 mg/day, with high intakes up to about 8 mg/day. High intakes of fruit and vegetables, especially tomato products, may result in occasional intakes of 20 mg/day or more. The applicant's proposed use levels of lycopene from *B. trispora* in foodstuffs would lead to an additional intake of up to about 2 mg/day. The proposed use level of lycopene in food supplements would give rise to an additional intake of 20 mg/day.

To date, no long-term feeding studies conducted with lycopene extracted from the microorganism *B. trispora* have been performed. The toxicological data on α-tocopherol-containing oil suspensions of lycopene from *B. trispora* (90-day oral feeding study) are not sufficient to derive an acceptable daily intake (ADI).

It is concluded that α-tocopherol-containing oil suspension of lycopene, obtained from *B. trispora*, for use as a novel food ingredient in foodstuffs leading to an additional intake of up to about 2 mg/day is not of concern from the safety point of view. However, 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.

KEY WORDS

Lycopene, novel food ingredient, CAS Registry Number 502-65-8.

BACKGROUND

In December 2003 the company Vitatene submitted a request for placing on the market in the EU lycopene from *Blakeslea trispora* as a novel food ingredient to the authorities of the United Kingdom.

On 6 April 2004, the authorities of the United Kingdom forwarded to the Commission their initial assessment report of the product concerned carried out by the Advisory Committee on Novel Foods and Processes (UK), which had reached the conclusion that this lycopene was acceptable as a food ingredient.

In accordance with Article 6(4) of the Novel Foods Regulation, the Commission forwarded the initial assessment report to Member States on 27 April 2004. Member States submitted their comments and/or presented reasoned objections within the 60 day period provided for the authorisation procedure.

It emerged that views differed on the issue. Several Member States supported the conclusion of the initial assessment report carried out by the authorities of the United Kingdom. However, some Member States opposed the same conclusion, raising scientific concerns with regard to the risk assessment and/or claiming lack of relevant data.

In view of the divergent opinions of the Member States and the Community interest in this matter, the European Commission has decided to seek the opinion of EFSA.

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

- Details on the production process, the absence of harmful substances formed during manufacturing and storage and the specifications of the final product should be clarified.

- A clear distinction should be made between the assessment of the safety of lycopene used as an ingredient added to foodstuffs, and that of lycopene used in food supplements. This is because the level proposed for food supplement use (20 mg per day) is expected to be higher than the normal intake.

- There should be clarification regarding the nutritional benefit of adding lycopene to the diet.

- There were insufficient toxicology studies and it is inappropriate to transfer the results of studies on lycopene products derived from tomatoes, which contain a high proportion of other naturally occurring compounds.

- Comparative studies were required regarding the bioavailability of lycopene obtained from the fungus *B. trispora*, along with a study of some factors that may possibly

contribute to its bioavailability and an evaluation of its interaction with other carotenoids present in the diet.

- No test for genetic mutation in mammalian cells has been conducted and in addition, the test material used in the tests for genetic mutations in bacteria and for chromosome aberrations in human lymphocytes is not consistent with the formulation that is to be used in foods and food supplements.

- An issue was raised regarding the possibility of lycopene increasing the incidence of lung tumours in heavy smokers.

- A complete risk assessment of the intake of lycopene from all sources should be made.

- Finally, it was considered that in order to adequately inform the consumer there should be an additional labelling indication that lycopene is obtained from the *B. trispora* fungus.

In addressing these issues and considering the overall safety of lycopene from *Blakeslea trispora* 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 and the response from the applicant to the issues raised by the Member States.

Existing authorisations and evaluations

Lycopene, extracted from tomatoes, is authorised as 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 (SCF, 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 does not differ significantly from the amount consumed through the relevant foodstuffs. 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 allow for an acceptance. 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 is unacceptable at present."

Synthetic lycopene is currently not approved for colouring matters within the EU and it is considered Generally Recognised as Safe (GRAS) for use as a food ingredient in the US (GRAS notice No GRN 000119).

An application for approval of lycopene from *B. trispora* as a food colour additive has recently been filed with the European Commission and EFSA.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) Nº 178/2002, the European Commission requests the European Food Safety Authority to issue a scientific opinion on the use of lycopene from *Blakeslea trispora* as a food ingredient in the context of Regulation (EC) Nº 258/97.

The Authority is asked to specify whether the authorisation of lycopene from *Blakeslea trispora* as a food ingredient is likely to have an effect on public health and, in particular, to focus on the elements of a scientific nature in the comments/objections raised by the Member States to the Initial Assessment Report.

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.

I.   Specification of the novel food (NF)

Lycopene is a carotenoid with the formula $C_{40}H_{56}$. It has a molecular mass of 536.85 and the CAS Registry Number of all-*trans* lycopene is 502-65-8. Its structural formula is:
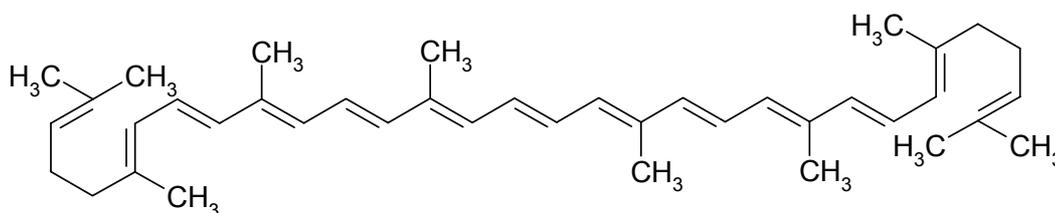
Lycopene occurs in food predominantly in an all-*trans* form (Cronin 2000; Boileau *et al.*, 2002). It is soluble in fats and some organic solvents, but virtually insoluble in water, methanol and ethanol.

Blakeslea trispora is a fungus found on a number of tropical plants, and strains of *B. trispora* are able to synthesise large quantities of carotenoids. Lycopene is biosynthesised by the fungus through the same pathway as lycopene produced in the tomato. Specifications of lycopene obtained from dry, milled biomass of the fungus *B. trispora* have been provided by the applicant. They indicate that the lycopene crystals contain not less than 95% lycopene of which at least 90% is *trans*-lycopene. The remainder consists of a number of low level contaminants such as the extraction solvent, isobutyl acetate (not greater than 1%), and other colouring materials (not greater than 5%). Sulphated ash was not greater than 1%. In the final product lycopene is formulated as 20% or 5% lycopene in high oleic acid sunflower oil

suspension with α-tocopherol at 1% of the lycopene level to minimise oxidation. The novel food will be available in this oil suspension form (5% and 20%) only.

Table 1 presents a comparison of the chemical compositions of synthetic lycopene, lycopene from *B. trispora* and lycopene from tomatoes.

Table 1.   Comparison of the chemical compositions of synthetic lycopene, lycopene from tomatoes and lycopene from *B. trispora*

	Synthetic lycopene*	**Lycopene from tomatoes***	**Lycopene from *B. trispora***
Purity	≥ 96%	≥ 5% of total colouring matters	≥ 95%
Impurities, other pigments	Up to 0.3% of C_{25} aldehyde	Other pigments, oils, fats, waxes and natural flavours	Other carotenoids
All-*trans* isomer	>70%	94-96%	≥ 90%
5-*cis* isomer	<25%	3-5%	1-5%
9-*cis* isomer	<1%	0-1%	
13-*cis* isomer	<1%	1%	
Other cis-isomers	<3%	<1%	
Formulation	10% lycopene with ascorbyl palmitate (5%) and α-tocopherol (1.5%)	Oleoresin: 2-3% lycopene Powder: 5% lycopene	5 or 20% oil suspension with α-tocopherol (1% of lycopene level)

* SCF, 1999.

The applicant also carried out mycotoxin assays to determine whether aflatoxin B1, mycotoxin T2, ochratoxin and zearalenone were present. The results, for both crystalline and oil suspended lycopene were all negative.

II.   Effects of the production process applied to the NF

The manufacturing process has been described by the applicant and is carried out in two phases: the fermentation step and the isolation of the biosynthesised product via extraction.

The two mating type strains of the fungus *B. trispora* used in the fermentation phase for the production of lycopene are the same as those approved for the production of β-carotene. The two strains are grown separately in fermentation tanks in sterile culture media and then combined into one tank for the co-fermentation step and lycopene production. The biosynthesis of lycopene proceeds via the same pathway as that involved in the formation of β-carotene. However in the co-fermentation tank imidazole or pyridine is added to the culture broth because these compounds are known to inhibit the enzyme lycopene cyclase. This results in the accumulation of lycopene because the biosynthetic steps leading to the production of β-carotene cannot proceed, since there is no cyclation of the two β-ionone rings (Lopez Nieto *et al.*, 2004).

The extraction and purification processes are identical to those used in the production of β-carotene from *B. trispora*, which have been examined and approved by the SCF (SCF, 2000).

Following completion of the fermentation, isobutyl alcohol is added to the culture to remove lipophilic substances and oils from the fungal cell walls. This improves the purity of the lycopene in the final product. The biomass is separated from the broth/isobutyl alcohol by centrifugation and the solvent content of the biomass is further reduced using a turbo or vacuum drier which simultaneously mills the biomass. Lycopene is extracted from the dry, milled biomass with isobutyl acetate and the lycopene-rich solvent is separated from the biomass by centrifugation. Isobutyl acetate has recently been considered safe and acceptable by the SCF for use as an extraction solvent in the production of β-carotene from *B. trispora* (SCF, 2003). The lycopene concentration is increased by vacuum distillation. The crystallisation of lycopene (i.e. its removal from solution) is completed using isopropyl alcohol. The lycopene suspension is filtered, washed with isopropyl alcohol and dried under vacuum or nitrogen atmosphere at a temperature of less than 30°C. The final lycopene product is formulated as an oil suspension prior to packaging.

Due to the chemical structure 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). To overcome these stability issues, all the recovery, formulation, and packaging processes are carried out in the dark, at controlled temperature and under nitrogen atmosphere conditions. It is a continuous process in which lycopene crystals are not accumulated but immediately suspended in high oleic acid sunflower oil containing α-tocopherol.

Stability of the α-tocopherol-containing lycopene oil suspensions

The stability of 5% and 20% lycopene oil suspensions containing α-tocopherol, stored in aluminium bottles filled to the top without air displacement by nitrogen, has been evaluated by the applicant. Stability was evaluated at 25°C ± 2°C and 60% ± 5% relative humidity (RH) for 3, 6 and 12 months, at 40°C ± 2°C and 75% ± 5% RH for 0.5, 1, 3 and 6 months, and under conditions of intended use at 5°C ± 3°C for 3, 6 and 12 months. In addition the stability of lycopene 20% oil suspension, used during the sub-chronic toxicity study (Jonker *et al.*, 2003) and stored in an aluminium bottle filled to the top without air displacement by nitrogen, has been evaluated under conditions of intended use at 5°C ± 3°C over a 2.5 year period.

In all stability trials the stability of the lycopene oil suspensions was judged on the basis of comparison of the initial level of lycopene as compared to the residual amount of lycopene quantified by HPLC.

The results of the stability trials including that of a batch of material continually monitored (same container opened several times) over a 2.5 year period, indicates the stability of lycopene when stored in oil containing α-tocopherol and kept refrigerated. A product stored at 40°C ± 2°C and 75% ± 5% RH for 6 months likewise provided evidence of the stability of lycopene when stored in oil containing α-tocopherol.

It is expected that any breakdown products derived from lycopene from *B. trispora* in the oil suspension in the presence of α-tocopherol would be identical to those that would occur with lycopene from other natural sources.

The stability of the lycopene oil suspension 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 up to 4 days at room temperature, and following storage for up to 4 weeks in the freezer at <18°C. Based on measured lycopene concentrations following storage under the aforementioned conditions, lycopene was considered to be stable in the rat feed of 0.25, 0.50 and 1.0% in the prepared diets (Jonker *et al.*, 2003).

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

The current taxonomic placement of *B. trispora* is as follows: Kingdom Fungi; Phylum Zygomycota; Class Zygomycetes; Order Mucorales; Family Choanephoraceae; Genus Blakeslea and Species trispora.

Information to support the safety of the source organism and that the resultant "foods" obtained are not detrimental to human health has been provided both by the SCF (SCF, 2000) and JECFA (JECFA, 2001).

The SCF concluded that β-carotene from *B. trispora*, produced via a similar biosynthetic route and process, as lycopene from *B. trispora* is acceptable for use as a colouring agent for foodstuffs (SCF, 2000). A similar position regarding β-carotene from *B. trispora* has been taken by the JECFA (JECFA, 2001), who concluded that β-carotene isolated from different sources, including *B. trispora*, is acceptable for food additive use.

Lycopene is produced by co-fermentation using the two mating type strains VKPM F-744 (-) and VKPM F-816 (+) of the fungus *B. trispora*. According to the SCF (SCF, 2000) the mould has been shown to be non-pathogenic and non-toxigenic, by a literature search, a standard pathogenicity experiment in mice, and by analysis of extracts of several fermentation mashes for fungal toxins and of the final product, the β-carotene crystals, by enzyme immunoassays for 4 mycotoxins. The strains can also be considered to be non-toxigenic and non-pathogenic on the basis of a 28-day oral feeding study conducted with the biomass for lycopene production (Jonker, 2000). Furthermore, *B. trispora* belongs to risk group 1 of the German "Gentechnik-Sicherheitsverordnung" (Regulations on the Safety of Gen-technology) (Robert Koch Institute, 2002), which is comprised of microorganisms that present no risk for humans and other vertebrates.

IX.   Anticipated intake and extent of use of the NF

Lycopene oil suspension is intended for use as a food ingredient in fat spreads (2.0-5.0 mg/kg), milk and milk products (3.0-6.0 mg/kg), condiments, seasonings, relishes and pickles (all at 6.0 mg/kg), mustard (5.0 mg/kg), savoury sauces and gravies (7.0 mg/kg), soups and soup mixes (6.0 mg/kg) and sugar, preserves and confectionary (5.0 mg/kg), and dietary supplements. The proposed level of use for lycopene in food supplements is 20 mg per day.

Lycopene provides the familiar red colour to tomato products and is one of the major carotenoids in the diet of Europeans and North Americans (Clinton, 1998). Lycopene is also present in watermelon and pink grapefruit (Reed Mangels *et al.*, 1993; O'Neil *et al.*, 2001).

O'Neil *et al.* (2001) compared the carotenoid intakes in five European countries (France, Republic of Ireland, Spain, The Netherlands and UK) of 70 to 76 individuals between 25 and 45 years of age in each country. Lycopene intake was estimated by a food frequency

questionnaire and a carotenoid database. Median intakes per country ranged from 1.64 mg (Spain) to 5.01 mg (UK) per day. High intakes ranged from 2.64 mg (Spain) to 8.31 mg (France) per day. The authors state that this food frequency questionnaire method probably overestimates the intake (O'Neil *et al.*, 2001). Goldbohm *et al.* (1998) published another intake estimate for lycopene, based on a semi-quantitative food frequency questionnaire collected within in the Dutch Cohort Study on diet and cancer in 1986 among subjects aged 55-69 years. The mean lycopene intake was 1.0 mg and 1.3 mg per day for men and women, respectively. Corresponding high intakes (97.5 percentile, calculated from the SD) were 4.2 mg and 5.1 mg per day, respectively. In a British study conducted with elderly females the daily consumption of lycopene-rich food including tomatoes, was reported to result in a daily lycopene intake of 1.07 mg/day per person (Scott *et al.*, 1996). Müller analysed the lycopene content of 27 daily duplicate diets collected from a canteen in Germany in 1981 (Müller, 1996). The average content was 0.55 mg lycopene per day (median 0.12 mg/day), with individual daily diets containing up to 7.08 mg/day. In a Finnish population study covering the dietary habits during 1967-72, the mean daily intakes of lycopene were calculated to be 0.70 mg/day for females and 0.87 mg/day for males on the basis of a dietary history interview (Järvinen, 1995).

The applicant also provided an evaluation of the estimated daily intake of lycopene from natural sources in France using French data derived from the Institute National de la Recherche Agronomique (INRA). The INRA food consumption survey data were based on household purchases (Combris *et al.*, 1998). Data were collected weekly from each of over 3,000 households and the results averaged over one year. The human intake estimate based upon the INRA data that are based on consumption of tomatoes and grapefruit products gave an average of 4.8 mg/day.

Intake estimations from countries outside Europe, e.g. Australia, Canada and the US, show mean lycopene intakes in the range of 1.9-6.3 mg/day, with medians considerably lower (Manzi *et al.*, 2002; Johnson-Down *et al.*, 2002; Nebeling *et al.*, 1997; Forman *et al.*, 1993; Yong *et al.*, 1994). Most of these studies used food frequency questionnaires and cover either the general adult population or selected population groups such as premenopausal women, elderly and males. In studies of 57 US males (Forman *et al.*, 1993) and 98 females (Yong *et al.*, 1994), lycopene intake was assessed with both a food frequency questionnaire and a 7-day dairy. Calculated mean intakes from the two dietary assessment methods were similar, amounting to 3.9 and 3.7 mg/day, respectively, in men and 3.4 and 3.1, respectively, in women.

The applicant refers to intake estimations in North America showing that the intakes of lycopene from natural sources could be higher than the reported intakes, namely up to 11.3 mg/person/day in the US (McGirr and Copeland, 2000), and up to 25 mg/person/day in Canada (Argawal *et al.*, 2001). Tomato products are rich in lycopene and regular consumption of such foods may therefore result in high intakes in individual subjects (Lee *et al.*, 2000) (Table 2).

The applicant also argues that the calculated intakes from natural sources in France using the INRA food consumption survey in combination with the more recent results from North America, indicate that the proposed level of use for lycopene in food supplements (20 mg per day) may not in fact be considerably higher than normal dietary intake, especially in those individuals consuming tomato juice.

Table 2. Concentration of lycopene in tomatoes, tomato-based food products, and several fruits and vegetables (Khachik *et al.*, 2002)

Food type	Lycopene concentration in mg/100g
Tomato paste	55.45
Tomato sauce	17.98
Catsup	17.23
Tomato puree	16.67
Spaghetti sauce	15.99
Tomato juice	10.99
Vegetable juice	9.66
Tomato	9.27
Grapefruit pink	3.36
Papaya	2.52
Vegetarian vegetable soup	1.93
Minestrone soup	1.48

An increased consumption of fruit and vegetables can potentially result in considerably higher lycopene intakes than the current. In the DASH study (Most, 2004) the calculated lycopene content of the DASH diet was on average 3.3 mg per 1000 kcal, corresponding to about 7-9 mg per day for an adult women and man. Using values for the SD of the lycopene content of the DASH diet, theoretical estimated high intakes (97.5 percentile) could be up to 17-22 mg per day. However, these calculations are based on an average consumption of 4-5 servings of fruit and 4-6 servings of vegetables per day, which is far from the current situation in Europe.

The calculations from the DASH study indeed indicate that lycopene intakes from dietary sources could be substantially higher than current intakes, but this requires that the consumption of fruit and vegetables be increased several fold. Although certain individual foods may contain high levels of lycopene, available dietary data indicate that habitual intakes above 10 mg/day are rare.

It is stated by the applicant that supplements containing lycopene from other sources are currently on the market in the EU, and that it is likely that lycopene from *B. trispora* in food supplements would replace those supplements already being consumed. In that case overall consumption levels would not increase. Incorporation of lycopene into foods would result in additional intake.

Overall, the Panel concludes that average dietary intakes of lycopene in different populations are estimated to be between 0.5 and 5 mg/day, with high intakes up to about 8 mg/day. Individuals consuming large amounts of fruit and vegetables, especially tomato products, may occasionally have intakes up to about 20 mg/day or more, but these estimates are mainly based on experimental diets and do not reflect habitual intakes.

The applicant also provided estimated daily intakes of lycopene from *B. trispora* in the UK, based on the proposed use levels for use in novel foods, and food consumption data from the UK Food Standard Agency's DSP. In this estimate, proposed maximum use levels for all foods to which lycopene may be added were used to predict the potential intake. The dietary intake for children (1.5-4.5 years), young people (4-10 years), male and female teenagers and male and female adults were estimated. Given that the proposed range of foodstuffs is wide,

the applicant noted that the percentage of potential users was high amongst all age groups (>98%).

According to the applicant, the mean estimated daily intakes calculated for the different age groups were 0.22 mg/day (children), 0.37 mg/day (young people), 0.40 mg/day (female teenagers), 0.42 mg/day (male teenagers), 0.46 mg/day (female adults) and 0.60 mg/day (male adults). The estimated 97th percentile daily intakes amounted to 0.65 mg/day (children), 0.93 mg/day (young people), 1.02 mg/day (female teenagers), 1.18 mg/day (male teenagers), 1.23 mg/day (female adults) and 1.68 mg/day (male adults). According to the applicant, these figures may overestimate the actual consumption because they are based on the assumption that consumers always select foods that are fortified at the maximum level. Based on these data, the applicant concludes that average intake levels from use of lycopene as a food ingredient in food would be in the same range as the levels of lycopene consumed in general foodstuffs.

Overall, the Panel concludes that intake from the proposed levels of use would lead to intake levels that will not substantially increase the overall dietary intake of lycopene, except for the proposed level of use for lycopene in food supplements that amounts to 20 mg per day and could lead to an increase in dietary intake leading to daily intakes that exceed the normal range.

XI.   Nutritional information on the NF

The lycopene from *B. trispora* is considered by the Panel to be nutritionally equivalent to natural dietary lycopene. Since the other components of the final lycopene oil suspension (α-tocopherol and oleic acid sunflower oil) are similarly present in food, it is expected that the lycopene oil suspension derived from *B. trispora* is nutritionally equivalent to naturally occurring lycopene.

Unlike some of the carotenoids, lycopene cannot be converted to vitamin A due to its lack of a β-ionone ring structure (Agarwal and Rao, 2000; Rao and Agarwal, 2000). However, its consumption has been claimed to provide nutritional and health benefits beyond those associated with vitamin A precursors (Nguyen and Schwartz, 1999). Lycopene is considered one of the most potent antioxidants among the carotenoids due to its singlet-oxygen quenching ability (Di Mascio *et al.*, 1989; Khachik *et al.*, 1997; Agarwal and Rao, 2000; Rao and Agarwal, 2000). Although non-oxidative mechanisms of effects observed for lycopene have been identified (i.e. gene function regulation, regulation of gap-junction communication, hormone and immune modulation, and regulation of metabolism), the antioxidant properties are currently proposed to be the basis for lycopene's potential health benefits, such as prevention of cardiovascular disease and cancer (Agarwal and Rao, 2000; Rao *et al.*, 2002; Giovannucci, 1999; Rissanen, 2002; Arab and Steck, 2000).

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

Bioavailability

There is no information on the bioavailability of lycopene from the α-tocopherol-containing oil suspensions of lycopene from *B. trispora*.

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 bioavailability of these forms of lycopene is presented hereafter.

In toxicokinetic studies in rats and monkeys, less than 10% of an orally administered dose of radiolabelled 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 bioavailability 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. Three studies used tomato extracts as lycopene source. A 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 of Hininger *et al.* (2001) 175 healthy male volunteers received 15 mg lycopene (from tomato extracts) or placebo during 3 months. Plasma lycopene concentrations increased by 0.54 µmol/L and LDL lycopene concentrations increased by 65 ng/mg LDL cholesterol in the lycopene supplemented group. Hoppe (2003) described a study aimed at determining the relative bioavailabilities 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 from 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 the range of 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 oxidative 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 applicant provides data that indicate that typical food borne microbes (e.g. moulds, yeasts, *Salmonella, Escherichia coli*) do not appear in the lycopene crystals, lycopene 20% oil suspension, and lycopene 5% oil suspension products.

XIII.   Toxicological information on the NF

The available toxicity studies are in most cases carried out with synthetic lycopene (Zbinden and Studer, 1958; Mellert *et al.*, 2002), or unspecified material (Milani *et al.*, 1970) and therefore to some extent difficult to extrapolate to lycopene from a natural source and, thus, lycopene from *B. trispora*.

Acute oral toxicity

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

Subacute and semi-chronic toxicity

Lycopene-rich biomass obtained from *B. trispora* was used in a semi-chronic 28-days toxicity study. The Advisory Committee for Novel Foods and Processes in the UK summarised the results of this study of the applicant and presented conclusions, as follows:

> "Four groups of 40 rats (20 per sex) were exposed to 0 (control), 0.1, 0.3 or 1% lycopene in the total diet. These percentages correspond to daily doses of 90, 272, and 906 mg/kg body weight in males and 87, 260 and 868 mg/kg body weight in females, respectively. The lycopene-enriched diet was administered for 28 days. Clinical observations, neurobehavioural observations, growth, food consumption and food conversion efficiency were assessed throughout the study and haematology, clinical chemistry, organ weights and macroscopic and microscopic examinations were carried out at necropsy. No treatment-related differences were found in mean body weights and relative/absolute organ weights between the control and treatment groups. Food consumption and food conversion efficiency were also not adversely affected by the treatment. No treatment-related clinical signs or neurotoxic indications were found as

a result of the lycopene biomass administration. These were assessed using neurobehavioural observations and motor activity assessments. Haematological measurements showed a statistically significant decrease in mean corpuscular volume and prothrombin time in the high dose male group only. However no significant changes were noted for other red blood cell counts, packed cell volume or haemoglobin concentrations and the authors considered the decrease in mean corpuscular volume as an incidental finding and of no toxicological significance. The decrease in prothrombin times was found to be small (6%) and within the limits of historical controls. No adverse effects were noted in the clinical chemistry variables and macroscopic and microscopic examinations at necropsy revealed no treatment-related changes except a statistically significant decreased incidence of increased hyaline droplet nephropathy in the high dose male group. Again the authors of the study attached no toxicological significance to this finding."

The Advisory Committee for Novel Foods and Processes in the UK requested further information on the relevance of a significant change in the incidence of hyaline droplets in the semi-chronic study. The applicant responded to these comments highlighting that the increase in hyaline droplet nephropathy seen in male rats is not a toxicologically significant finding, noting that the mechanism of action is of no relevance to humans. The Panel was content with the applicant's conclusions.

In a 90-day oral toxicity study, conducted with oil suspensions of lycopene extracted from biomass of *Blakeslea trispora* (20% lycopene in sunflower oil) at dose levels of 0 (control), 0.25, 0.5 or 1.0% lycopene in the experimental diet, with 20 male and 20 female Wistar rats and performed in accordance with the OECD guidelines (Jonker *et al.*, 2003), it was concluded that dietary levels of lycopene up to 1.0% do not provide any evidence of toxicity. Lycopene intake was calculated to be 0, 156, 312 and 616 mg/kg body weight/day in control through high-dose females, and 0, 145, 291 and 586 mg/kg body weight/day in control through high-dose males. Parameters included clinical observations, neurobehavioural observations, motor activity assessments, body weight and food consumption measurements, ophthalmoscopic examinations, haematology, clinical chemistry, urinalysis, organ weights, gross pathology, or histopathology. Discoloration was limited to the contents of the gastrointestinal tract and did not extend to any tissue. The No Observed Effect level (NOEL) was 1% in the diet, the highest dietary concentration tested, that amounted to 616 and 586 mg/kg body weight/day for females and males, respectively. This was equivalent to a dose of 600 mg/kg body weight per day.

The applicant calculates, using the average NOEL of about 600 mg/kg body weight per day of lycopene from *B. trispora*, derived from the rat sub-chronic 90-day study, and the maximum level of intake from dietary use of 20 mg/day for a 60 kg person, a margin of safety of at least 1800. The applicant also points out that taking into account the fact that the NOEL from this 90-day study was actually the highest dose level tested, one may even conclude that the margin of safety with a daily intake of 20 mg/day for a 60 kg person would be greater than 1800.

Synthetic lycopene was well tolerated in rats at a daily dose of 1000 mg/kg body weight, for 100 consecutive days (only dose tested) (Zbinden and Studer, 1958). This study was evaluated by the SCF (SCF, 1999). No abnormalities were observed in blood parameters or histopathology.

Other semi-chronic oral administrations to experimental animals were reported not to cause any treatment-related adverse effects on body weight gain, food consumption, organ weights or behaviour. These studies include for example the following semi-chronic oral administrations:

- mice at concentrations of 0 (control) and 700 mg LycoRed lycopene/kg diet, corresponding to approximately 35 mg/kg body weight/day for 28 weeks (Black, 1998), in a study investigating the radical interception by carotenoids and their effects on UV carcinogenesis;

- rats at concentrations of 0 (control) and 300 mg lycopene oleoresin from tomato/kg diet, corresponding to approximately 15 mg/kg body weight/day, for 15 days (Gradelet *et al.*, 1996), in a study on the effects of various carotenoids on liver xenobiotic-metabolizing enzymes;

- rats at concentrations of 0, 50, 124, 248, 496 and 1240 mg tomato extracted lycopene (corresponding to approximately 0, 2.5, 6.2, 12.4, 25 or 60 mg/kg body weight/day) for 10 weeks (Zhao *et al.*, 1998), in a study investigating tissue disposition;

- rats at doses of 0 or 300 mg lycopene from a tomato oleoresin/kg diet, the latter corresponding to approximately 15 mg/kg body weight/day, for 16 days (Jewell and O'Brien, 1999), in a study on the effects of various carotenoids on xenobiotic-metabolizing enzymes in the liver, lung, kidney and small intestine;

- rats exposed to 0, 1, 5, 50 or 100 mg/kg body weight/day (gavage of a solution of pure lycopene in corn oil) for 14 days (Breinholt *et al.*, 2000), in a study on the effects of lycopene on selected drug-metabolizing and antioxidant enzymes; and

- rats exposed to 0, 50, 500 or 5000 mg lycopene from beadlets/kg food for 8 weeks, corresponding to approximately 0, 2.5, 25 and 250 mg/kg body weight/day (Boileau *et al.*, 2000) in a study on tissue and serum lycopene concentrations upon dietary administration.

- Exposure to 0.07 mg lycopene/kg body weight/day for 10 months did not adversely affect body weight, nor were there any adverse treatment-related changes in oestrus cycle, mammary gland growth, urine analyses, mammary gland thymidine kinase activity, or endocrine organ weights. Lycopene induced changes included reduced prolactin and free fatty acid levels and reduced thymidylate synthetase activity; however, these changes were considered beneficial and preventative against spontaneous mammary tumours and were therefore not considered toxic endpoints (Nagasawa *et al.*, 1995).

These studies were however not designed to examine adverse health effects of lycopene.

In another study (Mellert *et al.*, 2002), the 13-week oral toxicity of synthetic lycopene products was assessed in rats. The dose levels selected for this study were: 0, 500, 1500 and 3000 mg/kg body weight/day, as well as 3000 mg/kg body weight/day of the matrices used to formulate and stabilize each product. This study was performed according to OECD guideline 408 (OECD, 1998). No statistically significant or dose-related effects were observed. The only clinical finding at necropsy was the presence of red pigment in the faeces and gastrointestinal tract that was associated with the red-pigmented test materials.

In the SCF opinion on synthetic lycopene (SCF, 1999) more studies are reported some of them published in the open literature at later dates. A short summary of these studies reported by the SCF is as follows:

- In a 4-week study in HanIbm Wistar rats a synthetic lycopene beadlet formulation was well tolerated at a daily oral dose of 1000 mg/kg body weight. A red discolouration of the faeces and a brown-orange discolouration of the liver were observed, the latter being due to disposition of a brown-yellow fine granulated pigment in hepatocytes. Pigment deposition was more pronounced in females but not associated with morphologic alterations. Slight effects on food consumption and body weight development were not considered substance-related. (See also McClain and Bausch, 2003)

- In a 14-week feeding study, also in rats, the highest dose of 500 mg (beadlet) lycopene/kg body weight/day did not produce relevant toxic effects. Deposition of lycopene or a metabolite resulted in a dose-related orange-red discolouration of the liver and adipose tissue, again more pronounced in females, with only limited elimination of these deposits after a recovery period of 5 weeks. Slight variations in haematological and clinical chemical parameters were observed in the high and occasionally the mid dose (150 mg/kg body weight/day). Changes in the relative weight of the thyroid and brain at the high dose were found in one sex only. They were not associated with morphological alterations and did not persist beyond the recovery period. (See also McClain and Bausch, 2003).

- A series of special studies on pigment deposition in rat liver over up to two years confirmed the absence of gross and microscopic liver changes associated with pigment deposition. After 13 weeks of depletion, the liver deposits disappeared completely.

- The pigment deposition in the liver was also reported in an experiment with one dog (Zbinden and Studer, 1958; SCF, 1999).

Reproductive and developmental toxicology

No reproductive and developmental toxicity studies with α-tocopherol-containing oil suspensions of lycopene extracted from *B. trispora* have been performed.

In a one generation reproduction study in rats with synthetic lycopene the only dose tested (1 g synthetic lycopene/kg feed) did not result in toxicity in the parents or the offspring. However, the histopathology findings revealed pigment deposition of lycopene in the liver, mostly in females (Zbinden and Studer, 1958). Zhao *et al.* (1998) reported that the coat appearance of the rats was unaffected by diets containing tomato extracted lycopene up to 1240 mg/kg, equivalent to doses up to 60 mg lycopene/kg body weight/day (the highest dose tested), with the exception of discoloured tails in a few experimental animals. In a reproductive toxicity study male and female rats were fed 10 to 20 mg lycopene/kg body weight/day for approximately 200 days prior to mating and subsequently through pregnancy. Despite a slightly reduced litter size, there were no significant treatment-related effects on fertility, pregnancy, number of litters, pup growth, or incidence of foetal malformations (Zbinden and Studer, 1958; SCF, 1999). In a recent study, no substance-related maternal or developmental toxicity was reported in rats or rabbits following oral exposure at 0, 500, 1500

and to up to 3000 (rats) or 2000 (rabbits) mg/kg body weight/day of synthetic lycopene (Christian *et al.*, 2003).

In the SCF opinion on synthetic lycopene (SCF, 1999) the following is reported:

- In a study on embryotoxicity and teratogenicity in rats, the beadlet formulation at a dose of 1000 mg lycopene/kg body weight/day, administered from day 6-18 of gestation, induced no relevant effects apart from an increase in the number of complete additional thoracic ribs (14th rib) in the pups. These results were however not taken into account as indicative, because the study protocol was considered by both SCF and JECFA too limited to draw a definite conclusion (SCF, 1999; JECFA, 1978). (See also McClain and Bausch, 2003).

Chronic toxicity and carcinogenicity

To date, no long-term feeding studies conducted with lycopene extracted from the microorganism *B. trispora* have been performed.

Mutagenicity

The applicant conducted mutagenicity studies with the lycopene from *B. trispora*. These studies included a *Salmonella* mutation test and a mammalian cell chromosomal aberration test. Both studies were designed according to international regulatory agencies. The bacterial mutation test was conducted with a lycopene 20% cold water dispersible (CWD) formulation from *B. trispora* in *Salmonella enterica var.* Typhimurium strains TA1535, TA1537, TA98, TA100, and in *Escherichia coli* strain WP2 uvrA in either the absence or presence of S9 mix. No genotoxicity was observed. In addition, the incidence of chromosomal aberrations was 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 a lycopene 20% CWD formulation from *B. trispora*. In these mutagenicity studies the CWD formulation was used because results from a test using an oil suspension, which would be immiscible with the assay media, would suffer from lack of exposure of the target cells to the test material. Other mutagenicity studies with lycopene from *B. trispora* in mammalian cells were not performed.

The beadlet formulation, containing synthetic lycopene together with 1.5% dl-α-tocopherol and 5.0% ascorbyl palmitate as well as a number of carrier substances was negative in the Ames test, in a gene mutation assay with *E. coli*, in the mouse lymphoma assay, in a test on chromosome aberrations with human peripheral blood lymphocytes, in the mouse bone-marrow micronucleus test and in a study on unscheduled DNA synthesis in rat liver cells after *in vivo* administration (SCF, 1999; McClain and Bausch, 2003).

In the mammalian microsome reverse mutation assay with *Salmonella* (Ames test) and *E. coli* synthetic lycopene of a high degree of purity of >99% was negative or showed at most only a marginal positive trend, whereas lycopene of 96% purity gave clearly positive results that were most pronounced in strains TA100 and TA97 without metabolic activation (S9 mix). The formation of mutagenic activity during storage of a lycopene solution was reduced in the presence of the antioxidant α-tocopherol. Re-purified batches of crystalline lycopene showed only marginal activity and pure crystalline lycopene was not mutagenic in the Ames test (McClain and Bausch, 2003). These additional examinations suggest that mutagenic

degradation products are formed by oxidative processes during storage of unformulated synthetic lycopene (SCF, 1999; McClain and Bausch, 2003). These data refer to highly concentrated lycopene in its unformulated form, that is in the absence of antioxidants, a form in which lycopene is highly sensitive to oxygen and light (SCF, 1999).

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 enterica var.* Typhimurium with and without metabolic activation (He and Campbell, 1990; Aizawa *et al.*, 2000; McClain and Bausch, 2003), and in *Eschericia coli* with and without metabolic activation (Aizawa *et al.*, 2000).

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).

Addition of a lycopene-rich tomato oleoresin, at levels of 47.5 to 95 mg/kg body weight/day for 9 months, to the diet of benzo[a]pyrene (BaP) treated lacZ mice, enhanced mutagenesis in the colon and lung (Guttenplan *et al.*, 2001). Spontaneous mutagenesis was not affected in these organs. The authors suggest that ingestion of large amounts of dietary lycopene in individuals exposed to carcinogens requires some caution. The authors state that the reason for the enhancement of BaP-induced mutagenesis is speculative but may be related to the pro-oxidant effects exerted by high levels of antioxidants, and/or to the enhancement of BaP metabolism to genotoxic products due to the pre- or co-treatment with lycopene. Such a change in BaP metabolism upon pre- or co-treatment with lycopene may result for example from an effect of lycopene on biotransformation enzymes. Lycopene intake was reported to induce ethoxyresorufin O-dealkylase and benzyloxyresorufin O-dealkylase activities in the liver (Breinholt *et al.*, 2000) and to decrease benzyloxyresorufin O-dealkylase activity in the lungs (Jewell and O'Brien, 1999) and nitrosodimethylamine N-demethylase activity in the liver (Gradelet *et al.*, 1996).

The dosage material in this study with lacZ mice was a lycopene-rich tomato oleoresin. In addition to lycopene as a 3.7% suspension, the dosage material also contained 1.2% β-carotene, 0.44% z-carotene, 0.3% phytofluene, and 0.47% 2,6-cyclolycopene-1,5-diol. Since the dosage material contained several substances, including β-carotene, the effects (if any) cannot be solely ascribed to lycopene. Furthermore, the results reported to show a pro-mutagenic effect (colon and lung) must be interpreted with caution since the study also reported large inter-animal variations in the number of mutants per animal, with a resultant high standard deviation. Since there were large inter-animal variations in mutant fraction values the authors used a non-standard, non-parametric statistical test to determine a level of statistical significance. Due to the large inter-animal variations, standard statistical analyses would have failed to identify any statistical significance between the treated groups. Without having the individual animal results it is also difficult to assess whether the variation may have been caused by a couple of outliers, while the rest of the animals may have been within the control range. In addition, it is important to note that this is an experimental study of a non-regulatory nature and that there is no standard protocol for this type of study and secondly the finding has not been corroborated under other similar experimental conditions.

Furthermore, treatment with lycopene-enriched tomato oleoresin at dosages higher than those administered in the lacZ mice study (Guttenplan *et al.*, 2001) (up to 833 mg/kg) was shown to

have no effect on lung tumour multiplicity following lung tumour induction in the A/J mouse by the tobacco smoke carcinogens benzo[a]pyrene plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Hecht *et al.*, 1999).

Human data on lycopene

Pigment deposition in liver and skin was observed in two cases. In the first case it was reported that a middle-aged woman showed discoloration of her 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 upon excessive and prolonged consumption of tomato juice (2 L juice/day for several years) (Reich, 1960; SCF, 1999). According to Clinton (1998) this amount corresponds to 100-232 mg lycopene/day, excluding other sources of lycopene in the human diet.

Another case refers to a 19-year old Italian girl, diagnosed with lycopenaemia (La Placa *et al.*, 2000). A yellow-orange pigmentation was observed on the forehead, palms and soles. She reported recurrent abdominal pain. All laboratory parameters (blood cell counts, liver enzymes) were within the normal range. Her dietary history revealed a lack of carrots and green vegetables, but a heavy daily intake of four to five big red tomatoes and pasta with tomato sauce for 3 years. When dietary intake of tomatoes was restricted, a complete regression of pigmentation followed within 6 weeks and the abdominal pain disappeared. Because no amounts were reported in the study it is not possible to make an accurate assessment of lycopene intake. It is however obvious that the lycopene intake of this girl was far above the normal range.

Other studies also reported incidences of orange-yellow skin discoloration in individuals consuming diets rich in tomatoes or tomato products (Bonnetblanc *et al.*, 1987; Gandhi *et al.*, 1988).

A 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). No significant side effects, except for carotenodermia in 25% of the subjects in the Spanish cohort supplemented with 13.3 mg lycopene/day, or changes in biochemical or haematological indices were observed throughout the study. In a study of 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.

Numerous epidemiological studies are available reporting a positive association between decreased risk of chronic diseases such as cancer and cardiovascular disease and lycopene intake through dietary intake of tomatoes and tomato products (Rissanen *et al.*, 2003; Agarwal and Rao, 2000; Bramley, 2000; Giovannucci, 1999; Erhardt *et al.*, 2003; Ito *et al.*, 2003; Sesso *et al.*, 2003). No adverse effects related to tomato and tomato product intakes were reported. However, these studies had not been designed to evaluate the safety of lycopene.

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, also these studies have not been designed to evaluate the safety of lycopene.

Similarity of lycopene to beta-carotene

Considering the structural similarity of lycopene and β-carotene the possibility of lycopene increasing the incidence of lung tumours or colon tumours in heavy smokers (as previously reported for β-carotene) (ATBC Study, 1994 a and b; Omenn *et al.*, 1996 a and b; Baron *et al.* 2003) was considered by the Panel as well.

The following data and arguments provide insight into this matter.

- Lycopene is a non-vitamin A carotenoid, and due to its lack of a β-ionone ring, its structural characteristics are such that it is unlikely that its chemical degradation products would resemble retinal.

- In contrast to β-carotene, lycopene is not a substrate for the 15,15'-cleavage enzyme, and hence is not a provitamin A substance (Wirtz *et al.*, 2001).

- Jewell and O'Brien (1999) studied the effect of 16-day intake of 300 mg lycopene/kg diet (approximately 15 mg/kg body weight/day) on xenobiotic metabolising enzymes in the liver, lung and small intestine of male Wistar rats. Modified cytochrome P450 enzyme patterns may be a mechanism underlying modified risks of heavy smoking. Cytochrome P450 activity was assessed using the substrates ethoxyresorufin for CYP 1A1, methoxyresorufin for CYP 1A2, pentoxyresorufin for CYP 2B1/2 and benzyloxyresorufin for CYP 1A1/2. 2B1/2 and 3A. Lycopene did not have an inducing effect on any of the activities. Lycopene decreased the benzyloxyresorufin O-dealkylating activity in the lung.

- Gradelet *et al.* (1996) studied the effect of various carotenoids including lycopene (5% lycopene oleoresin from tomato) on several biotransformation enzymes in the liver of male rats fed for 15 days diets containing 300 mg lycopene/ kg diet, leading to an intake of approximately 15 mg/kg body weight/day. Lycopene was reported not to induce any of the parameters measured including liver P450 content, ethoxyresorufin O-dealkylation, methoxyresorufin O-dealkylation, benzyloxyresorufin O-dealkylation, and erythromycin N-demethylation activity. Nitrosodimethylamine N-demethylation activity was decreased upon lycopene exposure.

- Breinholt *et al.* (2000) reported that upon dietary exposure to 0 (control), 1, 5, 50 or 100 mg lycopene/kg body weight/day for 2 weeks there was a significant induction in the liver of benzyloxyresorufin O-dealkylation at all doses, and a significant induction of ethoxyresorufin O-dealkylation at the two highest dose levels. Pentoxyresorufin O-dealkylation and methoxyresorufin O-dealkylation were not affected. The authors concluded that the observed induction pattern suggest an isoenzymic specificity of lycopene towards CYP1A and 3A4 at the dose levels indicated. The plasma concentrations of lycopene at these dietary levels of 1, 5, 50 and 100 mg/kg body

weight were estimated to be 16, 32, 71 and 67 nM, which was stated by the authors to be barely within the lower range of the mean human plasma concentration of lycopene, which ranges from 70-1790 nM.

- Lycopene, in the same tobacco smoke exposed ferret model as the one used for β-carotene, prevented lung metaplasia instead of enhancing it (Liu *et al.*, 2003). The differences noted in the ferret studies between β-carotene and lycopene regarding lung metaplasia following exposure to tobacco smoke supports the conclusion that generalizations regarding carotenoids and lung tumour development cannot be made.

In summary, based upon the differences in the structures of β-carotene and lycopene, the difference in the route of metabolism, and the fact that the ferret animal model study with lycopene showed a preventive effect, it is concluded that lycopene is unlikely to act in a manner similar to that of β-carotene, or that data on β-carotene and heavy smokers can be extrapolated to lycopene.

Allergenicity

There are no reports on allergic reactions due to *Blakeslea trispora*. In addition, the amount of protein detected in lycopene preparations made from this fungus is very small. The Panel concludes that it is currently unlikely that the final product poses a risk of triggering allergic reactions.

DISCUSSION

Lycopene is biosynthesised by the fungus *B. trispora* through the same pathway as lycopene produced in the tomato. The predominant lycopene isomer in the material is all-*trans* lycopene. The lycopene product is formulated into a 20% or 5% sunflower oil suspension with α-tocopherol at 1% of the lycopene level.

It is expected that any breakdown products derived from lycopene from *B. trispora* in the oil suspension in the presence of α-tocopherol would be identical to those that would occur with lycopene from other natural sources.

Lycopene oil suspension from *B. trispora* is intended for use as a food ingredient in fat spreads (2.0-5.0 mg/kg), milk and milk products (3.0-6.0 mg/kg), condiments, seasonings, relishes and pickles (all at 6.0 mg/kg), mustard (5.0 mg/kg), savoury sauces and gravies (7.0 mg/kg), soups and soup mixes (6.0 mg/kg) and sugar, preserves and confectionary (5.0 mg/kg), and dietary supplements. The proposed level of use for lycopene in food supplements is 20 mg per day.

Average dietary intakes of lycopene from foods in different populations are, according to dietary surveys, estimated to be between 0.5 and 5 mg/day, with high intakes up to about 8 mg/day. High intakes of fruit and vegetables, especially tomato products, may result in occasional intakes of 20 mg/day or more.

The lycopene from *B. trispora* is considered by the Panel to be nutritionally equivalent to natural dietary lycopene. However, the Panel did not carry out an assessment of the possible nutritional benefits of lycopene.

Results from human intervention studies with tomato extract as lycopene source indicate that lycopene from this source is bioavailable. It is expected that lycopene from *B. trispora* when used in foodstuffs of comparable composition is bioavailable to a similar extent.

A physiological pharmacokinetic model, validated by a phase I study in healthy male subjects (five per dose), described 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. This suggests that lycopene bioavailability is saturated at doses above 10 mg/person.

Toxicity studies in rodents, mostly with synthetic lycopene, show no relevant effects other than red discolouration of the faeces and reversible pigment deposition in the liver, which was not of toxicological significance.

To date, no long-term feeding studies conducted with lycopene extracted from the microorganism *B. trispora* have been performed. The toxicological data (90-day oral feeding study) on α-tocopherol-containing oil suspensions of lycopene from *B. trispora* are not sufficient to derive an acceptable daily intake (ADI).

Although the majority of human studies performed with lycopene from tomatoes or synthetic lycopene have not been designed to assess the safety of lycopene, they have not revealed adverse effects. There are case reports of gastrointestinal symptoms and orange-yellow skin discolouration upon long-term high intakes of tomatoes and/or tomato products.

The differences in the structures of β-carotene and lycopene, the difference in the route of metabolism, and the fact that the ferret animal model study with lycopene showed a preventive effect supports the conclusion that lycopene is unlikely to act in a similar manner to β-carotene on heavy smokers.

There are no reports on allergic reactions due to *B. trispora*. In addition, the amount of protein detected in lycopene preparations made from this fungus is very small. The Panel concludes that it is unlikely that the final product poses a risk of allergic reactions.

There are no indications for groups at extra risk for lycopene from *B. trispora*.

CONCLUSIONS AND RECOMMENDATIONS

Average dietary intakes of lycopene from foods in different populations are, according to dietary surveys, estimated to be between 0.5 and 5 mg/day, with high intakes up to about 8 mg/day. High intakes of fruit and vegetables, especially tomato products, may result in occasional intakes of 20 mg/day or more. The applicant's proposed use levels of lycopene from *B. trispora* in foodstuffs would lead to an additional intake of up to about 2 mg/day. The

proposed use level of lycopene in food supplements would give rise to an additional intake of 20 mg/day.

To date, no long-term feeding studies conducted with lycopene extracted from the microorganism *B. trispora* have been performed. The toxicological data on α-tocopherol-containing oil suspensions of lycopene from *B. trispora* (90-day oral feeding study) are not sufficient to derive an acceptable daily intake (ADI).

It is concluded that α-tocopherol-containing oil suspension of lycopene, obtained from *B. trispora*, for use as a novel food ingredient in foodstuffs leading to an additional intake of up to about 2 mg/day is not of concern from the safety point of view. However, 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.

DOCUMENTATION PROVIDED TO EFSA

Application on lycopene from *Blakeslea trispora* as a novel food. Dossier submitted by Vitatene pursuant to the Novel Foods Regulation (EC) Nº 258/97. October 2003.

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