

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

LYCOPENE FROM *BLAKESLEA TRISPORA*

ISSUE

An application has been submitted to the UK Competent Authority for authorisation of a cold water dispersible formulation of lycopene from *Blakeslea trispora* under the novel foods regulation (EC) No 258/97. The Committee is asked to consider and comment on the application dossier, which the UK Competent Authority intends to refer to the European Commission for further assessment, in view of the current review of the use of lycopene (as a food colour) being undertaken by the European Food Safety Authority.

BACKGROUND

Annex 1, pp1-3

1. An application has been submitted by Vitatene to the UK Competent Authority for authorisation of lycopene cold water dispersible (CWD) products derived from the fungus *Blakeslea trispora* for use as a novel food ingredient. The application was accepted by the UK Competent Authority on 28 August 2007. In accordance with Article 6(3) of Regulation (EC) No 258/97, the UK has 3 months to prepare an initial assessment report on the above application. The application dossier is attached as **Annex 1**. Sections IIa and IIb are confidential as they contain commercially sensitive details of the production process.
2. The UK issued a positive initial opinion in 2004 for the use of a lycopene from *B. trispora* produced by the same applicant company and marketed in the form of a suspension in sunflower oil (opinion attached at **Annex 2**). A number of Member States subsequently objected to the UK opinion and as a consequence the Commission referred the application to the European Food Safety Authority (EFSA). EFSA's opinion (attached at **Annex 3**) concluded that "the toxicological data are not sufficient to derive an Acceptable Daily Intake . . . use as a novel food ingredient leading to an additional intake up of to about 2 mg/day is not of concern . . . However, this does not hold for the proposed levels of use . . . that would give rise to an additional intake of 20 mg per day." An authorisation was issued in 2006 (Commission Decision 2006/721/EC and, in line with the EFSA opinion, the permitted levels of incorporation are intended ensure that the level of consumption of lycopene do not exceed 2mg/day).
3. The current application is for an extension of use of the product described in the earlier application, as the basic ingredient (lycopene obtained from the fermentation of *B. trispora*) is being formulated in an alternative "cold water dispersible" formulation that permits its addition to a range of different foodstuffs where the vegetable oil suspension could not be used.

4. Members will also recall that since 2004 they have reviewed two other lycopene applications, a lycopene rich oleoresin from tomatoes¹ and a synthetic oil suspension, which both remain under consideration at EU level. Tomato oleoresin sold as a supplement is not within the scope of (EC) 258/97 and is available in products with a daily dose that significantly exceeds 2mg. Lycopene rich oleoresin from tomatoes is also approved for use as an additive (E160d²).
5. In 2005 Vitatene also submitted an application for lycopene from *B. trispora* (in both forms i.e. the oil suspension and the CWD form) for authorisation as an additive (colour). In accordance with the regulatory procedures for food additives, this application was reviewed by EFSA 2005. (Opinion attached at **Annex 4**) EFSA again highlighted a paucity of safety data for lycopene *per se* and on this basis was unable to conclude whether the proposed use as a food colour was safe, given the relatively high permitted levels for lycopene in food additive legislation. Previously in 1999 the EC Scientific Committee on Food rejected an application for use of a synthetic lycopene citing an incomplete set of toxicological studies.
6. EFSA has recognised that the current authorisations for a number of food colours are based on relatively old scientific data. In view of this they have set out a programme to evaluate all available scientific data and, where possible, to establish Acceptable Daily Intake (ADI) values. The first group of colours to be evaluated includes lycopene from all sources, including *B. trispora*. In addition to setting an ADI, this review will also recommend the maximum levels of incorporation to achieve the desired technological function. It is expected that this review may also enable Member States to determine appropriate conditions of use for lycopene as a food ingredient and to reach decisions on the two outstanding novel food applications mentioned above. EFSA's opinion on lycopene is expected to be available in late 2007 / early 2008.
7. Given that the EFSA opinion is pending it would not be appropriate for the Food Standards Agency, as the UK Competent Authority, to undertake a parallel review of the safety of the NI at the proposed levels of incorporation. Instead, the Agency will refer the application to the European Commission for further assessment, as provided for in Article 6(3) of the novel food regulation. This will allow a decision on this dossier to be taken in the light of EFSA's advice on lycopene from all sources, alongside the two outstanding novel food applications mentioned in paragraph 4 above. Before doing this, the Agency would welcome any comments that the Committee may have on other aspects of the application dossier, such as the formulation of the NI, the intended food categories, potential intake in different user groups, or issues arising from the production process.
8. The present application for authorisation of lycopene from *B. trispora* was prepared pursuant to Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food

¹ The Committee issued a positive initial assessment for tomato oleoresin in 2005 and commented on a Dutch assessment of synthetic lycopene in 2006.

² The use of this food colouring is regulated by Commission Directive 94/36/EC which permits the use lycopene oleoresin *per se* as a colour in a range of foodstuffs at levels up to 500mg/kg.

ingredients. Lycopene from *B. trispora* has been classified as a complex novel food from a non-GM source (class 2.2). The requirements for a submission for this class are as follows:

I	Specification of the NF	X
II	Effect of the production process applied to the NF	X
III	History of the organism used as the source of the NF	X
<i>IV</i>	<i>Effect of the genetic modification on the properties of the host organism</i>	-
<i>V</i>	<i>Genetic stability of the GMO</i>	-
<i>VI</i>	<i>Specificity of expression of novel genetic material</i>	-
<i>VII</i>	<i>Transfer of genetic material from GM microorganisms</i>	-

<i>VIII</i>	<i>Ability to survive in and colonise the human gut</i>	-
IX	Anticipated intake/extent of use of the NF	X
X	Information from previous human exposure to the NF or its source	-
XI	Nutritional information on the NF	X
XII	Microbiological information on the NF	X
XIII	Toxicological information on the NF	X

The information presented in the dossier is structured accordingly and is considered below under these schemes. (Although information on item X is not a requirement for this class of novel ingredient, the applicant has included information on previous exposure to lycopene).

I. Specification of the novel food

Annex 1, pp 4-7

9. Lycopene (C₄₀H₅₆) is an aliphatic branched hydrocarbon with a molecular weight of 536.9 Daltons. It exists predominantly in the trans- form and is a red crystalline powder soluble in fats and organic solvents, but virtually insoluble in water, methanol or ethanol.
10. The applicant intends to market the NI as a nutritional food ingredient in a wide range of food categories. As would be expected these categories are different from those considered in the original application. The purified, crystalline lycopene mixture, is mixed with an octenyl succinic anhydride (OSH) starches to produce a homogenous mixture which is then dried. The NI will be available two concentrations, 10% and 20%.
11. Detailed compositional analyses of the NF are given in the application dossier. The company has tested both crystalline lycopene and CWD forms (Annex 1 Tables 1.e-1 to 1.e-3). The Applicant's specification of the novel food states that it should contain not less than 95% lycopene of which at least 90% is trans-lycopene. The applicant notes the presence of a number of low level contaminants, such as the extraction solvent, isobutyl acetate (not greater than 1%), sulphated ash (not greater than 1%) and subsidiary colouring matters (not greater than 5%). Analytical data have been provided on three non-consecutive, representative lots indicating that none of these met the company's specification.

II. Effect of the production process applied to the novel food

Annex 1, pp 9 - 18

12. The production process is identical to that described in the previous novel food application (ACNFP/63/3) up to the stage where the crystalline lycopene is formulated for food use.
13. Lycopene from *B. trispora* is obtained by the co-fermentation of 2 sexual mating types of the fungus, obtained using classical strain selection techniques to increase the efficiency of lycopene production. The mating types are stable and are preserved and maintained using GLP methods and are deposited in a culture collection. Based on a 28-day feeding study on the biomass, strains are non-toxicogenic and non-pathogenic. The strains used are the same as those approved for the production of β -carotene. Biomass has been tested for the presence of mycotoxins (negative).
14. Fermentation of the fungi to produce lycopene is a two-stage process. Flasks are inoculated with each of the mating types, and grown under controlled conditions. Once vegetative growth is established, the contents of the flasks are individually transferred aseptically to larger growth tanks containing sterile medium. Once sufficient cell mass has accumulated the strains are transferred aseptically into another tank where co-fermentation commences. It is at this point that the fungi start to produce lycopene. The process is further controlled by the addition of imidazole which inhibits the formation of carotene.
15. Lycopene rich biomass is subject to an initial purification process using isopropyl alcohol, which removes any oils and other lipophilic substances. The resultant biomass is evaporated to dryness, milled and then a lycopene extraction step is performed using isobutyl acetate. The resulting enriched solvent is separated and concentrated by vacuum distillation. The lycopene is then crystallised.
16. The production process for the CWD form differs from the oil suspension from this point, with the applicant using an octenyl succinic anhydride (OSH) starch solution to produce a water soluble form. The applicant states that use of OSH is in compliance with conditions described in the 'miscellaneous additives' directive (95/2/EC), and provides the specifications of OSH (Annex 1 Appendix B). Formation of the CWD form involves dissolving the crystalline lycopene in methylene chloride. A solution of OSH starch is added to the lycopene solution and the methylene chloride is removed from the resulting emulsion by vacuum evaporation. The resultant solution is then dried in a fluid bed granulator under controlled temperature. Each batch of the final product is assayed to check compliance with the specification.
17. The applicant has supplied data indicating that lycopene from *B. trispora* is predominantly present in the trans- form (at least 90%). The data also indicate that the purity of the fungal lycopene is comparable with synthetic lycopene (Annex 1 Table II c-1).
18. The applicant has provided data to demonstrate that the CWD formulation (10 and 20% forms) is stable for a period of at least at least 6 months at a range of temperatures (5°C, 25°C and 40°C) at up to 60% relative humidity with no appreciable deterioration in product quality. In all cases the tests took place in

conditions conducive to oxidation as, although the NF was sealed in bottles, the applicant did not flush the containers with nitrogen. The dossiers also refers to a study of stability in foods but this is not directly as it refers to the lycopene in oil suspension form.

III. History of the organism used as a source of the novel food

Annex 1, pp19-21

19. The applicant has based previous dietary exposure to *B. trispora* on its use as a source of β -carotene, noting that the safety of the organism was assessed by the SCF (2000) and the Joint Expert Committee on Food Additives (JECFA) (2001). The SCF concluded that, based on the information supplied, the organism is non-pathogenic and non-toxicogenic. A subsequent 28-day oral feeding study using Wistar rats, Jonker (2000) (see also Section XIII) also demonstrated that the organism was both non-toxicogenic and non-pathogenic.
20. JECFA concluded that β -carotene from *B. trispora* is acceptable for food additive use, providing that it met the specification of its synthetic counterpart. The applicant quotes this finding as being consistent with their view that the source organism is safe.
21. The applicant also carried out mycotoxin assays on each of three non-consecutive batches to determine whether aflatoxin B1, Mycotoxin T2, ochratoxin and zearalenone were present. The results, for both crystalline lycopene and *B. trispora* biomass were negative.

IX. Anticipated intake/extent of use of the novel food

Annex 1, pp23-28

22. The applicant intends to use the NI as a nutritional food ingredient. As the NI is in a CWD form the uses are different from the original application and subsequent authorisation, which was for fat spreads and predominantly dairy based products. A full list of the proposed uses is reproduced below. The use categories are similar to, but not as extensive as, those proposed for the lycopene rich oleoresin from tomatoes for which an authorisation decision is pending (see above).

Proposed food uses and use levels for the NI			
Food Category	Proposed Food Use	Use Level (ppm)	
Beverages	Fortified Juice Mixtures	5	
	Soft Drinks	5	
Cereal and Cereal Products	Biscuits	5	
	Buns, cakes and pastries	5	
	Processed Cheese	5	
	Fruit pies	5	
	Pudding powder	5	
	Pie and Pastry Fruit Filling	5	
Fruit and Nuts	Pie and Pastry Fruit Filling	5	
	Sugar, Preserves and Confectionery	Bakery Fillings	5
		Chocolate confectionery	5
	Sugar confectionery	5	

23. In order to estimate the intake of the NI the applicant has used the most up to date information available from UK dietary surveys. In order to compare the data over a 7-day period across the surveys that target different sub-groups of the UK population, the applicant has applied a weighting factor. This approach was reviewed in 2004 by the Agency's Dietary Exposure Assessment Team who were satisfied that the method is valid.

24. The intake estimates are summarised below. The largest consumers of the NI on an absolute basis are predicted to be male teenagers, whereas the lowest are female adults. However children and young people have the highest estimated intakes on a body weight basis (See also Annex 1 Tables IX.b-1 & 2). High level intake is represented in the dossier by the 95th percentile. The applicant has subsequently provided the values for the 97.5th percentile, which is used as the standard measure of high level intake of food chemicals in the UK.

Population Group (age)	ESTIMATED DAILY INTAKE ("all-users")					
	(mg/day)			(mg/kg bodyweight/day)		
	Mean	95 %ile	97.5 %ile	Mean	95 %ile	97.5 %ile
Children (1½-4½)	2.13	5.11	5.88	0.15	0.37	0.42
Young People (6-11)	3.41	7.23	8.42	0.13	0.31	0.35
Teenager (F) (11-18)	2.68	6.73	7.64	0.05	0.12	0.15
Teenager (M) (11-18)	3.54	8.16	9.49	0.07	0.16	0.18
Adult (F) (16-64)	1.71	4.19	5.09	0.02	0.06	0.08
Adult (M) (16-64)	1.55	5.39	6.80	0.02	0.06	0.08

25. These estimates refer to a 'worst case scenario', which assumes that all food types within a category contain the NI at the maximum level of incorporation (See also paragraphs 25 - 28).

26. The Secretariat notes that the intake levels are significantly higher than those proposed for the lycopene oil suspension, where the highest mean consumption was 0.6mg/day (male adults) (97.5 %tile 1.68 mg/day) (See Annex 2 para 17).

X Information from previous human exposure to the novel food or its source

Annex 1, pp29-31

27. Lycopene from *B. trispora* is an authorised novel food ingredient and is permitted in a range of food categories at levels that would not lead to intakes exceeding 2mg/day (Annex 4). In addition to its use as a food colour, lycopene is a normal constituent of the diet in a number of red fruits and vegetables such as tomatoes and watermelon. Levels of lycopene in tomato are dependent both on the species of tomato and the degree of ripening but are generally in the range 3.1-7.7 mg / 100g.

28. A UK study reported (1996) indicated that consumption of a lycopene-rich diet would lead to consumption of 1.03mg of lycopene/person/day. This is similar to the intake levels reported in Finland (0.70 and 0.87 mg/day for females and males respectively). An EU wide dietary survey published in 2001 suggested that levels of lycopene consumption differed widely across Europe (median daily intakes 1.64 mg (Spain), 5.01 µg(UK))
29. The applicant has summarised a number of North American dietary surveys that reinforce the European findings that consumption of lycopene is intrinsically varied and dependent on dietary preference. Consumption of lycopene in North America indicates a large variation dependent upon the method of data collection. However, in all cases the mean levels were significantly higher than those seen for UK subjects. Healthy young adults in Canada were estimated to consume 25.2 mg lycopene per day (calculated on the basis of tomato consumption). In the US, USDA studies show mean levels of intake in the diet to be 4.7 and 8.2 mg/day (2000 and 2004 figures respectively).
30. The permitted levels for the use of lycopene as a colour in the EU are high (500mg/day) but this figure is not necessarily indicative of usage.

XI. Nutritional information on the novel food

Annex 1, 32-33

31. The applicant is of the view that, although the source of lycopene is novel, the nutritional value of the novel ingredient is the same as that of dietary lycopene.
32. Lycopene is an effective antioxidant, and these antioxidant properties are perceived to be primarily responsible for the potential health benefits of dietary carotenes. The applicant lists the perceived nutritional benefits of increased lycopene intake but this information is not relevant in the context of a novel food assessment.

XII. Microbiological information on the novel food

Annex 1, pp35-36

33. Microbiological information supplied by the applicant indicates that three non-consecutive batches contained no moulds, yeast *Salmonella* or *Escherichia coli*. These findings applied to both the crystalline lycopene and the CWD form (10% and 20% forms).

XIII Toxicological Information on the Novel Food

Annex 1, 37-70

34. The applicant has provided a number of toxicological studies on both the novel food and the source organism. These studies were reviewed by the Committee in the context of the previous application for lycopene from *B. trispora* in an oil suspension, when members accepted the data as providing sufficient reassurance of safety (Annex 2).
35. The majority of the studies that were carried out on lycopene from *B. trispora* used the crystalline or oil suspension forms. The Secretariat notes that there is little difference in the production process employed for the CWD form, other than

the final formulation step. The Secretariat has obtained additional information from the company on the particle size of lycopene from *B. trispora* in the two formulations. The applicant has stated that the particle sizes are approximately 2 µm in the oil suspension and 0.25 µm (250nm) in the CWD formulation. The applicant has also pointed out that the test material is at a much lower concentration when it is added to the animal feed used in the feeding trials and the lycopene will be dissolved in the fat fraction of the diets.

Safety Assessment

36. In view of the pending EFSA review which is likely to set an ADI for lycopene, Members are not required to comment on the toxicological data provided by the applicant, which are the same as presented in their previous dossier. For completeness, the available data are summarised below.

Summary of studies

37. The applicant has assessed the sub-chronic toxicity of the source of the novel food by testing the lycopene-rich biomass extracted from *B. trispora*. Supplementary information to demonstrate the safety of the source organism has been supplied from an independent scientist, the SCF and JECFA. A 90-day oral toxicity study has been carried out on lycopene from *B. trispora* (20% oil suspension).
38. The applicant has also provided details of acute, sub-chronic and chronic, carcinogenicity, mutagenicity and genotoxicity, reproductive toxicity trials and human safety data for lycopene from other sources.

Toxicological assessment of *B. trispora* (Annex 1 p37)

39. The two mating strains of *B. trispora* are stable cultures that are preserved under conditions that adhere to good manufacturing practices. The strains are considered to be non-toxicogenic and non-pathogenic on the basis of 28-day oral feeding study described above. The applicant also notes that *B. trispora* is formally classified in Germany as “risk group 1”, organisms that pose no risk for humans and vertebrates
40. The production of lycopene by *B. trispora* is an intermediary of the beta-carotene synthetic pathway and the SCF considered the use of *B. trispora* as a source of beta-carotene as acceptable. The committee concluded that the “source organisms and the production process yielded no grounds to suppose that the final crystalline product, beta-carotene, differs from the chemically synthesised beta-carotene used as a food colourant” (SCF, 2000)

Final Product (Annex 1 p38, 48)

41. A 90-day oral toxicity study was carried out to assess the toxicity of the 20% lycopene oil suspension in male and female Wistar rats. Following a 13-day acclimatisation period the rats were divided into 4 groups with 20 rats per sex in each group. The groups received a diet containing 0, 0.25, 0.5, or 1.0 % lycopene in the form of a sunflower oil suspension. These percentages corresponded to daily doses of 0, 145, 291 and 586 mg/kg bodyweight for males and 0, 156, 312 and 616 mg/kg bodyweight for females.
42. The animals were monitored for viability, clinical signs of toxicity, body weights and food consumption. Prior to necropsy, neurobehavioural testing and

ophthalmoscopic examinations were performed and blood and urine analyses were obtained. Following necropsy, gross and histopathological examinations of various tissues were performed and organ weights recorded.

43. A pink discolouration of the fur was noted in all animals in the high dose group and many in the mid-dose group. This was attributed to the direct contact of the animals to the red staining lycopene mixture in the diet. No adverse effects were noted from the examinations described above and as a result the no observed effect level (NOAEL) was set at 1% in the diet. This was equivalent to a dose of 601mg / bodyweight per day, averaging the doses received by the male and female groups.
44. The genotoxicity of a 20% cold water dispersal of lycopene from *B. trispora* was assessed using a bacterial mutation test and an *in vitro* chromosome aberration test. As a result of these studies the investigator concluded that lycopene is not genotoxic.

Margin of safety (Annex 1 p58)

45. Comparing the NOEL of 601 mg lycopene/kg bodyweight/day from the sub-chronic rat study with the anticipated intake from food use, the applicant calculates that there is a 4000 – 10000 fold safety margin for high level (95th percentile) and mean consumers in the male teenager group.

Toxicological assessment of lycopene from sources other than *B. trispora*

46. The applicant has supplied details of additional toxicological studies with lycopene derived from natural tomato extracts, tomato paste and synthetically produced lycopene in a number of forms including cold water dispersible (CWD) and water-soluble (WS) beadlet formulations and dietary supplements. Studies supplied include acute toxicity studies, sub-chronic and chronic toxicity studies, carcinogenicity studies mutagenicity / genotoxicity studies, reproductive toxicity studies and human safety data.

Allergenicity

Annex 1, p 71

47. The applicant is of the view that the primary source of allergenic material, the source organism, is not present in the final products to any significant degree. This fact is borne out by the microbiological information (See para.30 above). Protein assays carried out on both the novel food (5% and 20% suspensions) and the sunflower oil were negative at the limit of detection (1µg protein/ml or 1µg protein in 400mg lycopene oil suspension). The applicant concludes that this is indicative of the absence of allergenic potential.

Labelling

Annex 1, p 29

48. The applicant proposes that the ingredient would be described on food labels as "lycopene" without describing the source (*B. trispora*) to the consumer. The applicant confirms that labelling of products containing the NF will comply with current EU regulations and may include the statement 'contains an additional source of lycopene'. This is consistent with the 2004 application.

Consumer access and choice

49. The Secretariat has considered the issues of access and choice in relation to lycopene from *B. trispora*. If authorised, the NF would be available for use in products across the UK and subsequently in other EU Member States. In practical terms, access to foods fortified with lycopene from *B. trispora* could be limited by a high price or by limited geographic distribution, which are both driven by commercial considerations that cannot be predicted at this stage.
50. It is envisaged that the introduction of foods fortified with lycopene from *B. trispora* will increase existing consumer choice, and although it is not anticipated that other ingredients will be displaced, the source of the ingredient could change compared with products currently on the market. The consumer would be aware of the presence of lycopene through the ingredient list and, most likely, through special marketing that highlights its contribution to the nutrient composition of the food.

COMMITTEE ACTION REQUIRED

51. The Committee's comments on this dossier will be included in the UK Competent Authority's report on this novel food application, recommending additional assessment.

**Secretariat
August 2007**

Annexes attached:

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| Annex 1 | Application Dossier |
| Annex 2 | UK Initial Opinion on use of Lycopene from <i>B. trispora</i> (oil suspension) as a novel food ingredient |
| Annex 3 | EFSA Opinion on use of Lycopene from <i>B. trispora</i> (oil suspension) as a novel food ingredient & Commission decision 2006/721/EC |
| Annex 4 | EFSA Opinion on use of Lycopene from <i>B. trispora</i> (oil suspension and CWD) as a food colour |

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Application Dossier

**Secretariat
August 2007**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

UK Initial Opinion on use of Lycopene from *B. trispora* (oil suspension) as a novel food ingredient

**Secretariat
August 2007**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

EFSA Opinion on use of Lycopene from *B. trispora* (oil suspension) as a novel food ingredient

**Secretariat
August 2007**

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

EFSA Opinion on use of Lycopene from *B. trispora* (oil suspension and CWD) as a food colour

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