APPLICATION FOR THE APPROVAL OF LYCOPENE COLD WATER DISPERSIBLE (CWD) PRODUCTS FROM *BLAKESLEA TRISPORA*

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

March 23, 2007

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ADMINISTRATIVE DATA

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The application is submitted by:

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GENERAL INTRODUCTION

Vitatene proposes to market lycopene cold water dispersible (CWD) products, derived from the fungus *Blakeslea trispora*, for use as a nutritional food ingredient in Europe. Approval is sought under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredient*s (hereafter referred to as EC 258/97), and accordingly, this submission has been prepared pursuant to the *Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients* (hereafter referred to as the Commission Recommendation of 1997) (European Parliament and the Council of the European Union, 1997).

Article 1(2.) of EC 258/97 states that the regulation "…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under the following categories…(d) foods and food ingredients consisting of or isolated from microorganisms, fungi or algae". Lycopene CWD products from *B. trispora* are thus considered a novel food/food ingredient due to the source organism.

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee on Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the six classes identified, lycopene CWD products from *B. trispora* would be classified in Class 2 as a "Complex Novel Food from non-GM source", since the production strains of *B. trispora* have been developed by conventional techniques, with no use of genetic modification. While the components of the final product (a cold water dispersible product) have a history of food use in the Community, the source organism does not. Accordingly, lycopene CWD products from *B. trispora* would be further allocated under Sub-Class 2.2: "the source of the novel food has no history of food use in the Community". The essential information requirements corresponding with this classification are outlined in a detailed list below, and are expanded upon in separate sections throughout the document, forming the basis of the application.

I. Specification of the Novel Food
II. Effect of the production process applied to the Novel Food
III. History of the organism used as the source of the Novel Food
IX. Anticipated intake/extent of use of the Novel Food
X. Information from previous human exposure to the Novel Food or its source[1]
XI. Nutritional information on the Novel Food
XII. Microbiological information on the Novel Food
XIII. Toxicological information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the SCF, which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

As detailed herein, the safety of lycopene CWD products from *B. trispora* is supported by the purity of lycopene CWD products from *B. trispora* (>95%), the conformity between biosynthetically-derived lycopene in nature and chemically-derived lycopene CWD products from *B. trispora*, the historical consumption of lycopene as a normal component of the diet

[1] Although this category is not required for Class 2.2 novel foods and food ingredients, it has been included in this application since all the components of the final product are present in the diet, thus rendering this a relevant category.

(*e.g.*, red fruits and vegetables including tomatoes, watermelon, pink grapefruit, apricots), minimal exposure under the conditions of intended use, safety data provided by Vitatene for the final lycopene CWD products and for the biomass, additional safety data for the biomass, and published toxicological and clinical data conducted with lycopene (from sources other than *B. trispora*). Furthermore, lycopene from *B. trispora* in oil suspension form has been authorized as a novel food in the EU (Council Directive 2006/721/EC) (Commission of the European Communities, 2006).

I. SPECIFICATIONS OF LYCOPENE CWD PRODUCTS FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- "…is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"
- "Is the information representative of the novel food when produced on a commercial scale?"
- "Is there an appropriate specification (including species, taxon *etc*. for living organisms) to ensure that the novel food marketed is the same as that evaluated?"

These questions have been addressed collectively in Sections I.a through I.e.

I.a Common Name or Usual Name

Lycopene CWD products

Vitatene's lycopene CWD products are derived from the fungus *Blakeslea trispora*. The proposed trade name for lycopene CWD products derived from *B. trispora* is LYCONAT.

I.b Chemical Name and Chemical Abstract Service (CAS) Number

The predominant occurring lycopene isomer in the final manufactured material is *trans* lycopene, which has the following CAS number:

all-*trans* lycopene [502-65-8]

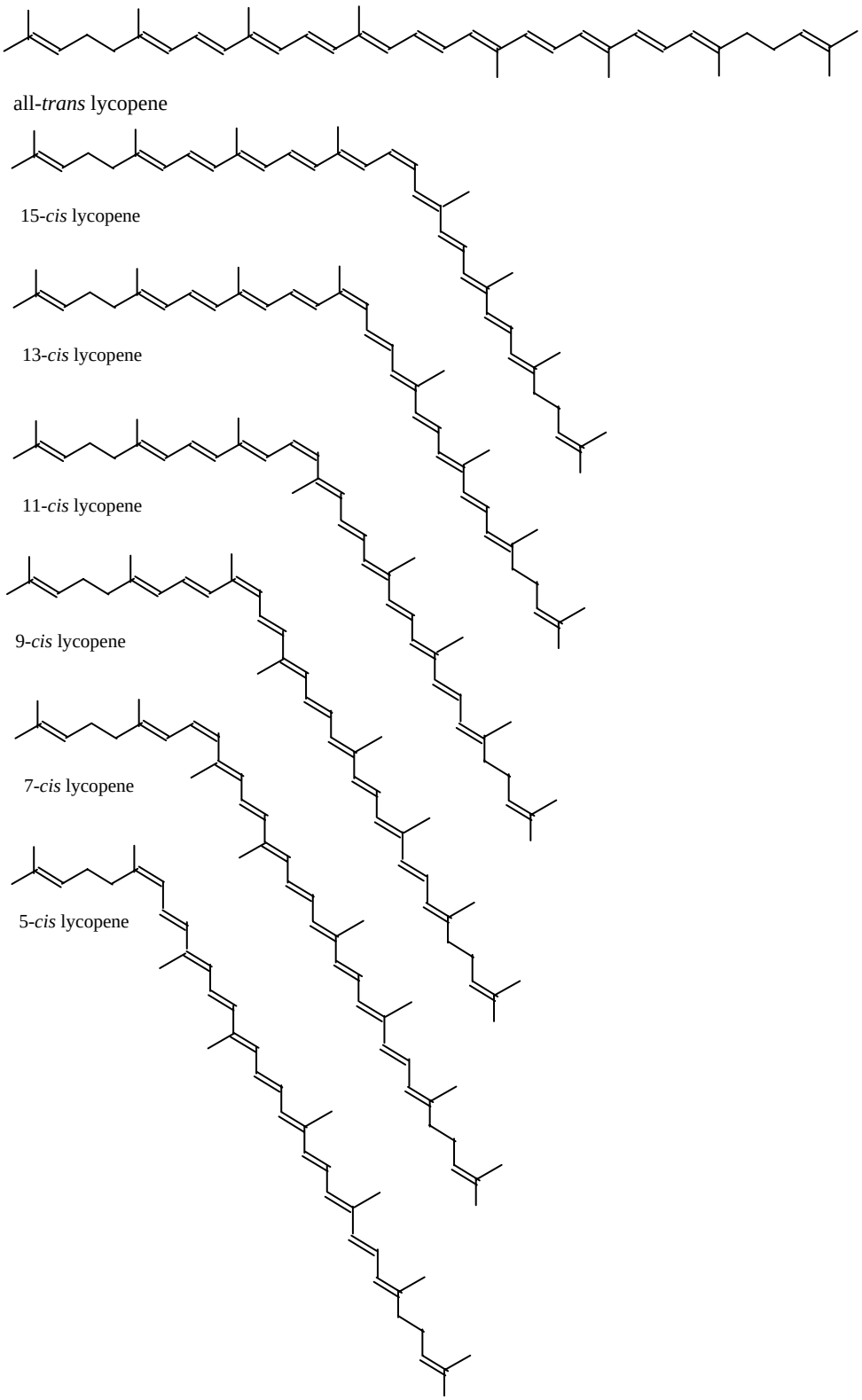
I.c Empirical Formula

Lycopene is a nonpolar hydrocarbon chain with two open-end rings, a molecular weight of 536.87 Daltons, and empirical formula $C_{40}H_{56}$ (Merck, 2001).

I.d Structural Formulae

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

Figure I.d-1 Structural Formulae of Lycopene



I.e Product Specifications and Analyses for Lycopene CWD Products

Lycopene crystals are described as a red crystalline powder. Lycopene produced following a fermentation process is extracted from the biomass, purified and crystallised. The lycopene CWD product is a dark red powder. The product specifications and the analyses of 3 non-consecutive, representative lots of lycopene crystals, 10% CWD product and 20% CWD product, produced to demonstrate a reproductive and representative process capable of meeting the proposal specification, are outlined in Tables I.e-1, I.e-2, and I.e-3, respectively. The product specifications for lycopene crystals are similar to those established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2006), with the exception of the following parameters: identification test for carotenoids and loss on drying, which are not included in the product specifications outlined below.

Table I.e-1 Product Specifications and Analyses of Lycopene Crystals

Test	Specification*	Batch Number		
		LC 052	LC 054	LC 057
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	471.5	471.5	471.5
Assay (%) (472 nm)	≥95	101.4	98.6	100.5
Total lycopene (%)	≥95	100.1	100.4	100.8
Trans-lycopene (%)	≥90	95.7	94.9	96.8
Subsidiary colouring matters (%)	≤5	ND	ND	ND
Sulphated ash (%)	≤1	0	0.26	0.09
Imidazole (ppm)	≤1	<1	<1	<1
Isopropanol (%)	≤ 0.1	0.0051	0.0099	<0.001
Isobutyl acetate (%)	≤ 1.0	0.22	0.25	0.23
Heavy Metals				
Arsenic (ppm)	≤1	<0.6	<0.6	<0.6
Lead (ppm)	≤1	<0.4	<0.4	<0.4
Mercury (ppm)	≤1	<0.150	<0.173	<0.150
Cadmium (ppm)	≤1	<0.02	<0.02	<0.04

*Certificates of analysis and analytical methods for specifications can be found in Appendix B

Table I.e-2 Chemical Specifications and Analyses of Lycopene 10% CWD Product				
Test	**Specification***	**Batch Number**		
		152	**174**	**176**
Colour	Dark red powder	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	471	471	471
Assay (%) (472 nm)	≥ 10	10.13	10.91	11.29
Loss on drying (%)	≤ 8	2.96	2.48	1.69
Heavy Metals				
Arsenic (ppm)	≤ 1	<0.6	<0.6	<0.6
Lead (ppm)	≤ 1	<0.4	<0.4	<0.4
Mercury (ppm)	≤ 1	<0.15	<0.15	<0.15
Cadmium (ppm)	≤ 1	<0.04	<0.04	<0.04

*Certificates of analysis and analytical methods for specifications can be found in Appendix A

Table I.e-3 Chemical Specifications and Analyses of Lycopene 20% CWD Product				
Test	**Specification***	**Batch Number**		
		180	**182**	**184**
Colour	Dark red powder	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	469	470	471
Assay (%) (472 nm)	≥ 20	22.6	22.95	23.29
Loss on drying (%)	≤ 8	2.18	1.43	1.62
Heavy Metals				
Arsenic (ppm)	≤ 1	<0.6	<0.6	<0.6
Lead (ppm)	≤ 1	<0.4	<0.4	<0.4
Mercury (ppm)	≤ 1	<0.15	<0.15	<0.15
Cadmium (ppm)	≤ 1	<0.04	<0.04	<0.04

*Certificates of analysis and analytical methods for specifications can be found in Appendix A

The manufacturing process and extraction system is shown to produce material of a highly purified nature. Analysis of representative batches of the lycopene crystal and the resultant lycopene CWD products also demonstrate that the manufacturing process and final product formulation are both reproducible and capable of producing material that meets specification.

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO LYCOPENE CWD PRODUCTS FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- "Does the novel food undergo a production process?"
- "Is there a history of use of the production process for the food?" If no, "does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?"
- "Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"
- "Are the means identified for controlling the process to ensure that the novel food complies with its specification?"
- "Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?"
- "After processing is the novel food likely to contain microorganisms of adverse public health significance?"

These questions have been addressed collectively in Sections II.a through II.d.

II.c Comparison of Lycopene from *Blakeslea trispora* with Synthetic and Naturally Occurring Lycopene

Research has indicated that the formation of β-carotene and lycopene is universal in bacteria, fungi and plants. The synthesis involves a condensation reaction followed by four desaturation steps and the cyclisation of both ends to beta-ionone rings (Sandmann, 2001). Studies conducted by Rodríguez-Sáiz *et al*. (2003) and López-Nieto *et al*. (2003) have demonstrated similarities between *B. trispora* and carotenogenic fungi (*e.g*., *Mucor circinelloides*) in terms of gene sequences, and between *B. trispora* and carotenogenic fungi, algae, and higher plants (*e.g*., tomatoes and red peppers) in terms of metabolic pathways involved in the production of lycopene. The lycopene biosynthetic process is very different from the synthetic pathway that involves the formation of several aldehydes.

The chemical composition of lycopene produced from *B. trispora,* naturally occurring lycopene from tomatoes and synthetic lycopene are outlined in Table II.c-1. The results indicate that the purity of lycopene from *B. trispora* is similar to that of synthetic lycopene, but is greater than that which is extracted from tomatoes. The difference between synthetic lycopene and lycopene from *B. trispora* is related to the presence of impurities, in particular lycopene-C_{25}-aldehyde, which is recognized by the SCF to have mutagenic properties. Lycopene from *B. trispora* has a composition of geometric isomers similar to that of lycopene from tomatoes.

Table II.c-1 Chemical Comparison of Lycopene from *B. trispora* with Synthetic Lycopene and Lycopene from Tomatoes

	Synthetic Lycopene[1]	**Lycopene from Tomatoes**[1]	**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	10-20% CWD product

[1] SCF/CS/ADD/COL/160 Final 6/12/99 Opinion on Synthetic Lycopene as a colouring matter for use in foodstuffs (SCF, 1999)

II.c.1 Current Regulatory Situation

Lycopene obtained by solvent extraction from tomatoes is currently permitted as a food colour in the EU and is listed as E 160d in Directive 94/36/EC (European Parliament and the Council of the European Union, 1994). Lycopene from *B. trispora* in a tocopherol-containing oil suspension is authorized as a novel food in the EU (Council Directive 2006/721/EC) (Commission of the European Communities, 2006). The European Food Safety Authority (EFSA) concluded that lycopene from *B. trispora* is nutritionally equivalent to natural dietary lycopene. In its evaluation of lycopene from *B. trispora* as an oil suspension and a CWD product for use as a food colour, EFSA stated that it was unable to conclude whether the proposed use levels of lycopene would be safe due to the limited toxicity data and the high estimated intake levels (up to 43 mg/day) compared to background dietary intakes of lycopene (EFSA, 2005a). Synthetic lycopene is not permitted for use as a colour additive since the SCF concluded in 1999 that its use in food was currently unacceptable due to insufficiencies in the database.

Lycopene from *B. trispora* and synthetic lycopene have recently been evaluated by JECFA (JECFA, 2007). Biosynthetically-produced lycopene from *B. trispora* was considered toxicologically equivalent to chemically synthesized lycopene, thus a group ADI (for both forms of lycopene) of 0 to 0.5 mg/kg body weight was assigned. This value was based on the 50 mg/kg body weight dose tested in a chronic toxicity study conducted on synthetic lycopene in rats with a safety factor of 100, and was supported by negative results for 2 genotoxicity tests and a lack of adverse effects in a short-term toxicity study conducted on lycopene from *B. trispora*. JECFA estimated a high exposure for synthetic lycopene of 30 mg/person per day, equivalent to 0.5 mg/kg body weight per day. This value includes background exposure and additional exposure from food additive uses. The exposure estimate for lycopene from *B. trispora* was the same as for synthetic lycopene.

II.d Stability of Lycopene

Due to its chemical structure (*i.e.*, long chain of conjugated carbon-carbon double bonds), lycopene is susceptible to chemical changes such as isomerisation and degradation when exposed to light and heat (Lee and Chen, 2002). To overcome these stability issues, lycopene production is carried out under dark, controlled temperature, and nitrogen atmosphere. It is a continuous process in which lycopene crystals are not accumulated but are immediately utilized in the manufacturing of the CWD product.

II.d.1 Stability of Lycopene CWD Products

The stability of lycopene CWD products (average values for batch numbers 152, 174, and 176 for the 10% formulation and batch numbers 180, 182, and 184 for the 20% formulation), stored in aluminium bottles filled to the top without air displacement by nitrogen, has been evaluated. Stability was evaluated at 25°C ± 2°C and 60% ± 5% RH (Table II.d.1-1), at 40°C ± 2°C and 75% ± 5% RH (Table II.d.1-2), and under conditions of intended use at 5°C ± 3°C (Table II.d.1-3). Testing was conducted for periods of up to 6 months. The results of the

stability trials indicate the stability of lycopene in a CWD formulation under all conditions tested.

Table II.d.1-1 Stability of 10% and 20% Lycopene CWD Formulations Following Storage at 25°C ± 2°C and 60% ± 5% RH for up to 6 Months**

Test	10% Lycopene CWD Formulation					20% Lycopene CWD Formulation				
	Duration (months)					Duration (months)				
	Initial	0.5	1	3	6	Initial	0.5	1	3	6
Assay (%)	10.78	-	-	10.88	9.97	22.83	-	-	22.41	21.43

**See Appendix C

Table II.d.1-2 Stability of 10% and 20% Lycopene CWD Formulations Following Storage at 40°C ± 2°C and 75% ± 5% RH for up to 6 Months**

Test	10% Lycopene CWD Formulation					20% Lycopene CWD Formulation				
	Duration (months)					Duration (months)				
	Initial	0.5	1	3	6	Initial	0.5	1	3	6
Assay (%)	10.78	10.75	10.71	10.96	9.94	22.83	22.54	22.57	21.93	21.49

**See Appendix C

Table II.d.1-3 Stability of 10% and 20% Lycopene CWD Formulations Following Storage at 5°C ± 3°C for up to 6 Months**

Test	10% Lycopene CWD Formulation					20% Lycopene CWD Formulation				
	Duration (months)					Duration (months)				
	Initial	0.5	1	3	6	Initial	0.5	1	3	6
Assay (%)	10.78	-	-	10.78	10.26	22.83	-	-	22.15	21.93

**See Appendix C

II.d.2 Stability of Lycopene in Food

Since the lycopene produced from *B. trispora* is similar to that which occurs naturally in food (see Table II.c-1), it is expected that any breakdown products derived from the lycopene from *B. trispora* in CWD formulations are similar to those that would occur naturally. However, the presence of macromolecules in a food system have been suggested to offer additional protection for lycopene (Lee and Chen, 2002); therefore, the lycopene stability data presented above are expected to be a conservative representation of the stability of lycopene under the proposed conditions of intended use.

The stability of the lycopene in food is supported by a stability experiment conducted with rat feed containing lycopene at 0, 0.25, 0.50, and 1.0% (Jonker *et al.*, 2003). For each concentration level, diets were sampled immediately after preparation, following storage for 1 and 4 days at room temperature, and following storage for 7, 14, and 29 days at <-18°C.

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

III. HISTORY OF *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism:

- "Is the novel food obtained from a biological source, *i.e*., a plant, animal or microorganism?"
- "Has the organism used as the source of the novel food been derived using GM?"
- "Is the source organism characterized?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

These questions have been addressed collectively in Sections III.a through III.c.

III.a Taxonomic Classification of *Blakeslea* sp.

Lycopene is produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *B. trispora*. The current taxonomic placement of *B. trispora* is summarized below:

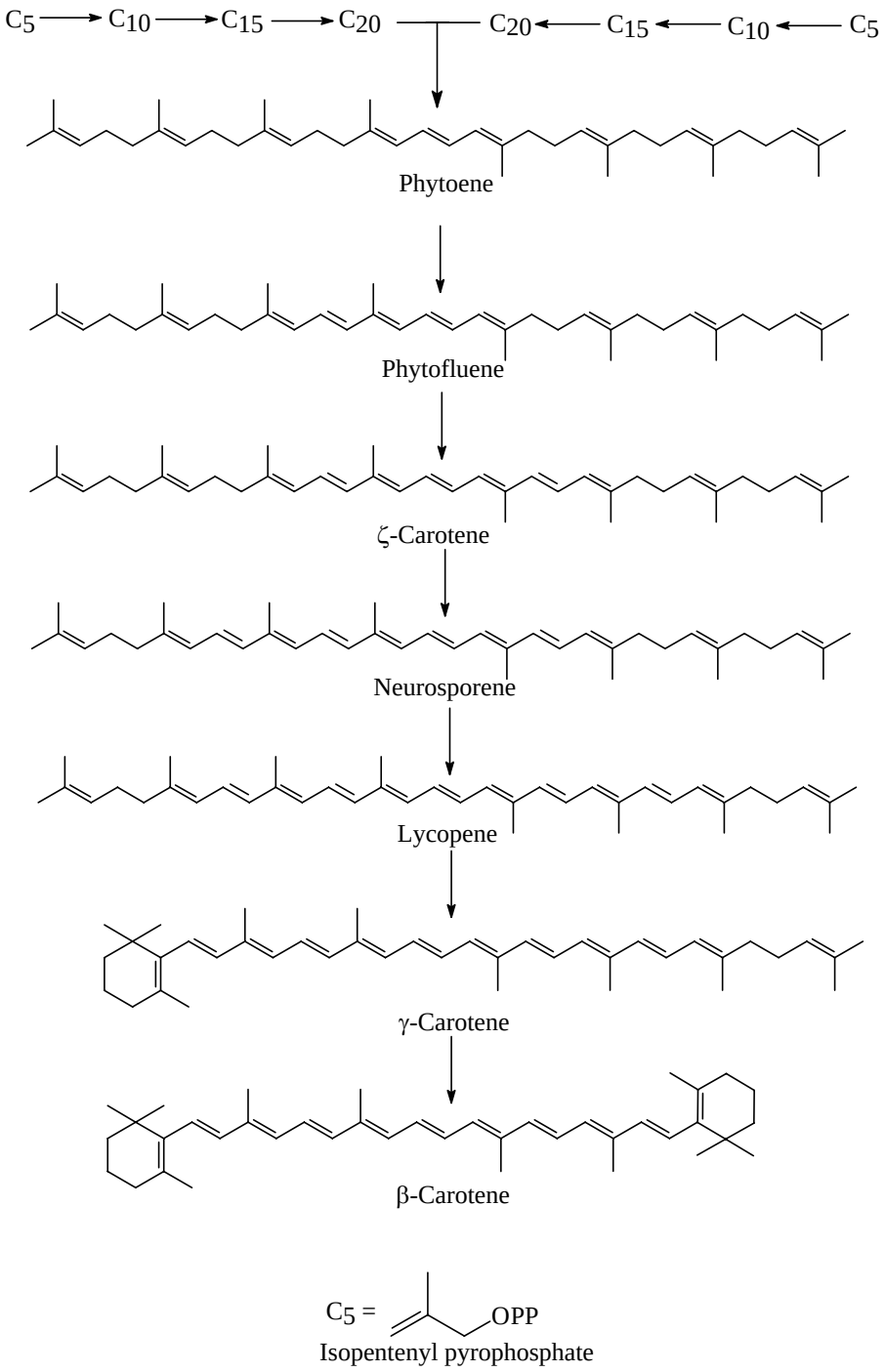
Kingdom:	Fungi
Phylum:	Zygomycota
Class:	Zygomycetes
Order:	Mucorales
Family:	Choanephoraceae
Genus:	Blakeslea
Species:	trispora

III.b Other Dietary Exposures to *Blakeslea 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. The SCF has previously considered that β-carotene from *B. trispora*, produced *via* an identical biosynthetic route and process as lycopene from *B. trispora* (see Figure III.b-1), is acceptable for use as a colouring agent for foodstuffs. The SCF considered the safety of *B. trispora*, was supported by a literature search, a standard pathogenicity experiment in mice, and by analyses of extracts of several fermentation mashes for fungal toxins, all of which revealed that the mould is non-pathogenic and non-toxigenic. In addition, the final product, the β-carotene crystals, was shown to be non-pathogenic and non-toxigenic by enzyme immunoassays for 4 mycotoxins (aflatoxin B1, mycotoxin T2, ochratoxin, and zearalenone). Following review of the available safety

information, the Committee concluded that the “source organism and the production process yielded no grounds to suppose that the final crystalline product, differs from the chemically synthesised β-carotene used as a food colorant” (SCF/CS/ADD/COL 158 Final – correction, 2000).

Figure III.b-1 Lycopene Biosynthetic Route and Process are Identical to β-Carotene from *B. trispora*



A similar position regarding β-carotene has recently been taken by the Joint Expert Committee on Food Additives (JECFA, 2001), who concluded that β-carotene isolated from different sources, including that from *Blakeslea trispora*, is acceptable for food additive use, as long as it is of sufficient purity to meet the specifications for synthetic β-carotene. Therefore neither the SCF nor JECFA have expressed any concerns regarding the use of *Blakeslea trispora* in the production of β-carotene.

III.c Safety of *Blakeslea trispora*

Lycopene is produced by co-fermentation of two strains, VKPM F-744 (-) and VKPM F-816 (+) of the fungus *B. trispora*. The strains are considered to be non-toxigenic and non-pathogenic on the basis of a 28-day oral feeding study conducted with the biomass (Jonker, 2000) (see Section XIII.a.1). Furthermore, *B. trispora* belongs to risk group 1 of the German "Gentechnik-Sicherheitsverordnung" (Regulation on the Safety of Gen-technology) (Robert Koch Institute, 2002), which is comprised of microorganisms that present no risk for humans and vertebrates. The safety of *B. trispora* is further supported by a safety assessment conducted by Dr. Michael Pariza, in which he concluded that *B. trispora* is both non-toxigenic and non-pathogenic (see Appendix D).

III.d Mycotoxins

Enzyme immunoassays for the four mycotoxins identified above for β-carotene (aflatoxin B1, mycotoxin T2, ochratoxin, and zearalenone), were conducted likewise on the lycopene crystal (Table III.d-1) and a sample of the biomass of *B. trispora* (Table III.d-2). Aflotoxin B2, Aflotoxin G1 and Aflotoxin G2 were also analysed for the biomass (Table III.d-2).

Table III.d-1 Product Specifications and Analyses of Lycopene Crystals

Test	Specification*	Batch Number		
		LC 052	LC 054	LC 057
Aflatoxin B1 (µg/kg)	Absent	Absent	Absent	Absent
Mycotoxin T2 (µg/kg)	Absent	Absent	Absent	Absent
Ochratoxin (µg/kg)	Absent	Absent	Absent	Absent
Zearalenone (µg/kg)	Absent	Absent	Absent	Absent

*Analytical methods for specifications can be found in Appendix B

Table III.d-2 Product Specifications and Analyses of the Biomass of *Blakeslea trispora*					
Test	**Specification***	**Batch Number**			
		BC 709	**BC 710**	**BC 711**	**LC 069**
Aflatoxin B1 (µg/kg)	Absent	Absent	Absent	Absent	Absent
Aflotoxin B2 (µg/kg)	Absent	Absent	Absent	Absent	Absent
Aflotoxin G1 (µg/kg)	Absent	Absent	Absent	Absent	Absent
Aflotoxin G2 (µg/kg)	Absent	Absent	Absent	Absent	Absent
Mycotoxin T2 (µg/kg)	Absent	Absent	Absent	Absent	Absent
Ochratoxin (µg/kg)	Absent	Absent	Absent	Absent	Absent
Zearalenone (µg/kg)	Absent	Absent	Absent	Absent	Absent

The results of the mycotoxin analysis reveal that aflatoxin B1, mycotoxin T2, ochratoxin and zearalenone were below the levels of detection in the lycopene crystal. Furthermore, an extended analysis regimen also failed to detect the presence, of mycotoxins in the biomass of *B. trispora*. The analyses were conducted on the biomass that was used in the production process of both lycopene and β-carotene. The exact same biomass is used to produce both carotenoids except that in this case of lycopene the carotenoid synthesis process is terminated at an earlier stage in the production pathway.

IX. INTAKE/EXTENT OF USE OF LYCOPENE CWD PRODUCTS FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the intake/extent of use of the novel food:

- "Is there information on the anticipated uses of the novel food based on its properties?"
- "Is there information to show anticipated intakes for groups predicted to be at risk?"
- "Will introduction of the novel food be restricted geographically?"
- "Will the novel food replace other foods in the diet?"

These questions have been addressed collectively in Sections IX.a through IX.c.

IX.a Conditions of Intended Food Use

Lycopene CWD products are intended for use as a nutritional food ingredient. The individual proposed food-uses for lycopene CWD products from *B. trispora* in the EU are summarized in Table IX.a-1.

Table IX.a-1 Summary of the Individual Proposed Food Uses and Use-Levels for Lycopene (as CWD) in the U.K.

Food Category	Food-Use	Use-Level (ppm)	Use-Level (%)
Beverages	Fortified Juice Mixtures	5	0.0005
	Soft Drinks	5	0.0005
Cereals and Cereal Products	Biscuits	5	0.0005
	Buns, Cakes, and Pastries	5	0.0005
	Cereal Bars	5	0.0005
	Fruit Pies	5	0.0005
	Pudding Powder	5	0.0005
Fruit and Nuts	Pie and Pastry Fruit Filling	5	0.0005
Milk and Milk Products	Fruit Preparation for Fromage Frais	5	0.0005
	Fruit Preparation for Yoghurt	5	0.0005
Sugar, Preserves, and Confectionary	Bakery Fillings	5	0.0005
	Chocolate Confectionary	5	0.0005
	Sugar Confectionary	5	0.0005

IX.a.1 Food Labelling Instructions

Lycopene shall be displayed on the labelling of the food product as such or in the list of ingredients of foodstuffs containing it.

The food product may also incorporate on the label the words “contains an additional source of lycopene” in a typeface, which is at least the same size as the list of ingredients itself.

IX.b Estimated Consumption of Lycopene CWD Products from *Blakeslea trispora* from Proposed Food Uses

IX.b.1 Estimated Daily Lycopene Intake from All Proposed Food-Uses

The Ministry of Agriculture, Fisheries, and Food (MAFF) and the Department of Health were responsible for the joint commission of the National Diet and Nutrition Survey (NDNS) program in 1992. The responsibility for the program was subsequently transferred from MAFF to the FSA upon its inception in April 2000. The NDNS programme itself consists of four different surveys targeting specific age groups, which were conducted every 3 years in succession. Separate survey data are available from the U.K. Data Archive (UKDA) for the NDNS: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for

National Statistics, 2005), the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS 1997) (UKDA, 2001), and the National Diet and Nutrition Survey: People Aged 65 Years and Over, 1994-1995. Although all 4 surveys are available, only the former three were utilized in the generation of estimates in the current intake analysis. When combined, the survey results provide the most current data for use in the evaluation of food-use, food-consumption patterns, and nutritional status for individuals residing within the U.K. Weighted 4- or 7-day food records for individuals were selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001; Office for National Statistics, 2005) as the primary sampling unit.

NDNS data were collected from individuals as well as households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighted dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents or by parents or guardians in the case of the children's survey for the duration of the survey period. NDNS 2000-2001 contains 7-day weighed dietary records for more than 1,724 individuals aged 16 to 64, while NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700 youth aged 4 to 18 (UKDA, 1995, 2001; Office for National Statistics, 2005). Initial postal questionnaires and interviews were employed to identify eligible children, youth, or adults, respectively, for the surveys. Overall, response rates of 93%, 92%, and 73% were achieved; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were, 88%, 80%, and 61%, respectively, while only 81%, 64%, and 47% of surveyed individuals completed a full dietary record (Gregory *et al*., 1995; UKDA, 2001; Office for National Statistics, 2005).

The NDNS programme collects physiological, anthropometric and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption in addition to collecting information on the types and quantities of foods being consumed. Further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples was made possible by the inclusion of this information. In order to compensate for the potential under-representation of intakes from specific population groups resulting from sample variability due to differential sampling probabilities and differential non-response rates [particularly the lower response rate among males aged 15 to 18 years (UKDA, 2001)], sample weights were developed and incorporated with the youth survey (NDNS, 1997).

Weighting the children's survey data to 7 days facilitated the comparison of adult and youth 7-day dietary survey data to dietary data obtained in the 4-day children's survey. This change was based on the assumption that intake patterns on non-recording weekdays were

similar to the intakes on recorded weekdays. The 2 weekend days were not re-weighted. All food and drinks consumed on the 2-recorded weekdays were averaged to obtain a daily intake value, which was then multiplied by 5 to approximate intakes for all weekdays. This data was combined with consumption data from weekend dietary records. The full details of the weighting method employed are provided in Appendix J of the report on the children's diet and nutrition study (Gregory *et al*., 1995).

Estimates for the intake of lycopene (as CWD) by the U.K. population were generated and collated by computer, using consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days. Estimates for the daily intake of lycopene (as CWD) represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data. The distribution from which mean and percentile intake estimates were produced was comprised of these average amounts. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.* when using youth data to estimate intakes, as described in Section 2.1) in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of lycopene (as CWD) averaged over all individuals surveyed regardless of whether they consumed food products in which lycopene (as CWD) is currently proposed for use, and therefore includes "zero" consumers [those who reported no intake of food products containing lycopene (as CWD) during the 7 survey days]. All-user intake refers to the estimated intake of lycopene (as CWD) by those individuals consuming food products in which the use of lycopene (as CWD) is either currently under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which lycopene (as CWD) are proposed for use on one of the 7 survey days.

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

Table IX.b.1-1 summarizes the estimated total intake of lycopene (as CWD) (mg/person/day) from all proposed food-uses by U.K. population group, while Table IX.b.1-2 presents the data on a per kilogram body weight basis (µg/kg body weight/day). The percentage of users was high among all age groups evaluated in the current intake assessment as would be expected for a 7-day survey. Greater than 90.9% of the population groups consist of users of food products in which lycopene (as CWD) is currently proposed for use (Table IX.b.1-1). The population group with the greatest percentage of users was that of young people at 99.5%. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates, and as a consequence, only the all-user intake results will be discussed in detail.

Male teenagers were determined to have the greatest mean and 95th percentile all-user intakes of lycopene (as CWD) on and absolute basis of the individual population groups, with values of 3.54 and 8.16 mg/person/day, respectively (Table IX.b.1-1). Female adults had the lowest absolute intakes with mean and 95th percentile all-user intakes of 1.31 and 4.17 mg/person/day, respectively.

Table IX.b.1-1 Summary of the Estimated Daily Intake of Lycopene (as CWD) from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

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

Table IX.b.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Lycopene (as CWD) from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

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

Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 95th percentile all-user lycopene (as CWD) intakes of 0.15 and 0.36 mg/kg body weight/day, respectively. Female and male adults had the lowest mean and 95th percentile intakes, at 0.02 and 0.06 mg/kg body weight/day (Table IX.b.1-2), respectively.

A complete intake report is provided in Appendix E.

IX.c Conclusions

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

In summary, on an all-user basis, the highest mean and 95th percentile intakes of lycopene (as CWD) by the U.K. population, as observed in male teenagers, were estimated to be 7.00 mg/person/day (0.07 mg/kg body weight/day) and 8.16 mg/person/day (0.16 mg/kg body weight/day). On a body weight basis, children consumed the greatest amount of lycopene (as CWD), with mean and 95th percentile all-user intakes of 0.15 and 0.37 mg/kg body weight/day, respectively. These values are within the range of the dietary intakes of lycopene as estimated by EFSA in its evaluation of a lycopene oil suspension from *B. trispora* (average intake between 0.5 and 5 mg/day, and high intakes up to approximately 8 g/day) (EFSA, 2005b).

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO LYCOPENE

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous human exposure to the novel food:

- “Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”

- “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to nutritional, microbiological, toxicological and/or allergenicity problems?”

These questions have been addressed collectively in Sections X.a and X.b.

X.a Natural Occurrence of Lycopene in the Diet

Lycopene is a normal constituent of the human diet due mainly to its presence in red fruits and vegetables, including tomatoes, watermelon, pink grapefruit, apricots and pink guavas, as well as in algae and fungi (Feofilova, 1994; Nguyen and Schwartz, 1999). The lycopene content of tomatoes can increase with ripening and can vary with different varieties of tomatoes and growing conditions (Clinton, 1998). In the common variety of tomatoes, lycopene is found in concentrations ranging from 3.1 to 7.7 mg/100 g of ripe fruit, and in certain species, levels are as high as 40 mg/100 g tissue (Nguyen and Schwartz, 1999). The consumption of lycopene in the normal human diet has led to it being found as a constituent of mature breast milk (can contain from 9.9 to 60.7 nM lycopene, depending on dietary intake) (Giuliano *et al.*, 1994), and a predominant carotenoid in human plasma (Johnson, 1998; Rao and Agarwal, 1998a,b), contributing between 21 and 43% of total serum carotenoids, with similar levels detected in men and women (Sies and Stahl, 1998).

X.a.1 Intake of Lycopene in Europe

A number of studies have been completed where intakes of dietary lycopene have been assessed in various populations (Forman *et al.*, 1993; Olmedilla *et al.*, 1994; Yong *et al.*, 1994; Jarvinen, 1995; Scott *et al.*, 1996; Agarwal *et al.*, 2001). In a British study conducted with elderly females, the daily consumption of lycopene-rich food such as tomatoes and baked beans in tomato sauce (measured by weight of foods eaten) was equivalent to a daily lycopene intake of 1.03 mg per person (Scott *et al.*, 1996). There was a significantly higher intake of lycopene during the summer and autumn, with similar seasonal variation occurring in the plasma lycopene concentrations (Scott *et al.*, 1996). These results were not supported by Olmedilla *et al.* (1994), who revealed no seasonal variations in lycopene or serum carotenoid levels in a Spanish population. Using data obtained from a dietary history interview conducted with a Finnish population, mean daily intakes of lycopene were calculated to be 698 µg for females and 872 µg for males, which classified it as among the predominant dietary carotenoids, along with β-carotene and lutein (Jarvinen, 1995). In a comparison of various European countries (Spain, France, Ireland, United Kingdom and the Netherlands), median daily lycopene intakes ranged from 1.64 mg (Spain) to 5.01 µg (United Kingdom) (O'Neill *et al.*, 2001). Within the Irish population, O'Neill *et al.* reported daily lycopene intakes of 4.43 mg, whereas Carroll *et al.* (1999) reported values of 7.642 mg in males and 8.045 mg in females. These findings indicate that lycopene intakes vary among countries and even among populations within the same country.

X.a.2 Intake of Lycopene in Canada and the U.S.

Using a tomato products consumption frequency questionnaire, the average daily dietary intake of lycopene in the Canadian population (represented by male and female healthy subjects with mean age of 29 years) was calculated to be approximately 25.2 mg per person. Fresh tomatoes accounted for 50% of the daily lycopene intake and the various tomato products, including tomato paste, sauce and juice, accounted for the remaining 50%

(Agarwal *et al*., 2001). A separate study investigating lycopene intake in the Canadian population used the data compiled by the Nutrition Coordinating Center and the United States Department of Agriculture (USDA-NCC Carotenoid Database) together with data from one 24-hour recall/person (Johnson-Down *et al*., 2002). Mean and median intakes of 6.3 and 1.3 mg lycopene/day, respectively were reported for Canadian adults, age 18 to 65 years (Johnson-Down *et al*., 2002). In two separate studies from the United States conducted with males (Forman *et al*., 1993) or pre-menopausal females (Yong *et al*., 1994), assessment of food frequency questionnaires and food diaries revealed a daily lycopene intake of approximately 3.7 mg (males) or 3.1 mg (females) per person. Using the United States Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals (USDA CSFII 1989-91), and correcting the intake estimates on the basis of longer term consumption data (14-day food consumption records from the Institute of Europe, 1998) (McGirr and Copeland, 2000), the chronic mean and 90th percentile lycopene intakes for the general U.S. population approximated 4.7 mg/day (0.08 mg/kg body weight/day) and 11.3 mg/day (0.19 mg/kg body weight/day), respectively (McGirr and Copeland, 2000). Matulka *et al*. (2004) reported estimated mean and 90th percentile lycopene intakes of 8.2 and 15.7 mg/day, respectively, based on food consumption data from the USDA CSFII 1994-1996 database and food lycopene content data from the USDA-NCC Carotenoid database.

X.a.3 Intake of Lycopene as a Colour and Dietary Supplement

Lycopene from *B. trispora* as an oil suspension is authorised as a novel food ingredient in Europe (2006/721/EC) at levels within the range of background dietary lycopene intake (Commission of the European Communities, 2006).

In addition to its presence in foods (*e.g.*, red fruits and vegetables), lycopene extracted from tomatoes is authorised as a colour (E 160d Directive 94/36/EC) and is available as a dietary supplement throughout Europe (European Parliament and the Council of the European Union, 1994); however, there are no reliable estimates of the amounts of dietary supplements consumed either in Europe or the United States and Canada (IOM, 2000), whereas synthetic lycopene is currently not approved for colouring matters within the EU (SCF/CS/ADD/COL/160 Final 6/12/99) (SCF, 1999) but is considered Generally Recognised as Safe (GRAS) for use as a food ingredient in the U.S. (GRAS Notice No. GRN 000119) (U.S. FDA, 2005).

X.b Potential Allergenicity Concerns

The potential allergenicity of lycopene from *B. trispora* has been addressed and is covered in Section XIII.

XI. NUTRITIONAL INFORMATION ON LYCOPENE FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- "Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"

This question has been addressed in Sections XI.a through XI.b.

XI.a Nutritional Equivalence to Existing Foods

The dietary sources of lycopene have been identified in Section X.a, and the equivalence of lycopene from *B. trispora* to natural (dietary) lycopene has been discussed in Section II.c. Since the other components of the final lycopene CWD products (α-tocopherol and octenyl succinic anhydride starches) are similarly present in food, it is expected that the lycopene CWD products derived from *B. trispora* are nutritionally equivalent to naturally occurring lycopene.

XI.b Nutritional Benefits of 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 shown 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 unsurpassed singlet-oxygen-quenching ability (Di Mascio *et al.*, 1989; Khachik *et al.*, 1995; Agarwal and Rao, 2000; Rao and Agarwal, 2000). Although non-oxidative mechanisms of lycopene have been identified (*i.e.*, gene function regulation, regulation of gap-junction communication, hormone and immune modulation, and regulation of metabolism), it is the antioxidant properties that are currently considered to be primarily responsible for lycopene's potential health benefits (Agarwal and Rao, 2000; Rao *et al.*, 2002).

Chronic diseases causally related to oxidative stress include cancer, cardiovascular disease, age-related macular degeneration, Parkinson's disease, and inflammatory conditions such as sepsis and cystic fibrosis; however, the focus of the majority of lycopene studies to date has been on the former 2 diseases (Gerster, 1997; Weisburger, 1998; Giovannucci, 1999; Arab and Steck, 2000).

Clinical trials and epidemiology studies have shown that lycopene from "natural" food sources is readily absorbed and is present in plasma and breast milk. Based on the epidemiological data, it is hypothesized that higher dietary intakes of tomatoes and tomato-based products, resulting in higher blood levels of lycopene, may help reduce the risk of certain cancers. Giovannucci (1999) conducted a review of 72 epidemiological studies including cohort, case-control, diet-based and biomarker-based studies, which investigated the consumption of tomatoes and related products, blood lycopene levels, and cancer incidence at various anatomical sites. A total of 57 studies demonstrated inverse associations between intakes of tomatoes or lycopene or blood lycopene levels and risk of cancer, 35 of which were statistically significant. These data indicate that high consumers of tomatoes and tomato products are at substantially decreased risk of numerous cancers, with the strongest associations observed for cancers of the prostate gland, lung, and stomach across numerous diverse populations. A summary of the epidemiologic literature relating the intake of lycopene or blood lycopene levels to prostate cancer risk is included in Appendix F, and is representative of the extensive literature exploring the role of lycopene in cancer

prevention. In general, despite a current lack of prospective long-term clinical trials, the role of lycopene in disease prevention is suggestive of a protective effect (Giovannucci, 1999; Agarwal and Rao, 2000).

Although the data are limited, scientific reviews prepared separately by Agarwal and Rao (2000) and Rissanen (2002) have examined the available epidemiological evidence pertaining to lycopene and cardiovascular disease. Together, the data are suggestive of an inverse relationship between lycopene intake (and biomarkers thereof), and risk of cardiovascular disease. The strongest population-based evidence comes from a multicentre case-control study in which subjects were recruited from 10 European countries (Kohlmeier *et al.*, 1997). Results demonstrated an inverse relationship between intimal wall thickness/risk of myocardial infarction and adipose tissue concentrations of lycopene (Kohlmeier *et al.*, 1997). Based on these findings, it has been concluded that increased lycopene intake may have a protective role in prevention of cardiovascular disease, and may contribute to cardiovascular health (Rissanen, 2002).

XII. MICROBIOLOGICAL INFORMATION ON LYCOPENE FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

This question has been addressed in Tables XII.a-1 through XII.a-3.

XII.a Microbiological Specifications and Analyses for Lycopene Crystals and Lycopene Cold Water Dispersible (CWD) Products (10% and 20%)

As outlined in Tables XII.a-1, XII.a-2 and XII.a-3, typical food borne microbes (*e.g.*, moulds, yeasts, *Salmonella*, *E. coli*) are not present in the final lycopene crystals, lycopene 10% CWD, and lycopene 20% CWD products, respectively.

Table XII.a-1 Microbiological Specifications and Analyses of Lycopene Crystals

Test	Specification*	Batch Number		
		LC 052	LC 054	LC 057
Moulds (cfu/g)	≤ 100/g	Absent	Absent	Absent
Yeasts (cfu/g)	≤ 100/g	Absent	Absent	Absent
Salmonella (cfu/25g)	Absent in 25 g	Absent	Absent	Absent
Escherichia coli (cfu/g)	Absent in 5 g	Absent	Absent	Absent

*Certificates of analysis and analytical methods for specifications in Appendix A

Table XII.a-2 Microbiological Specifications and Analyses of Lycopene 10% CWD Product

Test	Specification*	Batch Number		
		152	174	176
Moulds (cfu/g)	≤ 100	<10	10	≤ 10
Yeasts (cfu/g)	≤ 100	<10	10	≤ 10
Salmonella (cfu/25g)	Absent in 25 g	Absent	Absent	Absent
Escherichia coli (cfu/5g)	Absent in 5 g	Absent	Absent	Absent

*Certificates of analysis and analytical methods for specifications in Appendix A

Table XII.a-3 Microbiological Specifications and Analyses of Lycopene 20% CWD Product

Test	Specification*	Batch Number		
		180	182	184
Moulds (cfu/g)	≤ 100	<10	<10	<10
Yeasts (cfu/g)	≤ 100	≤ 10	<10	<10
Salmonella (cfu/25g)	Absent in 25 g	Absent	Absent	Absent
Escherichia coli (cfu/5g)	Absent in 5 g	Absent	Absent	Absent

*Certificates of analysis and analytical methods for specifications in Appendix A

XIII. TOXICOLOGICAL INFORMATION ON LYCOPENE FROM *BLAKESLEA TRISPORA*

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"

- "Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?"

OR

- "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"

- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These questions have been addressed collectively in Sections XIII.a through XIII.b.

XIII.a Toxicological Assessment of *Blakeslea trispora*

As described in Section II.a, lycopene is produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *B. trispora*. Both mating types are stable cultures and were preserved under conditions consistent with Good Manufacturing Practices. The strains are considered to be non-toxigenic and non-pathogenic on the basis of a 28-day oral feeding study conducted with the biomass (Jonker, 2000) (see Section XIII.b.2.1). In addition, *B. trispora* belongs to risk group 1 of the German "Gentechnik-Sicherheitsverordnung" (Regulation on the Safety of Gen-technology) (Robert Koch Institute, 2002), which is comprised of microorganisms that present no risk for humans and vertebrates. The safety of *B. trispora* is further supported by a safety assessment conducted by Dr. Michael Pariza, in which he concluded that *B. trispora* is both non-toxigenic and non-pathogenic (see Appendix D).

In further support of the safety of *B. trispora*, the SCF has considered β-carotene from *B. trispora*, produced *via* an identical biosynthetic route and process as lycopene from *B. trispora* (see Figure III.b-1), acceptable for use as a colouring agent for foodstuffs. Following review of the available safety information, the Committee concluded that the "source organism and the production process yielded no grounds to suppose that the final crystalline product, β-carotene, differs from the chemically synthesised β-carotene used as a food colorant" (SCF, 2000). The fact that the SCF has already considered β-carotene from

B. trispora to be acceptable for use as a colouring agent, provides reassurance that lycopene, formed from the same starting organism as an intermediary product in the synthesis of β-carotene, is of no safety concern. Similarly, the JECFA concluded that "on the basis of the source organisms, the production process, and it composition characteristics, β-carotene from *B. trispora* does not raise specific concerns and from a toxicological point of view should be considered equivalent to chemically synthesized β-carotene…" (JECFA, 2002).

XIII.b Toxicological Assessment of Lycopene from *Blakeslea trispora*

Due to recent concerns regarding the suitability of the rat as an appropriate model for the study of β-carotene absorption and safety in humans (SCF, 2000), documentation is included in Appendix G that addresses the same concerns regarding lycopene. In brief, extensive literature searches were conducted, and the relevant data (*e.g.*, studies reporting post-prandial serum and organ levels of lycopene in rats and humans) were compiled in order to compare the uptake and tissue distribution of lycopene between humans and rats. The available data in rats indicate that lycopene is absorbed into the systemic blood supply and is distributed to the tissues in a similar distribution pattern as that seen in humans (Mathews-Roth *et al.*, 1990; Boileau *et al.*, 2000). Tissue levels in rats were comparable to those measured in human tissues, with the highest levels of lycopene occurring in the liver, adrenal glands, and testes. Based on these analyses, it was concluded that the rat can be regarded as a useful and appropriate animal model for the study of lycopene.

Although the source of the lycopene produced by Vitatene is fungal, toxicological data for both natural (fungal, dietary) and synthetic lycopene are included to ensure a comprehensive dataset. The safety of lycopene from *B. trispora* was assessed in two separate sub-chronic studies, one of which examined the safety of a lycopene oil suspension, and the other examined the safety of the biomass (see Section XIII.b.4.1). In addition, genotoxicity studies on lycopene CWD products from *B. trispora* have yielded negative results (see Section XIII.b.5.1). The safety of lycopene from sources other than fungal (*i.e.*, corroborative safety data) was assessed in various sub-chronic and chronic, carcinogenicity, genotoxicity/ mutagenicity, and reproductive toxicity studies, which are described in detail in Sections XIII.b.4.2, XIII.b.5, XIII.b.6, and XIII.b.7, respectively. The relevancy of the corroborative data is supported by the chemical similarities between lycopene produced from *B. trispora*, naturally occurring lycopene from tomatoes, and synthetic lycopene, which are outlined in Table II.c-1.

XIII.b.1 Metabolism/Toxicokinetics

The following provides an overview of the absorption, distribution, metabolism and excretion of lycopene. Given that the lycopene molecule produced biosynthetically from *B. trispora* is identical to that which is produced in the tomato, it is expected that there will be no differences in the bioavailability and metabolic breakdown products of the two compounds.

XIII.b.1.1 Absorption of Lycopene

Lycopene, like all carotenoids, is fat-soluble and therefore follows the same digestion and intestinal absorption pathways as dietary fat (Furr and Clark, 1997; Johnson, 1998; van den Berg, 1998). In general, optimal absorption of dietary carotenoids begins with their release from the food matrix and dissolution in the lipid phase, followed by incorporation into lipid micelles in the small intestine, which is required for mucosal uptake, and finally, transport to the lymphatic and/or portal circulation (Erdman *et al*., 1993). Lipid micelles are oil in water emulsions formed in the intestinal lumen, which consist of the lipids from the intestinal tract, such as free fatty acids, monoglycerides and phospholipids (non-polar centres), and bile salts (emulsifying agent) (Guyton and Hall, 1996). Movement of carotenoids from the mixed lipid micelle into the mucosal cells of the duodenum appears to occur *via* passive diffusion (Erdman *et al*., 1993; Parker, 1996), and subsequent transport from the enterocytes to the blood stream involves incorporation into chylomicrons (lycopene in the hydrophobic core), and secretion into the lymphatics (Parker, 1996; Johnson, 1998; van den Berg, 1999). Carotenoids that are taken up by the mucosal cells but not incorporated into chylomicrons are sloughed off when the enterocytes turn over, and are excreted into the lumen of the gastrointestinal tract (Boileau *et al*., 1999a).

Several studies in humans have examined serum concentrations of lycopene following dietary supplementation with tomato products and/or lycopene supplements, all of which have demonstrated increased plasma levels following dietary intake (Stahl and Sies, 1992; Rock *et al*., 1997; Paetau *et al*., 1998; Porrini *et al*., 1998; Rao and Agarwal, 1998b; Broekmans *et al*., 2000; Chopra *et al*., 2000; Stahl *et al*., 2001; Olmedilla *et al*., 2002; Hadley *et al*., 2003). The data demonstrate inter-individual variability in the uptake of lycopene (Stahl and Sies, 1992; O'Neill and Thurnham, 1998), which is dose-dependent but not linear with the dose (*i.e*., relatively more lycopene is absorbed when lower amounts are consumed, suggesting saturation of lycopene absorptive mechanisms at higher doses) (Stahl and Sies, 1992). In response to a single dose of lycopene, peak serum concentrations were reached between 24 to 48 hours following consumption of an unspecified amount of heated tomato juice (providing approximately 80 mg lycopene) (Stahl and Sies, 1992), and between 4 to 6 hours following consumption of encapsulated lycopene (approximately 38 mg lycopene) (O'Neill and Thurnham, 1998). In response to multiple doses of lycopene, plasma lycopene concentrations continued to increase throughout a 7-day intervention period with tomato products (Porrini *et al*., 1998), and steady state concentrations were reached by the 1st or 2nd week of a 4-week dietary intervention with lycopene supplements or tomato juice, respectively (Paetau *et al*., 1998).

The absorption of lycopene has also been demonstrated in various experimental animal models. Using the mesenteric lymph duct cannulated rat as an animal model, Clark *et al*. (1998) investigated the absorption of purified lycopene following continuous infusion with an emulsion containing 20 µmol/L of lycopene (2.5 mL/hour) *via* a feeding tube placed into the duodenum. Lymph samples were collected at 2, 4, 6, 8, 10, and 12 hours after the start of infusion. Based on reported body weights, exposure to lycopene at each time point

corresponded to 0.14, 0.29, 0.43, 0.57, 0.72, and 0.86 mg lycopene/kg body weight, respectively. Absorption, calculated by dividing the concentration of lycopene recovered in the lymph per hour by the concentration of lycopene infused into the duodenum per hour, ranged from 2 to 8%, with an average recovery of 6%. Lycopene was absorbed intact in a dose-dependent manner, with a steady-state reached in the lymph following 6 hours of continuous intraduodenal infusion (Clark *et al*., 1998). Oshima *et al*. (1999) examined the colonic absorption of lycopene that escapes intestinal absorption in Sprague-Dawley rats. Animals received a single dose of 12 mg lycopene (56 mg/kg body weight) administered directly into the colon or the stomach, and were euthanized 24 hours post-exposure for determination of lycopene content in the faeces, and tissues (jejunum, colon, liver). Based on HPLC analyses, lycopene was detected in the blood within 4 to 8 hours after the single dose, and was subsequently deposited in the liver, suggesting that lycopene is absorbed from the colon as well as the small intestine of rats (Oshima *et al*., 1999). Zaripheh *et al.* (2003) also reported increased serum ^{14}C in male rats within 72 hours of receiving an oral dose of radiolabelled lycopene, while Korytko *et al.* (2003) reported increased plasma lycopene levels in dogs following oral dosing.

XIII.b.1.2 Bioavailability of Lycopene

The term bioavailability is used to describe the degree of absorption of an ingested nutrient (*i.e*., the fraction of the ingested nutrient that appears in the portal circulation). Therefore, non-dietary and dietary factors that may interfere with the rate and/or completeness of any of the steps involved in carotenoid absorption will affect the overall bioavailability (Furr and Clark, 1997). In general, non-dietary factors that interfere with carotenoid bioavailability are largely related to the health status of an individual and include intestinal malabsorption diseases, intestinal parasites, liver or kidney disease, and drug interactions (Erdman *et al*., 1993; Castenmiller and West, 1998). Dietary factors affecting the bioavailability of lycopene are discussed separately below and include the digestibility of the food matrix, the level of fat present and/or absorbed from the diet, interactions with other carotenoids, and lycopene isoforms.

XIII.b.1.2.1 Digestibility of the Food Matrix

The efficiency of carotenoid release from food matrices is dependent upon several factors including the physical disposition of the carotenoid within the food matrix, the effectiveness of mastication and stomach action on reducing particle size, and the action of digestive enzymes (Parker, 1996; van den Berg, 1999). Several studies have shown that mechanical homogenization and heat treatment of tomato products increases lycopene bioavailability in humans, as evidenced by increased lycopene serum concentrations and increased chylomicron lycopene responses following ingestion of tomato paste *versus* fresh tomatoes, and heated *versus* unheated tomato juice (Stahl and Sies, 1992; Gärtner *et al*., 1997; Porrini *et al*., 1998). It is postulated that these responses are due to an increased extractability of lycopene from the vegetable matrix. In contrast, food form (vegetable juice *versus* raw or

cooked vegetables) had no effect on β-carotene bioavailability, as measured by serum values in female breast cancer patients (McEligot *et al*., 1999).

Regarding lycopene absorption from purified or synthetic sources (*i.e.*, supplemental lycopene), Böhm and Bitsch (1999) demonstrated that absorption from soft gel capsules containing tomato oleoresin was comparable to that from processed tomato products, and greater than that from raw tomatoes (Böhm and Bitsch, 1999). Hoppe *et al.* (2003) reported no significant differences between synthetic and natural (tomato-based) lycopene in serum total lycopene levels. In a recent study, the bioavailability of synthetic lycopene (in corn oil) was found to be approximately 3 times higher than that of lycopene from steamed and pureed tomatoes (Tang *et al.*, 2005a).

XIII.b.1.2.2 Dietary Fat

Once released from the food matrix, the incorporation of carotenoids into mixed micelles is dependent upon the presence of fat in the intestine, which stimulates the secretion of bile acids and increases the size and stability of micelles (Erdman *et al*., 1993; van het Hof *et al*., 2000). Therefore, reduced intestinal absorption of lipids would be expected to adversely affect the absorption of lycopene. For example, non-absorbable fat substitutes, such as Olestra and sucrose polyester, have been reported to significantly reduce the absorption of lycopene in male and female volunteers, as indicated by reductions in serum lycopene concentrations (Weststrate and van het Hof, 1995; Koonsvitsky *et al*., 1997). In the same regard, cholestyramine, a bile salt sequesterant drug known to decrease the absorption of lipids, and probucol, a cholesterol-lowering drug that reduces both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) particle size, each reduced serum lycopene levels by approximately 30% in a double blind, randomized trial reported by Elinder *et al*. (1995).

XIII.b.1.2.3 Nutrient Interactions

Interactions, both competitive and synergistic, between carotenoids may 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 (see Section XIII.b.1.2) (Furr and Clark, 1997; van den Berg, 1998, 1999). Castenmiller *et al*. (1999) and van het Hof *et al*. (1999) have shown in healthy adults that consumption of β-carotene and lutein from purified supplements as well as from spinach products decreases endogenous serum lycopene levels compared with control groups. Further evidence of competitive interactions between various carotenoids and lycopene was reported by Tyssandier *et al*. (2002) who demonstrated in healthy female volunteers that lycopene and lutein, but not lycopene and β-carotene, compete with each other for incorporation into chylomicrons. Postprandial chylomicron lycopene responses to tomato purée were diminished when a source of lutein (in its natural vegetable matrix and as a purified supplement) was ingested together with the lycopene source, and similarly, postprandial chylomicron lutein responses to chopped spinach were diminished when a

source of lycopene (in its natural vegetable matrix and as a purified supplement) was ingested together with the lutein source.

In a separate experiment reported by Tyssandier *et al*. (2002), plasma levels of lutein and lycopene were analyzed following 3 weeks of dietary supplementation with various combinations of carotenoid-rich vegetables and supplements. Results revealed that adding lutein to tomato purée enhanced, rather than diminished the plasma lycopene response, and that adding lycopene to chopped spinach had no effect on the plasma lutein response. Although inconsistent with the chylomicron responses reported in the same study (Tyssandier *et al*., 2002), and with results reported by Castenmiller *et al*. (1999) and van het Hof *et al*. (1999), evidence suggestive of a synergistic interaction between carotenoids has been reported elsewhere. Following 24 months of daily supplementation with β-carotene capsules, endogenous lycopene serum concentrations were significantly increased in male and female subjects with colorectal adenomas (Wahlqvist *et al*., 1994), and Johnson *et al*. (1997) demonstrated in healthy males that oral ingestion of gelatine capsules containing lycopene plus β-carotene (60 mg of each) significantly increased the serum lycopene response (measured by 24-hour area under the curve), and had no effect on the serum β-carotene compared with their ingestion alone. Null results were obtained by Nierenberg *et al*. (1997) who reported no change in endogenous lycopene serum concentrations following 4 years of oral supplementation with β-carotene, and by van den Berg and van Vliet (1998) who reported that supplementation with lycopene had no effect on β-carotene absorption (measured by chylomicron response) when ingested simultaneously (15 mg of each).

Two main types of studies have examined the effects of carotenoid interactions on lycopene bioavailability. Those studies examining endogenous serum concentrations of lycopene following dietary supplementation with lutein and/or β-carotene have revealed equivocal results, with synergistic (Wahlqvist *et al*., 1994), antagonistic (Castenmiller *et al*., 1999; van het Hof *et al*., 1999), and null (Nierenberg *et al*., 1997) interactions reported. Studies examining serum responses to dietary supplementation with lycopene plus lutein or β-carotene have shown synergistic effects (Johnson *et al*., 1997; Tyssandier *et al*., 2002), while chylomicron responses to dietary supplementation with lycopene plus lutein have revealed antagonistic results (Tyssandier *et al*., 2002), and chylomicron responses to dietary supplementation with lycopene plus β-carotene have revealed equivocal or null results (van den Berg and van Vliet, 1998). Taken together, these results demonstrate that interactions between both exogenous and endogenous carotenoids occur; however, the mechanisms *via* which this occurs is not clear, and due to equivocal nature of the results, definite relationships between specific carotenoids cannot be established.

In addition to the carotenoid interactions discussed above, the effects of other nutrients, specifically dietary fibre and plant sterol/stanol esters, on the bioavailability of lycopene have been investigated in humans. Riedl *et al.* (1999) tested the effects of various fibres (0.15 g/kg body weight of pectin, guar, alginate, cellulose, or wheat bran added to a standard meal) on the bioavailability of supplemental lycopene (0.7 mg/kg body weight) in healthy female volunteers. Each type of dietary fibre significantly reduced the plasma response

curve of lycopene, with a calculated decrease in relative absorption of approximately 40 to 74% (Riedl *et al*., 1999). The effects of sterol and stanol esters on serum carotenoid concentrations were investigated in male hypercholesterolemic subjects following ingestion of lycopene-containing diets supplemented with 2 g/day sterol or stanol esters (delivered *via* margarine mixtures) for 21 days (Raeini-Sarjaz *et al*., 2002). No effect of sterol or stanol ester on serum lycopene concentrations was reported, suggesting that neither compound had a negative effect on lycopene absorption (Raeini-Sarjaz *et al*., 2002).

Nutrient interactions in foods resulting from lycopene from *B. trispora* are unlikely to be different from those of lycopene occurring naturally in food products due to the structural similarity of lycopene from both sources (see Section II.c).

XIII.b.1.2.4 Cis/trans Isomers

Lycopene occurs in several geometrical isomers (Schierle *et al*., 1997). In most foods, lycopene occurs in the all-*trans* configuration (Schierle *et al*., 1997; Clinton, 1998; Hadley *et al*., 2003), whereas in human blood plasma and tissues, the *cis* isomers comprise approximately 50% or more of the total lycopene content (Stahl and Sies, 1992; Schierle *et al*., 1997; Hadley *et al*., 2003). Despite the fact that very little is currently known about the specific biological roles of *cis* or *trans* isomers (Clinton, 1998), several studies, the majority of which have been conducted with experimental animals, have examined the possible effects that isomeric form may have on lycopene bioavailability (Stahl and Sies, 1992; Boileau *et al*., 1999b, 2000; Ferreira *et al*., 2000).

Stahl and Sies (1992) measured the serum concentrations of various lycopene isomers (all-*trans*, 9-*cis* and 13-*cis* lycopene) in healthy male and female humans following tomato juice consumption, and reported that the *cis* isomers were absorbed to a slightly greater extent (or were metabolized to a lesser extent) compared with the all-*trans* isomer. Similarly, Müller *et al*. (1999) demonstrated that the consumption of tomato juice containing 95% *trans*-lycopene for 2 weeks resulted in a substantial increase in plasma levels of *cis*-lycopene, which remained elevated up to 4 weeks after the tomato juice intervention. Gustin *et al.* (2004) also found higher proportions of *cis* isomers in plasma and chylomicrons than would have been anticipated relative to the actual proportions in the lycopene formulation administered to healthy male volunteers.

Boileau *et al*. (2000) examined tissue lycopene isomer patterns following 8 weeks of dietary supplementation with lycopene in male F344 rats. Lycopene was incorporated into the diet as water-dispersible beadlets delivering from 0 to 0.50 g lycopene/kg body weight, with the 2 major isomers in each of beadlets and diet being all-*trans* and 5-*cis* lycopene. As dietary lycopene increased, so did the percentage of lycopene as *cis*-isomers in serum and tissues. In contrast, following continuous intraduodenal administration of increasing concentrations of all-*trans* lycopene prepared with olive oil or corn oil for 12 hours, no isomerisation of lycopene was observed in the lymph of male Holtzman albino rats (Clark *et al*., 2000). Following oral supplementation with 4.6 mg lycopene (93% all-*trans*, 7% *cis* isomers)/kg

body weight/day in a tomato oleoresin-corn oil mixture for 9 weeks, all-*trans* lycopene was reported to be the most predominant isomer in male F344 rat tissues, whereas in male ferrets receiving the same dietary treatment, *cis* lycopene was the predominate isomer isolated in tissues (Ferreira *et al*., 2000). In a separate study conducted with male ferrets, the *cis*-isomer composition of digestive fractions, as well as the serum, liver, and lungs were determined following a single oral administration of a soybean oil mixed with Lycored™ delivering 40 mg lycopene/kg body weight (Boileau *et al*., 1999b). The dose, stomach and intestinal contents contained from 6.2 to 17.5% *cis*-lycopene, suggesting a lack of significant isomerisation; however, the mesenteric lymph secretions contained significantly more *cis*-lycopene (77.4%), suggesting that *cis*-isomers of lycopene are more bioavailable than *trans*-lycopene (Boileau *et al*., 1999b). In a study conducted in male Beagle dogs, the proportion of *trans* isomers in all tissues was lower than that of *cis* isomers, despite the higher percentage of *trans* isomers in the administered lycopene (30% *cis* and 70% *trans*) (Korytko *et al.*, 2003).

Based on the limited data, definitive comparisons between the bioavailability of *cis*- and *trans*-lycopene cannot be made; however, the data is suggestive of increased bioavailability from the *cis* isoform, which indicates an enhanced solubility of *cis*-lycopene in bile acid micelles and possibly preferential incorporation into chylomicrons (Boileau *et al*., 1999b). Other possible explanations for increased plasma levels of *cis*-lycopene (*i.e*., other than preferential uptake and increased bioavailability) could be the conversion of *trans*- to *cis*-lycopene, or a higher degradation rate of *trans*- compared with *cis*-lycopene (Müller *et al*., 1999).

XIII.b.2 Distribution, Metabolism and Excretion of Lycopene

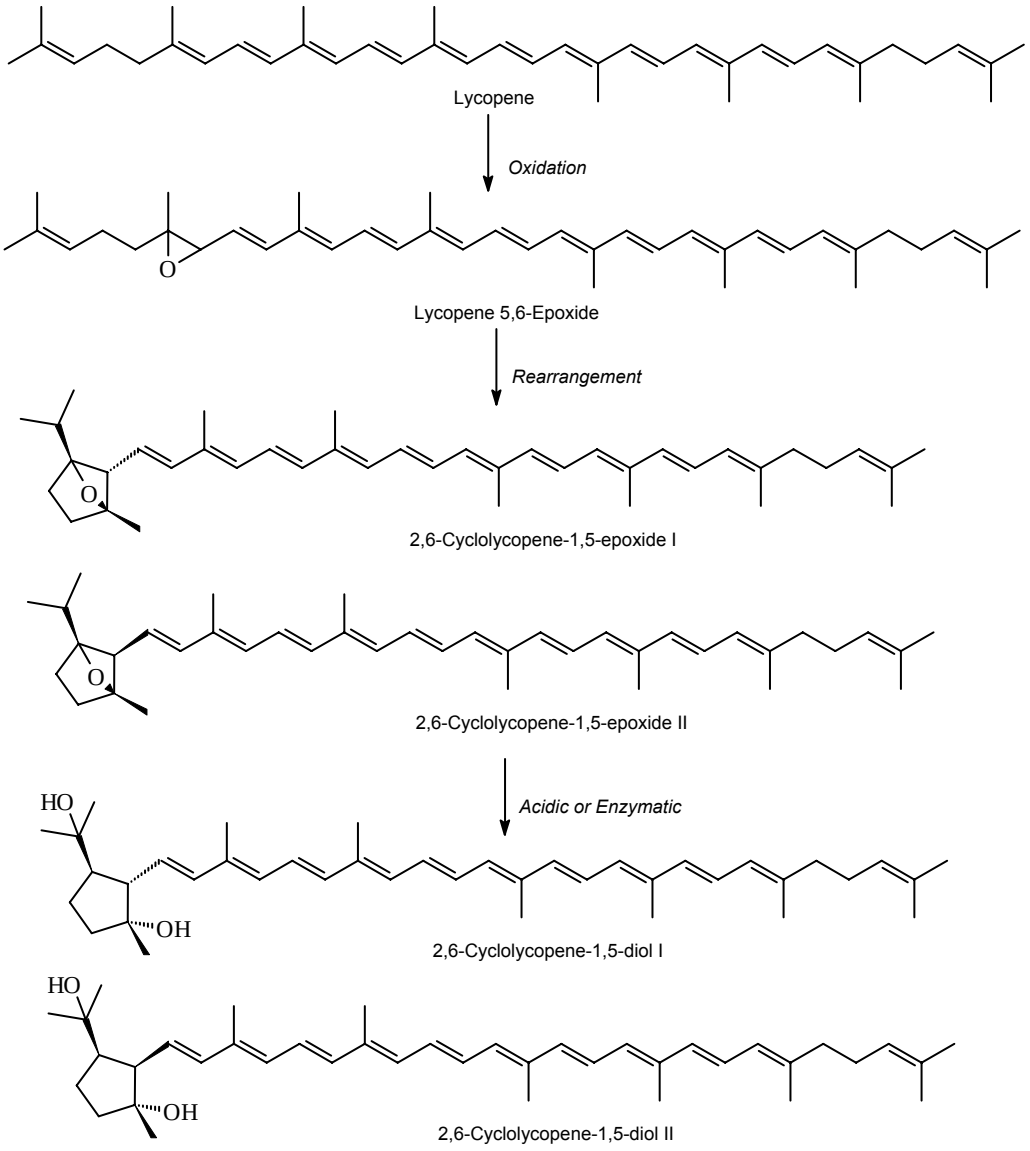
Carotenoids are transported in blood plasma exclusively *via* lipoproteins (Erdman *et al*., 1993; Parker, 1996). Once chylomicrons have entered the blood stream from the lymphatics (see Section XIII.b.1.1), they become decomposed through the action of lipoprotein lipases and circulating plasma lipoproteins (Erdman *et al*., 1993; van den Berg, 1999). The remaining chylomicron remnants are subsequently taken up by the liver where the internalized apolar carotenoids (including lycopene) are either stored or repackaged for re-release into the blood stream (Erdman *et al*., 1993; van den Berg, 1999). Following hepatic re-secretion, lycopene is primarily transported in LDL, from where it is presumably transferred to tissues through interactions with LDL receptors (Kaplan *et al*., 1990; Johnson, 1998; Paetau *et al*., 1998; Mayne *et al*., 1999; Chopra *et al*., 2000; Maruyama *et al*., 2001). It is unclear what factors come into play regarding uptake of carotenoids by tissues (Erdman *et al*., 1993); however, evidence that they accumulate in human tissues indicates that the transfer from plasma lipoproteins to tissues is effective (Johnson, 1998; Sies and Stahl, 1998). Analyses of human tissue samples have revealed that lycopene is found predominantly in the liver, adrenals and testes, with lower amounts found in the kidneys, lungs, ovaries, and fat tissue (Schmitz *et al*., 1991; Stahl *et al*., 1992). Other human tissues containing detectable levels of lycopene include skin, colon and breast (Nierenberg and Nann, 1992). Similarly, the liver was the major site of storage in rats and monkeys 48 to 72

hours after a single oral dose of radioactive lycopene, with smaller amounts of radioactivity noted in the colon, intestine, stomach, lungs, and ovaries (Mathews-Roth *et al*., 1990). In dogs, the highest lycopene concentration was found in the liver, followed by the adrenals, spleen and lymph node (Korytko *et al.*, 2003).

To date, very little is known of the metabolism or excretion of lycopene in mammals (Parker, 1996; Clinton, 1998; Bramley, 2000). As a non-provitamin A carotenoid, lycopene is not converted to retinoid metabolites *via* interaction with β-carotene-15,15'-dioxygnease (Ershov *et al.*, 1993; van Vliet *et al.*, 1996). However, in ovariectomized HotIbm:ROHO(spf) rats with established vaginal keratinisation on a vitamin A-deficient diet, oral doses of 1.86 or 3.72 µmol/rat over 2 days had small but significant pro-vitamin A activity (measured *via* epithelial protection tests) (Weiser and Kormann, 1993).

Paetau *et al*. (1998) identified 2,6-cyclolycopene-1,5-diol I as the major metabolite of lycopene in human plasma following chronic ingestion of lycopene-rich tomato juice or lycopene supplements (*e.g.*, lycopene beadlets). Similarly, Khachik *et al*. (1995, 1997a,b) reported the presence of 2,6-cyclolycopene-1,5-diol I (major component) and 2,6-cyclolycopene-1,5-diol II (minor component) in the milk and serum of healthy lactating females, as well as 5,6-dihydroxy-5,6-dihydrolycopene from human serum/plasma. It has been hypothesized that these metabolites are formed *via* the metabolic oxidation of lycopene to lycopene 5,6-epoxide, which subsequently undergoes enzymatic or acidic hydrolysis and forms the epimeric mixture of 2,6-cyclolycopene-1,5-diol I and II (Khachik *et al*., 1995, 1997a,b). This hypothesis has been supported by results obtained by Müller *et al*. (1999), who demonstrated increased plasma levels of lycopene epoxides following 2 weeks of dietary intervention with tomato juice. Figure XIII.b.2-1 outlines the proposed metabolic pathway described above, and includes two intermediate compounds (2,6-cyclolycopene-1,5-epoxides I and II) that are proposed to be formed from the rearrangement of lycopene 5,6-epoxide, and to serve as the precursors for 2,6-cyclolycopene-1,5-diol I and II, respectively (Khachik *et al*., 1997b).

Figure XIII.b.2-1 Proposed Metabolic Pathway for Oxidation of Lycopene (adapted from Khachik *et al*., 1997b)



As evidenced by symptoms of excessive lycopene intake (see Section XIII.b.8.1), lycopene appears to be excreted by the sudoral and sebaceous glands, and is in part reabsorbed by the horny layer of the skin; therefore, when ingested in excessive amounts, lycopene causes a yellow discoloration of the skin, which is diagnosed as lycopenodermia (La Placa *et al*., 2000). The kinetics of carotenoid depletion and elimination has been investigated in healthy men (Rock *et al.*, 1992) and women (Burri *et al.*, 2001) fed controlled low-carotenoid diets for approximately 13 and 10 weeks, respectively. In males, there were significant decreases in levels of carotenoids (including lycopene) between Days 2 and 3, and between Days 14 and 15, which may be indicative of 2 separate pools of carotenoids in the body; one that is

rapidly responsive to changes in carotenoid intake, and one that is more resistant. The mean plasma depletion half-life (t½) for lycopene was calculated to be between 12 and 33 days (Rock *et al.*, 1992). In females, the decline in serum concentrations of all carotenoids (including lycopene) followed apparent first-order kinetics, and the t½ for lycopene was 26 days (Burri *et al.*, 2001). In a third study conducted with subjects fed a low carotenoid diet (over a 2-week period), the plasma t½ of lycopene was estimated to be approximately 14 days (Brown *et al*., 1989). In subjects given a single serving of tomato juice, the t½ of lycopene was determined to be between 2 and 3 days (Stahl and Sies, 1992).

Experimental animal models have been employed for the evaluation of lycopene distribution. In ddY and ICR mice, the highest concentration of lycopene following parenteral administration was detected in lung tissue, followed by spleen and adrenal (Senanayake *et al*., 2000). In F344 rats, the highest concentration of lycopene, together with 2,6-cyclolycopene-1,5-diols I and II and their epoxides (identified using HPLC), was detected in the liver following dietary exposure to lycopene for 10 weeks. Lycopene was also detected at smaller levels in mammary tissue, prostate tissue, lung, and colon (Zhao *et al*., 1997). Similarly, in both monkeys and rats, the liver contained the largest amount of lycopene following administration of a single radiolabelled dose *via* gavage (Mathews-Roth and Krinsky, 1992).

Zaripheh *et al.* (2003) examined the distribution of radiolabelled lycopene in male F344 rats prefed lycopene in the diet (0.25 g/kg diet) for a period of 30 days. Of a single 12 mg dose of ^{14}C-lycopene (22 µCi) administered orally, 68% was excreted in the urine and faeces over a 7-day observation period. Approximately 72% of tissue radioactivity was present in the liver, with smaller concentrations in adipose tissue, spleen, adrenal glands, and seminal vesicles. The majority (80%) of ^{14}C in the liver was reported to be in the *cis* and all-*trans* forms at all time points; however, in the seminal vesicles, the majority of the radioactivity present in the all-*trans* plus 5-*cis* forms 3 hours after dosing had been converted to ^{14}C-polar products after 7 days. The primary form of radioactivity in the prostate was reported to be ^{14}C-polar products at all time points. The data suggest that lycopene may be metabolised differently by different tissues.

A study of the pharmacokinetics of lycopene in dogs following oral administration was conducted by Korytko *et al.* (2003). Single doses of 10, 30, and 50 mg lycopene/kg body weight resulted in a mean half-life of 36 hours. Following repeated dosing of 30 mg lycopene/kg body weight/day over a period of 28 days, steady-state plasma concentrations of 785 to 997 nmol lycopene/L were reported. The highest levels of lycopene in the tissues were observed in the liver, adrenals, spleen, lymph nodes, and intestinal tissues. The authors reported that although 70% *trans*-lycopene was administered to the animals, the majority of lycopene recovered was in the *cis* form.

XIII.b.3 Acute Toxicity Studies

The acute toxicity of lycopene extracted from tomatoes has been investigated in an unspecified number of mice following oral, subcutaneous and intraperitoneal administration of up to 3,000 mg lycopene/kg body weight (Milani *et al.*, 1970). Since no deaths or adverse side effects on the central nervous system were reported for any of the treatments, definite LD_{50} values for lycopene could not be determined. Treatment-related effects occurred only in mice treated subcutaneously at the highest dose, and were limited to a short-term (*e.g.*, <24 hours) increase in defecation and slight decrease in bodily tone. In the absence of relevant effects, the authors concluded that there is no acute toxicity associated with high doses of lycopene.

The acute toxicity of a natural tomato oleoresin extract (NTOE), containing 6% lycopene, also was evaluated in male and female Sprague-Dawley rats (Dreher, 1994a,b). One female rat died 4 hours after dosing and displayed clinical signs of hunched posture, lethargy, decreased respiratory rate, and laboured breathing. Necropsy revealed hemorrhagic lungs, dark liver, and dark kidneys. In the remaining animals, no clinical signs other than some incidence of brown-coloured staining of the fur and no abnormalities at necropsy were observed. The oral (gavage) LD_{50} for NTOE was reported to be >5,000 mg/kg body weight (equivalent to >300 mg lycopene/kg body weight).

The reported LD_{50} values for lycopene are presented in Table XIII.b.3-1.

Table XIII.b.3-1 Acute Toxicity of Lycopene

Species	Route of Administration	LD_{50} (mg/kg body weight)	Reference
Swiss mice	Oral	>3,000	Milani *et al.*, 1970
Swiss mice	Subcutaneous	>3,000	Milani *et al.*, 1970
Swiss mice	Intraperitoneal	>3,000	Milani *et al.*, 1970
Sprague-Dawley rats	Oral	>5,000[a]	Dreher, 1994a,b

[a] Value for tomato oleoresin extract, which contains 6% lycopene. LD_{50} value is therefore equivalent to 300 mg/kg body weight of pure lycopene.

XIII.b.4 Sub-Chronic and Chronic Toxicity Studies

XIII.b.4.1 Lycopene from Blakeslea trispora

The long-term safety of lycopene from *B. trispora* in experimental animals is largely supported by a 90-day oral toxicity study conducted with male and female Wistar rats in accordance with the OECD Principles of Good Laboratory Practise, Organisation of Economic Co-operation and Development (OECD), Paris, ENV/MC/CHEM(98)17 (Jonker *et al*., 2003). Following a 13-day acclimatization period, the animals were divided into groups of 4 (20 rats/sex/group) and received an experimental diet containing 0 (control), 0.25, 0.5, or 1.0% lycopene in the form of a sunflower oil suspension. The corresponding mean intake

of lycopene from each of the experimental diets was calculated to be 0, 145, 291, and 586 mg/kg body weight/day for males, and 0, 156, 312, and 616 mg/kg body weight/day for females. Throughout the 90-day study, animals were monitored for viability and clinical signs of toxicity, and body weights and food consumption were recorded. Prior to necropsy, neurobehavioral testing and ophthalmologic examinations were performed, and blood and urine samples were obtained for haematological analysis, clinical chemistry, and urinalysis. Following euthanization, gross and histopathological examinations of various tissues (including adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, testes, thymus, thyroid, uterus) were performed and organ weights were recorded. Clinical signs related to lycopene treatment were limited to pink discoloration of the fur of all the high-dose and many of the mid-dose animals of both sexes, which was reportedly due to the direct contact of the animals with the red staining lycopene present in the dietary admixture. Neurobehavioral testing and ophthalmologic examinations revealed no treatment-related effects, and there were no differences in mean body weight, organ weights or food intake, or in parameters of haematology, clinical chemistry or urinalysis between the treated and control groups. Finally, gross necropsy did not reveal any adverse effects in any organ system, and there were no lycopene-related lesions as demonstrated by histopathological examinations. Taken together, these results demonstrate that dietary levels of lycopene up to 1.0% are well tolerated by male and female Wistar rats, and are without signs of toxicity. The no-observed-effect level (NOEL) of lycopene was therefore considered to be 1.0% in the diet (Jonker *et al*., 2003).

The possible sub-chronic toxicity of lycopene biomass (biomass of *B. trispora*), extracted from the fermentation manufacturing process of lycopene, was examined in male and female Wistar albino rats (Jonker, 2000). The study was carried out in accordance with the OECD Principles of Good Laboratory Practise, Organisation of Economic Co-operation and Development (OECD), Paris, ENV/MC/CHEM(98)17. Animals were allocated to seven experimental groups of 20 rats/sex, of which 3 received lycopene biomass (low-, mid- and high-dose groups), one served as a control group, and the remaining 3 received carotene biomass. The dried lycopene biomass was administered in the diet at 0.1, 0.3, or 1.0% (*w*/*w*) for at least 28 consecutive days, and treatment was terminated with the necropsy of the rats. 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 assessed at scheduled necropsy. There were no treatment-related differences in mean body weights, or absolute or relative organ weights between treatment and control groups, and food consumption and food conversion efficiency were not adversely affected by the treatments. The overall mean daily intakes in the low-, mid- and high-dose groups were, respectively, 90, 272, and 906 mg/kg body weight in males, and 87, 260, and 868 mg/kg body weight in females. There were no treatment-related clinical signs, nor was there any indication of neurotoxic potential of lycopene biomass, as evidenced by the neurobehavioral observations and motor activity assessments. Haematological measurements revealed that mean corpuscular volume and prothrombin time were statistically decreased in males of the

lycopene high-dose group only; however, no significant changes were noted for other red blood cell or coagulation variables, or total and differential white blood cell counts. Since there were no changes in packed cell volume, red blood cell count, or haemoglobin concentration, the authors considered that the decreased mean corpuscular volume was an incidental finding of no toxicological significance. Likewise, the decrease (6%) in prothrombin times is not considered to be clinically significant due to the fact that the decrease was small and within the range reported for historical controls (35.6 to 43.9 seconds) for the TNO testing facility. The slight decrease in prothrombin time in high-dose males is likely explained by the increased intake of dietary fats and lipids associated with the biomass. The biomass was reported to contain approximately 34% triglycerides and from 8 to 15% lipids. Increased intakes of dietary fats, lipids and triglycerides have been well documented to result in transient and prolonged reduction of prothrombin times in both experimental animals (Kim *et al.*, 1976) and in humans (Higazi *et al.*, 1971; Larsen *et al.*, 1997; Miller, 1997; Zwaal *et al.*, 1998). Lycopene biomass treatment did not adversely affect any of the clinical chemistry variables, including selected enzyme activities, total protein, fasting glucose, or blood lipid levels. Macroscopic and microscopic examinations at necropsy did not reveal any treatment-related changes, with the exception of statistically significantly decreased incidence of increased hyaline droplet nephropathy in males of the lycopene high-dose group; however, no toxicological significance was ascribed to this finding. The authors concluded that the administration of lycopene biomass in the diet of male and female rats at levels of 0.1, 0.3, and 1.0% for a period of at least 28 days was well tolerated and had no effect on appearance, general condition, behaviour, body weight, absolute or relative organ weights, histopathology, food consumption or food conversion efficiency (Jonker, 2000). In addition, the reported slight decrease in prothrombin time in the high-dose male rats is not considered to be toxicologically significant, with respect to the final lycopene formulation, as the extraction and purification steps yields a very pure crystalline material (purity shown to be approximately 100%) with no indication of any contamination from the biomass.

XIII.b.4.2 Lycopene from Sources other than Fungal

The sub-chronic toxicity of synthetic crystalline lycopene was evaluated in a 13-week study conducted with male and female Wistar rats (Mellert *et al*., 2002). Animals were assigned to one of seven treatment groups (10 rats/sex/group) and were dosed daily by gavage with water, lycopene CWD (500, 1,500, or 3,000 mg/kg body weight/day), lycopene CWD formulation matrix (3000 mg/kg body weight/day), Lyco Vit product (3,000 mg/kg body weight/day) or Lyco Vit formulation matrix (3,000 mg/kg body weight/day). Lycopene CWD and Lyco Vit products each contain approximately 10% synthetic lycopene, which corresponds to doses of 50, 150, and 300 mg lycopene/kg body weight/day from lycopene CWD and 300 mg lycopene/kg body weight/day from Lyco Vit. Throughout the treatment period, food consumption and body weights were measured, signs of toxicity or mortality were assessed, and clinical observations were conducted. Ophthalmoscopic examinations were performed prior to dosing and at the completion of the study on animals in the high-dose groups (3,000 mg/kg body weight/day of Lycopene CWD or Lyco Vit), and behavioural

and reflex tests were performed on all the animals on days 83 to 85 of the study. At terminal sacrifice, haematology (leukocytes, erythrocytes, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, platelets, differential blood count, and prothrombin times), clinical chemistry (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-gamma-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, and magnesium), and urinalysis (urine volume, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment, colour and turbidity) were performed, and selected organs (including liver, kidneys, lungs, adrenal glands, testes, epididymides, uterus, ovaries, thymus, spleen, brain and heart) were examined macroscopically and microscopically. There were no significant differences in body weights, relative organ weights, or haematological evaluations across treatment groups, and, compared with baseline findings, there were no treatment-related effects seen in ophthalmoscopic evaluations. Similarly, reflex tests, motor activity assessments, clinical chemistry evaluations, and urinalysis did not reveal any biologically relevant findings. Treatment-related clinical findings were limited to a red discolouration of the faeces, which was observed in the Lycopene CWD and Lyco Vit treatment groups. Consistent with this finding was a red discoloration seen in the jejunum and caecum in the Lycopene CWD and Lyco Vit treatment groups. The tissue discoloration was not associated with gross lesions or histopathological changes. No other remarkable or substance-related abnormalities were observed in any of the other tissues examined, nor were there any significant histopathological findings. The results of this study demonstrate an absence of significant toxicological findings following oral administration of Lycopene CWD and Lyco Vit for 13 weeks, and the authors reported a no-observed-adverse-effect level (NOAEL) for each product of 3,000 mg/kg body weight/day (equivalent to approximately 300 mg synthetic lycopene/kg body weight/day) (Mellert *et al*., 2002).

As reported by McClain and Bausch (2003), 4- and 14-week studies investigating the oral toxicity of Lycopene 10% WS beadlet formulation have been conducted with Han-Ibm Wistar rats. In the 4-week study, animals (6/sex/group) were assigned to one of 3 treatment groups to receive 1,000 mg lycopene/kg body weight/day in beadlet formulation with <0.01, 0.3, or 2% *apo*-12'-lycopenal (impurity), or one of 3 placebo groups to remain untreated, or to receive placebo beadlet formulation (2 groups). Throughout the study, clinical signs, body weight, feed consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, and macro- and microscopic examination of tissues and organs were performed. Treatment-related changes observed in the lycopene groups were limited to a red discoloration of the faeces and brown-orange discoloration of the liver, the latter of which was correlated with deposits of brown-yellow fine granulated pigment in hepatocytes, but was not associated with any histopathological alterations. It was concluded that lycopene beadlet formulation administered orally as a dietary admix to rats for one month at a dose of 1,000 mg/kg body weight/day was well tolerated without any clinically significant adverse effect. In addition, the *apo*-12'-lycopenal impurity was not associated with any clinically significant findings (McClain and Bausch, 2003). In the 14-week study, animals

(26/sex/group) were assigned to 1 of 3 treatment groups to receive 50, 150, or 500 mg lycopene/kg body weight/day, or to one of 2 placebo groups to receive the beadlet formulation or the powdered diet. A total of 6 rats/sex were assigned to recovery groups for a 5-week treatment-free period. Based on observations and measurements made throughout the experimental period (clinical chemistry, organ weights, body weight, and macro- and microscopic examination of organs), a NOAEL of 500 mg lycopene/kg body weight/day was established (McClain and Bausch, 2003).

The sub-chronic toxicity of NTOE was investigated in a 13-week study in groups of 20 male and 20 female CD rats (East, 1995). Animals were administered the extract at doses of 0, 45, 450, and 4,500 mg/kg body weight/day by gavage, equivalent to 0, 2.7, 27, or 270 mg lycopene/kg body weight/day. Clinical signs were assessed twice daily, food consumption was assessed weekly, and haematology, clinical chemistry, ophthalmoscopy, urinalysis, and absorption analyses were performed at regular intervals throughout the study period. At the end of the study period, all animals were subjected to macroscopic evaluations, measurement of organ weights, and microscopic evaluation of the tissues. No significant differences between treated and control groups were reported in body weight gain, food intake, or food conversion efficiency. Compared to controls, animals that received 45 and 450 mg/kg body weight/day of NTOE were reported to have an increased incidence of orange-stained faeces, whereas rats that received 4,500 mg/kg body weight/day had an increased incidence of red-stained faeces. There were no significant effects on haematological, ophthalmological, or urinary parameters. Significantly lower alkaline phosphatase activity was reported in males at the mid- and high dose levels at week 6, while alanine aminotransferase activity was increased in males at the high dose level at the same time point. These levels were not statistically significantly different from controls at the end of the study period. Papillary hyperplasia of the urinary bladder was reported in one animal in the 450 mg/kg body weight/day group. This was found to be the result of a chronic infection of the genitor-urinary tract, and was not considered to be treatment-related. No other histopathological findings were reported. Based on the study findings, the NOAEL was considered to be 4,500 mg/kg body weight/day of NTOE, which is equivalent to 270 mg lycopene/kg body weight/day.

Several additional experimental studies conducted with mice, rats and dogs have examined the effects of long-term oral exposure to lycopene. Although the toxicity of lycopene has not been the main focus of the majority of these studies, safety endpoints including adverse effects, body weight gain, food intake, and organ weights have been recorded. These studies and their safety-related findings are summarized in Table XIII.b.4-1, and discussed in detail below. In addition to these studies, the safety of long-term exposure to lycopene in experimental animals is supported by anti-carcinogenicity studies conducted with lycopene reporting no adverse treatment-related effects (see Section XIII.b.5 and Table XIII.b.5-1).

Table XIII.b.4-1 Summary of Sub-chronic and Chronic Studies Examining Safety-Related Endpoints of Lycopene

Reference	Species	Duration	Dose (mg/kg body weight/day)	Safety-Related Findings
Nagasawa *et al*., 1995	SHN/Mei virgin mice (11 control/14 treated)	10 months	0.07 (dietary)	Lycopene *versus* control: No difference in body weight gain, no deleterious side effects detected
Black, 1998	Female SKH-Hr-1 hairless mice (30/group)	28 weeks	90 (dietary)	Lycopene *versus* control: No differences in mortality or body or liver weights.
Zbinden and Studer, 1958	Rat (10/sex/group)	100 days	1,000 (dietary)	No adverse effects reported
Zbinden and Studer, 1958	Rat (numbers not specified)	200 days	10 to 20 (dietary)	Slight accumulation of pigments in the liver
Erdman and Lachance, 1973	Male hypercholesterolemic rats (numbers not specified)	28 days	7 groups: ranged from approximately 1.95 to 95 (dietary)	All lycopene groups (except 41 mg/kg body weight/day) *versus* control basal diet: ↑'d serum cholesterol levels. Group receiving 9.6 mg lycopene/kg body weigh/day *versus* control hypercholemic diet: ↑'d liver cholesterol levels.
Gradelet *et al*., 1996	Male SPF Wistar rats (5/group)	15 days	45 (dietary)	Lycopene *versus* control: No difference in food intake, body weights or absolute and relative liver weights. 60% ↓'d activity of liver enzyme nitrosodimethylamine *N*-demethylase (NDMAD).
Zhao *et al*., 1998	Male and female Fischer rats (20 control/10 per treatment)	10 weeks	2.4, 6.0, 12, 24, or 60 (dietary)	Lycopene *versus* control: No toxic side effects. No adverse effects on weight gain, behaviour, or coat appearance (brown discoloration of the tail in a few rats).
Jewell and O'Brien, 1999	Male Wistar rats (8/group)	16 days	45 (dietary)	Lycopene *versus* control: No differences in food intake, body weight changes or organ weights (small intestine, liver, lung, kidney). ↓'d activity of lung enzyme benzyloxyresorufin-O-dearylation (BROD).
Breinholt *et al*., 2000	Female Wistar rats (4/group)	14 days	0, 1, 5, 50, or 100 (gavage)	Lycopene *versus* control: No differences in food intake, body weight changes or liver weights. ↑'d activity of liver enzymes benzyloxyresorufin-O-dealkylase (BROD) and ethoxyresorufin O-dealkylase (EROD).
Boileau *et al*., 2000	Male F344 rats (22/group)	8 weeks	0, 0.5, 5.7, 57.5 (dietary)	Lycopene *versus* control: No difference in food intake.
Zbinden and Studer, 1958	1 Dog	192 days	100 (capsules)	Pigment deposition in the liver and kidney

Sub-chronic and chronic oral administrations of lycopene to rats at doses up to 1,000 mg/kg body weight/day for 100 days and 20 mg/kg body weight/day for 200 days have been reported not to cause any treatment-related adverse effects on body weight gain, food

consumption, organ weights, or behaviour (Zbinden and Studer, 1958; Gradelet *et al*., 1996; Zhao *et al*., 1998; Jewell and O'Brien, 1999; Boileau *et al*., 2000; Breinholt *et al*., 2000). Coat appearance was unaffected by doses of dietary lycopene up to 60 mg/kg body weight/day, with the exception of discoloured tails in a few experimental animals (Zhao *et al*., 1998), and treatment-related effects on the major organs were limited to slight pigment accumulations in the liver or kidneys in small groups of rats fed 10 to 20 mg lycopene/kg body weight/day for 200 days, and in one dog administered 100 mg lycopene/kg body weight/day for 192 days (Zbinden and Studer, 1958).

Various studies have been conducted in rats to examine the effects of lycopene on the activities of drug-metabolizing enzymes involved in the protection against oxidative stress and cancer. Lycopene was reported to either induce (Breinholt *et al*., 2000) or have no effect (Gradelet *et al*., 1996; Jewell and O'Brien, 1999) on ethoxyresorufin (EROD) and benzyloxyresorufin (BROD) activities in the liver, and to decrease BROD activity in the lungs (Jewell and O'Brien, 1999) and nitrosodimethylamine N-demethylase (NDMAD) activity in the liver (Gradelet *et al*., 1996). Although statistically significant, the inductions of EROD and BROD activities in the liver were considered minor (Breinholt *et al*., 2000) and were not supported by additional studies (Gradelet *et al*., 1996; Jewell and O'Brien, 1999). In those studies demonstrating decreased activity of BROD and NDMAD enzymes, similar effects were also obtained from other carotenoids (*i.e.*, modifying effect was not specific to lycopene), and the relevance was unexplained (Gradelet *et al*., 1996; Jewell and O'Brien, 1999). Considering the variability of the results and the fact that there were no differences in growth, food intake or organ weights in any of the studies, the modifying effect of lycopene on the activities of drug-metabolizing enzymes in rats is not considered an adverse event.

The effect of lycopene on serum, liver and intestinal cholesterol in hypercholemic rats was reported in an abstract by Erdman and Lachance (1973). Following lycopene supplementation at doses ranging from 78 to 3,813 µg/day for 28 days, serum cholesterol levels were significantly elevated in all but one intermediate lycopene dose group (1,650 µg/day) compared with basal control animals. Liver cholesterol levels were increased in only one dose group (384 µg/day) compared with hypercholemic controls, and there were no changes reported in intestinal cholesterol levels compared with controls. Given the lack of dose-dependence in these findings, coupled with findings from a human clinical study in which no change in serum cholesterol was reported in male adults consuming 40 mg lycopene/day for 2 weeks (Müller *et al*., 1999), the increased liver and serum cholesterol levels in rats are not considered to be treatment related.

In mice, sub-chronic and chronic studies reporting safety-related endpoints of lycopene were limited to two studies, each designed to examine the health benefits of lycopene. Exposure to 90 mg lycopene/kg body weight/day in the diet for 2 weeks had no adverse effects on mortality, body weight, or liver weight in UV irradiated mice (Black, 1998). Similarly, 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).

XIII.b.4.3 Margin of Safety

Based on the average NOEL of 601 mg lycopene/kg body weight per day from the rat sub-chronic study conducted on the lycopene oil suspension (Jonker *et al.*, 2003), and the maximum level of intake from proposed food uses (see Section IX.b), it is possible to calculate the human safety margin following the consumption of lycopene from *B. trispora*. The difference between the NOEL and the highest mean and 95th percentile intakes of 3.54 and 8.16 mg/day in the U.K., respectively, provides an approximate 4,000- to 10,000-fold safety margin.

XIII.b.5 Carcinogenicity Studies

In its evaluation of lycopene (synthetic and from *B. trispora*), JECFA assessed a 2-year carcinogenicity study in which rats were fed diets mixed with a beadlet formulation containing 10% synthetic lycopene at doses of 2, 10, or 50 mg/kg body weight/day (JECFA, 2007). Although hepatocellular foci were reported in rats receiving doses greater than 10 mg/kg body weight/day, no increase in the incidence of liver tumours was observed. Furthermore, lycopene treatment was not associated with increased incidence of tumours in any other organ or tissue. JECFA also stated that hepatocellular foci are extremely rare in humans, and that many substances that induce liver foci in rodents do not similarly affect humans.

Several studies examining the potential chemopreventive effects of lycopene on the incidence of experimentally-induced tumours have also been performed. Chemoprevention studies conducted with mice and rats that include reports on safety-related endpoints of lycopene are summarized in Table XIII.b.5-1.

Table XIII.b.5-1 Summary of Anti-Cancer Studies Examining Safety-Related Endpoints of Lycopene

Reference	Tumour Induction	Lycopene Dose	Duration of Lycopene Treatment	Safety-Related Findings
Mice				
Kim *et al*., 1997	Sequential treatment with Diethylnitrosamine , N-methyl-N-nitrosurea , and 1,2-dimethylhydrazine (DMD) (intraperitoneal. injections)	5 or 10 mg/kg bw/day (in drinking water with and without prior initiation)	21 weeks (weeks 11 to 32)	♀ DMD+lycopene groups: ↑'d body weights and ↓'d liver and kidney weights *versus* DMD alone. ♂ DMD+lycopene groups: ↓'d water consumption *versus* DMD alone. No adverse effects of lycopene reported.
Kim *et al*., 1998	1,2-dimethylhydrazine (DMH) (subcutaneous injection)	Up to 6.5 mg/kg bw/day (in drinking water with and without prior initiation)	7 weeks (weeks 5 to 12)	No histopathological evidence of toxicity in the livers or kidneys was reported.
Tang *et al*., 2005b	Subcutaneous injection of DU145 human prostate carcinoma cells	10, 100 or 300 mg/kg bw *via* gavage (5 days/week)	8 weeks (24 hours after tumour induction)	No effects on body weights.
Rats				
Wang *et al*., 1989	Tumour cell (C-6 glioma cells) inoculation (subcutaneous injection)	10 mg/kg solution/day (i.p. injection before or after tumour cell inoculation)	5 days before or after tumour cell inoculation	No hepatic function disorders (constant levels of serum enzyme markers: GOT, GPT, ALP, and GGT)
Narisawa *et al*., 1996	*N*-methylnitrosourea (3 intrarectal doses)	0.06, 0.12, 0.24, 1.2, or 6.0 mg/day *via* gavage	2 weeks (after tumour induction)	No effect on body weights.
Okajima *et al*., 1997	N-butyl-N-(4-hyroxybutyl)nitrosamine (in drinking water)	1.8 mg/kg bw/day in drinking water following initiation)	12 weeks (weeks 8 to 20)	No differences in survival, food or water intake, body weights, or weights of liver, kidneys, prostate or testes.
Sharoni *et al*., 1997	7,12-dimethyl-benz[a]anthracene	10 mg/kg solution (2 i.p. injections/week)	18 weeks (2 weeks prior to initiation and 16 weeks after)	Lycopene injections were well tolerated. No signs of toxicity, no changes in weight gain, no macroscopical changes, no pathological alterations in liver, kidney, brain, or lungs.
Jain *et al*., 1999	Azoxymethane (single i.p. injection)	0.142 (dietary)	100 days	No differences in body mass, food intake or faecal output.
Imaida *et al*., 2001	3,2'-dimethyl-4-aminobiphenol (DMAB) (subcutaneous injection)	15 ppm (1.5 mg/kg bw) in diet	20 weeks (during tumour induction)	No differences in body weight gain, final body weight, survival rates. No adverse effects observed.

Table XIII.b.5-1 Summary of Anti-Cancer Studies Examining Safety-Related Endpoints of Lycopene				
Reference	**Tumour Induction**	**Lycopene Dose**	**Duration of Lycopene Treatment**	**Safety-Related Findings**
Imaida *et al.*, 2001	3,2'-dimethyl-4-aminobiphenol (DMAB) (subcutaneous injection)	5, 15 or 45 ppm 0.5, 1.5 or 4.5 mg/kg bw) in diet	40 weeks (after tumour induction)	No differences in final body weight, average food intake, and liver, kidney or prostate weights.
Imaida *et al.*, 2001	2-amino-1-methylimizado[4,5-*b*]pyridine (intragastric administration)	45 ppm (4.5 mg/kg bw) in the diet	50 weeks (after tumour induction)	No differences in final body weight, and liver, kidney or prostate weights.
Martinez-Ferrer *et al.*, 2006	Azoxymethane (subcutaneous injection at 7 and 8 weeks of age)	200 or 400 ppm (20 or 40 mg/kg bw) in the diet	46 weeks (from 4 to 50 weeks of age)	No differences in body weight gain.

Although the studies outlined above were not of the classical toxicity/carcinogenicity design, the safety of lycopene in the drinking water at levels up to 10 mg /kg body weight/day in mice and 1.8 mg/kg body weight/day in rats has been clearly demonstrated by a lack of treatment-related changes in survival, food or water intake, body weights, organ weights, or organ histopathology (Kim *et al*., 1997, 1998; Okajima *et al*., 1997; Jain *et al*., 1999). Studies in which lycopene was present in the diet at levels of up to 400 ppm also indicate no adverse effects, as well as no effect of treatment on body weight, organ weights, food intakes and survival (Imaida *et al.*, 2001; Martinez-Ferrer *et al.*, 2006). Further evidence supporting a lack of lycopene toxicity comes from studies in which intraperitoneal injections of 10 mg lycopene/kg solution were well tolerated, resulting in no treatment-related changes in organ function or pathology (Wang *et al*., 1989; Sharoni *et al.*, 1997), and a study in which oral gavage of up to 300 mg/kg body weight did not affect body weight (Tang *et al*., 2005b).

XIII.b.6 Mutagenicity/Genotoxicity Studies

Data pertaining to the mutagenicity and genotoxicity of lycopene are summarized in Table XIII.b.6-1. Based on the negative results reported in bacterial reverse mutation assays and a human lymphocyte chromosomal aberration test conducted on the lycopene CWD product from *B. trispora*, JEFCA concluded that there was no evidence of genotoxicity of lycopene from *B. trispora* (JECFA, 2007).

Table XIII.b.6-1 Summary of Studies Evaluation the Genetic Toxicity of Lycopene				
Reference	**Assay/End Point**	**Strain/Cell Type**	**Lycopene Dose**	**Result**
In Vitro Studies Conducted on Lycopene CWD formulations				
CTBR, 2003a	Ames: *Salmonella typhimurium*	TA98, TA100, TA1535, TA1537	Up to 5,000 µg lycopene 20% CWD formulation/plate (with and without metabolic activation)	Negative
CTBR, 2003a	*Escherichia coli*	WP2 UVR A	Up to 5,000 µg lycopene 20% CWD formulation/plate (with and without metabolic activation)	Negative
CTBR, 2003b	Chromosome aberrations	Human lymphocytes	1,2850 to 5,000 µg lycopene 20% CWD formulation/mL (with and without metabolic activation)	Negative
In Vitro Studies Conducted on Lycopene from Sources Other than Fungal				
He and Campbell, 1990	Ames: *Salmonella typhimurium*	TA98, TA100	100 µg lycopene from tomato paste/plate (with and without metabolic activation)	Negative
Thompson, 1994	Ames: *Salmonella typhimurium*	TA98, TA100, TA1535, TA1537, TA1538	18.75, 37.5, 75, 150 and 300 µg lycopene/plate (with and without metabolic activation)	Negative
Rauscher *et al*., 1998	Ames: *Salmonella typhimurium*	TA98, TA100	25, 50, 75, and 100 µg lycopene from fruits and vegetables/plate (with and without metabolic activation)	Negative
Aizawa *et al*., 2000	Ames: *Salmonella typhimurium*	TA98, TA100, TA1535, TA1537	0.05 to 5,000 µg lycopene from tomato paste/plate (each strain with and without metabolic activation)	Negative
McClain and Bausch, 2003	Ames: *Salmonella typhimurium*	TA1535, TA97, TA98, TA100, TA102	10 to 1,000 µg crystalline lycopene from 10% WS beadlet formulation/plate (with and without metabolic activation)	Negative
McClain and Bausch, 2003	Ames: *Salmonella typhimurium*	TA1535, TA97, TA98, TA100, TA102	10.5 to 1050 µg lycopene from 10% fluid suspension/plate (with and without metabolic activation)	Negative
Thompson, 1994	*Escherichia coli*	WP2 UVR A	18.75, 37.5, 75, 150 and 300 µg lycopene/plate (with and without metabolic activation)	Negative
Aizawa *et al*., 2000	*Escherichia coli*	WP2 UVR A	0.05 to 5000 µg lycopene from tomato paste /plate (with and without metabolic activation)	Negative

Table XIII.b.6-1 Summary of Studies Evaluation the Genetic Toxicity of Lycopene				
Reference	**Assay/End Point**	**Strain/Cell Type**	**Lycopene Dose**	**Result**
McClain and Bausch, 2003	Mutations at the tk locus	Mouse lymphoma cells	Lycopene 10% WS beadlet formulation up to cytotoxic concentrations (not specified) (with and without metabolic activation)	Negative
McClain and Bausch, 2003	Chromosome aberrations	Human lymphocytes	Lycopene 10% WS beadlet formulation up to cytotoxic concentrations (not specified) (with and without metabolic activation)	Negative
In Vivo Studies				
Rauscher *et al*., 1998	Micronucleus Assay (chromosomal damage)	Mouse bone marrow cells	180 mg lycopene from fruits and vegetables/kg body weight (single oral gavage dose)	Negative
McClain and Bausch, 2003	Micronucleus Assay (chromosomal damage)	Mouse peripheral blood	Lycopene 10% WS beadlet formulation in soft drinks (25 or 50 ppm lycopene). Tomato juice (54 ppm lycopene)	Negative
McClain and Bausch, 2003	Unscheduled DNA synthesis	Rat hepatocytes	Lycopene 10% WS beadlet formulation (dose and duration of exposure not specified)	Negative
Guttenplan *et al*., 2001	Spontaneous and benzo[a]pyrene (BaP)-induced mutant fraction	LacZ male mouse DNA from lung, prostate and colon	Approximately 47.5 or 95.0 mg/kg body weight/day in the diet (lycopene-rich) for 9 months	High dose lycopene ↑'d BaP-induced mutagenesis in lung and colon. No effect of lycopene on BaP-induced mutagenesis in prostate or spontaneous mutagenesis in lung, colon or prostate.
Pool-Zobel *et al*., 1997	Single cell microgel electrophoresis (Comet) assay (measure of DNA damage)	Human peripheral blood lymphoctyes	150 mg lycopene from tomato juice/day for 2 weeks	↓ in endogenous levels of strand breaks in lymphocyte DNA.
Collins *et al*., 1998	Single cell microgel electrophoresis (Comet) assay (measure of DNA damage)	Human lymphoctyes	15 mg capsulated lycopene/day for 12 weeks	No change in level of endogenous DNA damage compared with controls.
Riso *et al*., 1999	Single cell microgel electrophoresis (Comet) assay (measure of DNA damage)	Human lymphoctyes	16.5 mg lycopene from tomato purée for 21 days	↓ in level of hydrogen peroxide-induced (*ex vivo*) DNA damage.

XIII.b.6.1 Lycopene from B. trispora

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

XIII.b.6.2 Lycopene from Sources other than Fungal

Results of *in vitro* assays with bacterial test systems were consistently negative for mutagenicity. Although the assays conducted by He and Campbell (1990) and Rauscher *et al*. (1998) are considered limited due to the restricted number of strains used (only two) and the low dose of lycopene tested (100 µg/plate); the negative results reported by each study were confirmed in subsequent assays, which used the recommended 4 strains, and tested lycopene at doses up to 5,000 µg/plate (Aizawa *et al*., 2000; McClain and Bausch, 2003). Thompson (1994) reported negative results at doses of 312.5, 625, 1,250, 2,500 and 5,000 µg NTOE/plate, equivalent to 18.75, 37.5, 75, 150 and 300 µg lycopene/plate. McClain and Bausch (2003) reported results obtained from *in vitro* assays conducted with mammalian test systems. Lycopene (10% WS beadlet formulation), applied at doses up to the cytotoxic concentration (not specified), was negative for mutagenicity in mouse lymphoma cells, and negative for clastogenicity in human lymphocytes (McClain and Bausch, 2003).

In vivo assays were conducted in mice and humans. Lycopene did not induce chromosomal damage in mouse bone marrow cells (Rauscher *et al*., 1998) or DNA damage (in a Comet assay) in human lymphocytes (Pool-Zobel *et al*., 1997; Collins *et al*., 1998; Riso *et al*., 1999). In their summary of safety studies conducted with synthetic lycopene, McClain and Bausch (2003) reported no evidence of clastogenicity in mice or DNA damage in rats following oral dosing with Lycopene 10% WS beadlet formulations. Addition of lycopene to the diet of benzo[a]pyrene (BaP)-treated mice had organospecific effects on mutagenesis, with enhancing effects in the colon and lung, and no effect in the prostate. In contrast, spontaneous mutagenesis was not affected in any of the tested organs following lycopene

supplementation, suggesting that ingestion of relatively large amounts of dietary lycopene in individuals exposed to carcinogens may be hazardous (Guttenplan *et al*., 2001). In 2 of the 3 Comet assays conducted with human lymphocytes, dietary lycopene provided a protective effect against both endogenous (Pool-Zobel *et al*., 1997) and induced (Riso *et al*., 1999) DNA damage.

XIII.b.7 Reproductive Toxicity Studies

In an experimental study evaluating the reproductive toxicity of lycopene, an unspecified number of male and female rats were fed 10 to 20 mg lycopene/kg body weight/day in the diet for approximately 200 days prior to mating and subsequently throughout 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).

The potential developmental toxicity of synthetic lycopene formulations (Lycopene 10 CWD and LycoVit® 10%) was investigated in female rats and rabbits (Christian *et al*., 2003). Mated animals (23 rats/group and 25 to 34 rabbits/group) were assigned to one of three control groups (2 placebo control groups and one vehicle control group), one of three Lycopene 10 CWD groups (500, 1,500, or 2,000 or 3,000 mg/kg body weight/day for rabbits and rats, respectively), or the LycoVit® 10% group (2,000 or 3,000 mg/kg body weight/day for rabbits and rats, respectively). Dosages were administered *via* gavage on gestational days (GD) 6 through 19 (rats) or 6 through 28 (rabbits). All animals were observed daily for mortality, and body weights and feed consumption were recorded at selected time points throughout the treatment period. Caesarean section observations (GD 20 and 29 for rats and rabbits, respectively) included weight of gravid uterus, numbers of corpora lutea, live and dead foetuses, early and late resorptions, implantation sites, and the normality of placenta. Live foetuses of both species were euthanized and examined for gross external alterations (soft tissue and skeletal), gender, and body weights. No substance-related evidence of maternal or developmental toxicity was reported in rats or rabbits following oral exposure to up to 3,000 (rats) or 2,000 (rabbits) mg/kg body weight/day of Lycopene 10 CWD or LycoVit® 10% (Christian *et al*., 2003).

McClain and Bausch (2003) reported the results of a teratology study conducted in mated Wistar female rats with WS beadlet formulations of lycopene and lycopene plus added *apo*-12'-lycopenal impurity. Animals (14/group) were treated orally with 0 (beadlet control), 1,000 mg lycopene/kg body weight/day, or 1,000 mg lycopene/kg body weight/day plus 20 mg *apo*-12'-lycopenal/kg body weight/day from GD 6 to 18. Animals underwent C-sections on GD 21. No signs of maternal or foetal toxicity were observed, and there were no clinically significant changes in measured reproductive or developmental parameters. It was thus concluded that exposure to 1,000 mg lycopene/kg body weight/day (with and without *apo*-12'-lycopenal) throughout gestation resulted in no teratogenic effects in Wistar rats (McClain and Bausch, 2003).

XIII.b.8 Human Safety Data

The International Life Sciences Institute (ILSI) conducted a review in 1999 to identify the existing scientific evidence in support of the safety and physiologic actions of specific phytochemicals. Lycopene was among the compounds chosen for review, for which there was reportedly no data to indicate toxicity (ILSI, 1999). The overall safety of lycopene is supported by a series of clinical trials (discussed in Section XIII.b.8.2) and several epidemiology studies (see Section XIII.c.6.3) that were identified following comprehensive and detailed searches of the published scientific literature conducted through November 2006. Section XIII.b.8.3 discusses case studies reporting the effects of excessive lycopene intakes.

XIII.b.8.1 Case Studies

Excessive dietary intake of lycopene-containing food accompanied by high serum levels of lycopene has been reported to cause a rare cutaneous disease referred to as lycopenodermia (Reich *et al.*, 1960). Lycopenodermia is differentiated from carotenemia (excessive dietary intake of β-carotene) by the presence of lycopene deposits in the focal areas of the liver, often resulting in the formation of fatty cysts, and from jaundice by normal bilirubin levels (Reich *et al.*, 1960; La Placa *et al.*, 2000). Symptoms of lycopenodermia are temporary (*i.e.*, symptoms cease upon termination of lycopene ingestion) and include orange-yellow discoloration of the skin and abdominal pain due to hepatic accumulation of lycopene pigments (Reich *et al.*, 1960; La Placa *et al.*, 2000). The levels of lycopene intake associated with lycopenodermia in these case studies was not reported.

Reich *et al.* (1960) reported a case of lycopenodermia occurring in a 61-year-old female who consumed 2 litres of tomato juice per day for several years (exact duration not specified). The subject suffered from recurrent bouts of abdominal pain, associated with nausea, vomiting and diarrhoea, and presented with orange-yellow discoloration of the skin on the hands, forearms, face and soles of feet. Clinical and chemical investigation revealed unusually high serum lycopene levels, and hepatic storage of lycopene pigments, as evidenced by large, round vacuolated parenchymal cells, and the presence of fatty cysts and fine yellow masses. The authors concluded that the subject suffered from a variant of carotenemia, referred to as lycopenodermia, due to high levels of lycopene intake from tomato juice (Reich *et al.*, 1960). Similar symptoms were reported for a 19-year-old female who consumed 4 to 5 large tomatoes plus pasta with tomato sauce daily for 3 years (La Placa *et al.*, 2000). A yellow-orange pigmentation was observed on the forehead, nasolabial folds, palms of the hands, and soles of the feet, and recurrent abdominal pain was reported. Hepatic echographia revealed liver alterations due to deposits of lycopene, and when dietary intake of tomatoes was restricted, there was a complete regression of pigmentation and the abdominal pain disappeared. Based on clinical features and dietary history, the authors diagnosed the subject with lycopenodermia (La Placa *et al.*, 2000). Additional case studies documenting incidences of orange-yellow skin discolouration (*e.g.*, carotenodermia) in individuals consuming diets rich in tomatoes or tomato products (*e.g.*, tomato soup) have

been reported (Bonnetblanc *et al.*, 1987; Gandhi *et al.*, 1988), and both have identified the lycopene content of the tomatoes/tomato products as the probable cause of the discoloration.

XIII.b.8.2 Clinical Studies

In a critical review of the scientific literature related to the safety of lycopene, 19 clinical studies were identified that evaluated safety-related endpoints, including tolerance (see Table XIII.c.8.2-1). Anthropometric and biochemical measurements, including body weights, full blood counts, and immune function and liver function tests, did not reveal any abnormalities in subjects supplemented with lycopene at levels ranging from 0.5 mg/day for 4 weeks to 75.0 mg/day for 1 week (Carughi and Hooper, 1994; Agarwal and Rao, 1998; Müller *et al.*, 1999; Chopra *et al.*, 2000; Watzl *et al.*, 2000; Kucuk *et al.*, 2001; Olmedilla *et al.*, 2002), although Watzl *et al.* (2003) demonstrated time-delayed modification of immune function and following lycopene consumption, and Briviba *et al.* (2004) reported a significant decrease in IL-4 overproduction in smokers treated with tomato oleoresin extract capsules to levels similar to those found in non-smokers. The effects of lycopene supplementation on serum lipid profiles were investigated in several studies, which consistently reported no changes in levels of cholesterol (total, high density lipoprotein-, or low density lipoprotein-) or triglycerides following daily exposures to lycopene ranging from 0.5 mg/day for 4 weeks to 75.0 mg/day for 1 week (Carughi and Hooper, 1994; Agarwal and Rao, 1998; Olmedilla *et al.*, 2002). Wright *et al.* (1999) analysed the plasma fatty acid profile of healthy male volunteers following daily supplementation with 15 mg lycopene for a total of 26 days, and revealed significant decreases in linoleic acid levels and in the polyunsaturated to saturated fatty acid ratio. The biological significance of these changes is not clear; however, such alterations in plasma fatty acid levels could potentially lead to changes in cell membrane composition and hence to alterations in the physical properties and fluidity of cellular membranes. Since these potential alterations may or may not be beneficial, the authors concluded that further investigation of lycopene-induced changes in the plasma fatty acid profile is warranted (Wright *et al.*, 1999).

The tolerance of lycopene supplementation was assessed in healthy individuals (Micozzi *et al.*, 1992; Agarwal and Rao, 1998; Müller *et al.*, 1999; Hininger *et al.*, 2001; Hoppe *et al.*, 2003; Watzl *et al.*, 2003; Briviba *et al.*, 2004) as well as in prostate cancer patients (Chen *et al.*, 2001; Kucuk *et al.*, 2001; Ansari and Gupta, 2003, 2004; Clark *et al.*, 2006). Daily doses up to 75.0 mg lycopene were generally well tolerated, with no reports of any illnesses or adverse biological effects (Micozzi *et al.*, 1992; Agarwal and Rao, 1998; Müller *et al.*, 1999; Chen *et al.*, 2001; Hininger *et al.*, 2001; Kucuk *et al.*, 2001; Ansari and Gupta, 2003, 2004). Clark *et al.* (2006) reported that one patient discontinued lycopene supplementation because of diarrhoea, but treatment was otherwise well tolerated. Gastrointestinal intolerances (not specified) were reported in one study in which prostate cancer patients were supplemented with approximately 30 mg lycopene/day for 3 weeks (Chen *et al.*, 2001); however, the effects were considered minor, were limited to 9% of the treated population, were resolved within a few days, and were not reported in a separate study conducted with a similar protocol (*i.e.*,

same population, duration and treatment) (Chen *et al.*, 2001; Kucuk *et al.*, 2001). In a study reported by Mohanty *et al.* (2005), no adverse effects were reported in patients with high-grade prostate intraepithelial neoplasia (HGPIN, a precursor to prostate cancer) who received 4 mg lycopene twice daily for 1 year.

Olmedilla *et al.* (2002) reported no adverse effects and no significant changes in the general biochemical or haematological profiles of subjects supplemented with 15 mg/day lycopene for 16 weeks. However, self reported incidences of carotenodermia (*i.e.*, discolouration of the skin) in 25% of the subjects (healthy Spanish volunteers) supplemented with 15 mg lycopene/day for 16 weeks (compared with 40% and 95% of the subjects supplemented with lutein and carotene, respectively. No objective form of skin discolouration measurement was made in the Olmedilla *et al*. (2002) study. The effects of dietary carotenoids on skin pigmentation have however been investigated in a double-blind study conducted by Postaire *et al.* (1997) (not included in Table XIII.b.8.2-1). Using chromametry, skin colour and melanin concentration were assessed at selected skin sites (*e.g.*, 1 cm^2 region of skin from the back, forehead, hands, and internal side of forearm) of healthy subjects following 8 weeks of dietary supplementation with either 26 mg β-carotene plus 4 mg lycopene/day (high β-carotene/low lycopene dose group), or 6 mg β-carotene plus 6 mg lycopene (low β-carotene/high lycopene dose group) in capsule form. Despite a slight change from baseline in the yellow, but not red, pigmentation of selected skin areas in the high β-carotene dose group, there were no significant changes in skin colour following the carotenoid treatments; however, there was a significant increase in melanin concentration in each treatment group, demonstrating that carotenoids play a role in the pigmentation of skin (Postaire *et al.*, 1997). In summary the clinical data suggest that dietary lycopene supplementation is generally well tolerated, with the only effects limited to dermal discolouration in a small percentage of the treated population following long-term high exposure. Such effects (carotenodermia/ lycopenodermia) are considered to be harmless and readily reversible once the carotenoid ingestion is discontinued (IOM, 2000).

Table XIII.b.8.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
Micozzi *et al.*, 1992	30 healthy ♂ (6 groups of 5)	20 to 45 years	6 weeks	Placebo-controlled supplementation study	30 mg carotenoids/day from β–carotene capsules or 272 g carrots	Verbal communication regarding tolerance of supplemented doses assumed.	No gastric discomfort was reported.
					12 mg β–carotene /day from β–carotene capsules or 12 mg lycopene/day 180 g tomato juice		
					6 mg carotenoids/day from 300 g broccoli		
					Placebo (capsules)		
Carughi and Hooper, 1994	11 healthy ♂ and ♀volunteers	22 and 52 years	6 weeks (2 weeks depletion period, 4 weeks supplementation)	Supplementation study	8.5 mg β-carotene/day	Body mass index (BMI) and serum lipids	BMI and blood lipid indexes were within normal ranges (*i.e.,* no significant changes due to supplementation).
					3.5 mg α-carotene/day		
					0.5 mg lycopene/day		
Agarwal and Rao, 1998	19 healthy subjects (10 ♂ and 9 ♀)	25 to 40 years	8 weeks (1 week/treatment separated by 1-week wash-out periods)	Randomized, cross-over dietary intervention study	39.2 mg lycopene/day from spaghetti sauce	Plasma lipids (total, HDL- and LDL-cholesterol levels, triglyceride levels). Anthropometric measurements and verbal communication regarding tolerance of supplemented doses assumed.	No effects on plasma lipid levels. All subjects maintained their body weights and no adverse symptoms were reported throughout the duration of the study
					50.4 mg lycopene/day from tomato juice		
					75.0 mg lycopene/day from tomato oleoresin capsules		
					0 mg lycopene/day (placebo)		
Böhm and Bitsch, 1999	22 ♀ volunteers (3 groups of 6 to 8)	Mean 21 years	6 weeks	Randomized supplementation study	5 mg lycopene/day from tomato oleoresin soft gel capsules	Plasma lipids (total and HDL-cholesterol levels, triglyceride levels).	No effects on plasma lipid levels.
					5 mg lycopene/day from tomato juice		
					5 mg lycopene/day from raw tomatoes		

Table XIII.b.8.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene							
Study	**Population**	**Age**	**Duration**	**Study Type**	**Treatment**	**Safety-related Endpoints**	**Results**
Müller *et al.*, 1999	23 ♂ healthy volunteers	27 to 40 years	8 weeks (2 weeks/treatment preceded by 2-week depletion period)	Dietary intervention trial	40 mg lycopene from tomato juice followed by 15.7 mg α-carotene plus 22.3 mg β-carotene from carrot juice followed by 11.3 mg lutein plus 3.1 mg β-carotene from spinach powder.	Blood haematology	Intervention was well tolerated by all subjects, no incidences of illnesses reported. Blood haemoglobin concentration, white blood cells and serum electrolytes remained within normal range.
Wright *et al.*, 1999	23 ♂ healthy volunteers	18 to 60 years	26 days	Double-blind, placebo-controlled supplementation study.	15 mg lycopene/day (lycopene-rich tomato extract in capsule form) *versus* placebo (corn oil)	Plasma fatty acid profile	Compared with placebo, lycopene treatment significantly decreased plasma linoleic acid, and significantly decreased the polyunsaturated: saturated fatty acid ratio.
Chopra *et al.*, 2000	34 healthy ♀ volunteers (18 non-smokers and 16 smokers)	24 to 52 years	4 weeks (dietary monitoring during week 1, depletion period during week 2, treatments on weeks 3 and 4)	Dietary intervention trial	25 mg carotenoids/day from “red foods” (lycopene treatment) followed by 25 mg carotenoids/day from “green foods” (β-carotene and lutein treatment).	Biochemical measurements (full blood count, lipid profile, liver function tests, serum creatine kinase activity	No change in any of the biochemical variables tested throughout the study.

Table XIII.b.8.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
Watzl *et al.*, 2000	Healthy elderly subjects (33 ♀ and 20 ♂)	63 to 86 years	8 weeks	Placebo-controlled dietary intervention study	47.1 mg lycopene/day from tomato juice Mineral water	Anthropometric measurements. Immunomodulatory activity (concanavalin-stimulated lymphocyte proliferation, cytokine secretion, number and lytic activity of NK cells, assessment of delayed-type hypersensitivity)	No changes in body weights throughout the study. No differences in immune function between treatment groups.
Hininger *et al.*, 2001	175 healthy ♂ volunteers	25 to 45 years	3 months	Placebo-controlled single blind study	β-carotene (15 mg/day *via* capsules) Lutein (15 mg/day *via* capsules) Lycopene (15 mg/day *via* capsules) Placebo (15 mg/day *via* capsules)	Verbal communication regarding tolerance of supplemented doses assumed.	No adverse biological effects were reported.
Olmedilla *et al.*, 2002	175 ♂and 174 ♀ volunteers (French, Irish, Danish and Spanish cohorts)	25 to 45 years	16 weeks	Placebo-controlled supplementation study	Carotene-rich palm oil (15 mg/day *via* capsules) with and without vitamin E Lutein (15 mg/day *via* capsules) with and without vitamin E Lycopene (15 mg/day *via* capsules) with and without vitamin E Placebo (15 mg/day *via* capsules) with and without vitamin E	Plasma lipids (total, HDL- and LDL-cholesterol levels), biochemical and haematological profiles, adverse event report (Spanish cohort only; 32 male and 32 female).	No significant changes in plasma lipid levels, general biochemical, or haematological profiles. Carotenodermia was reported in 25% of the subjects supplemented with lycopene (compared with 95% of those supplemented with carotene and 40% of those supplemented with lutein).

Table XIII.b.8.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
Hoppe *et al.*, 2003	36 healthy subjects (12 ♂, 24 ♀)	26-57 years	28 days	Randomized, placebo-controlled trial	15 mg lycopene/day from synthetic or tomato-based beadlets 0 mg lycopene/day (placebo)	Adverse events established by trained medical investigator, spontaneous reporting.	No adverse events were reported.
Watzl *et al.*, 2003	22 healthy ♂ volunteers	Mean 28.7 years	10 weeks (2 weeks/treatment preceded and followed by 2-week wash-out period)	Randomized, cross-over dietary intervention study	37.0 mg lycopene/day plus 1.6 mg β-carotene/ day from tomato juice 21.7 mg β-carotene/day plus 13.1 mg lycopene/day from carrot juice	Plasma carotenoid levels; modulation of immune function	Time-delayed modulation of immune function.
Briviba *et al.*, 2004	55 ♂ volunteers (30 non-smoking, 25 smoking)	19.6-49.1 years	2 weeks	Double-blind, randomized placebo-controlled study	4.88 mg lycopene 3 times daily from tomato oleoresin extract capsules 0 mg lycopene (placebo)	Immunological functions (IL-2, IL-4 and TNFα production, lymphocyte proliferation, NK cell activity). Verbal communication regarding tolerance of supplemented doses assumed.	Significant decrease in IL-4 overproduction in smokers treated with lycopene. No side effects reported.
Chen *et al.*, 2001	32 prostate cancer patients	60 to 74 years	3 weeks	Non-randomized, whole-food intervention arm of a clinical trial	Approximately 30 mg lycopene/day from tomato sauce-based pasta dishes	Record of gastrointestinal adverse effects (checklist including constipation, burping, gas and/or flatulence, nausea, bloating, diarrhoea, cramping, and heartburn).	Intervention was well accepted by patients. 3 patients reported minor gastrointestinal problems (not specified), which resolved within a few days.

Table XIII.b.8.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
Kucuk *et al.*, 2001	26 patients with newly diagnosed prostate cancer	62 years (mean)	3 weeks	Randomized clinical trial	15 mg lycopene twice daily (15 patients) or no supplementation (11 patients)	Adverse event report, complete physical examination, complete blood count and chemistry profile	No adverse events were reported, no abnormalities were observed in blood counts or chemistries.
Ansari and Gupta, 2003	54 patients with metastatic prostate cancer	<60 years: 33-37%; 60-75: 37-41%; >75: 26%	2 years	Randomized clinical trial	Orchidectomy plus 2 mg lycopene twice daily (27 patients) Orchidectomy alone (27 patients)	Verbal communication regarding tolerance of supplemented doses assumed.	Treatment was well tolerated by all patients, no adverse reactions reported.
Ansari and Gupta, 2004	20 patients with metastatic hormone refractory prostate cancer (HRPC)	56-90 years, median age 72	3 months	Non-randomized study	10 mg lycopene per day	Patients were instructed to report side effects or toxicity related to the drug	No drug intolerance or toxicity was reported.
Mohanty *et al.*, 2005	40 patients with high-grade prostate intraepithelial neoplasia (HGPIN)	N/a	1 year	Randomized study	4 mg lycopene twice daily (20 patients) or no supplementation with recommended decrease in dietary tomato and melon intake (20 patients)	Verbal communication regarding tolerance of supplemented doses assumed.	No adverse events were observed.
Clark *et al.*, 2006	36 patients with biochemically relapsed prostate cancer	56-83 years, median age 74	1 year	Dose-escalating clinical trial	15, 30, 45, 60, 90 or 120 mg/day (6 patients in each group)	Toxicity was graded according to the revised National Cancer Institute Common Toxicity Criteria, v. 2.0.	Supplementation was well tolerated. Therapy was discontinued in 1 patient due to diarrhoea.

XIII.b.8.3 Epidemiology Studies

The purported health benefits of lycopene are numerous and have been explored in several epidemiology studies that examine the relationship between lycopene and chronic disease prevention, including prevention of certain cancers and cardiovascular disease (Giovannucci, 1999; Arab and Steck, 2000). A summary of the epidemiologic literature relating the intake of lycopene or blood lycopene levels to prostate cancer risk is included in Appendix F as a representative of the extensive literature exploring the role of lycopene in cancer prevention. In general, despite a current lack of long-term clinical trials, the role of lycopene in disease prevention is suggestive of being protective (Giovannucci, 1999; Agarwal and Rao, 2000); however, an epidemiology study conducted by Mares-Perlman *et al.* (1995) reported a positive correlation between dietary lycopene intake and severity of nuclear sclerosis in women. Diets of middle- to older-aged adults participating in the Beaver Dam Eye Study were assessed retrospectively, and relations between intakes of nutrients and nuclear opacities in the lens of the eye were evaluated using logistic regression analyses. Women in the highest lycopene intake quintile (mean intake of approximately 203 µg lycopene/day *versus* 38 µg for the lowest quintile) had a significantly greater risk of having severe nuclear sclerosis. A similar direct relationship was reported for one of the three food sources of lycopene (*e.g.*, statistically significant relationship for pasta with tomato sauce but not for tomatoes/tomato juice or pizza) in women; however, there were no statistical relationships reported between lycopene or lycopene food sources and severity of nuclear sclerosis in men. The biological significance of these findings is unknown given that they were observed only in females, and given the inherent limitations of the study design (*i.e.*, additional data from clinical studies are needed to support those obtained retrospectively from dietary questionnaires).

XIII.b.9 Additional Safety Considerations Related to Lycopene

Carotenoids are suggested to act as potential antioxidants in biologic systems, thereby scavenging free radicals and other oxidants involved in disease processes (Khachik *et al.*, 1995; Palozza, 1998; IOM, 2000). As mentioned in Section XI.b, lycopene is considered one of the most potent antioxidants among the carotenoids due to its unsurpassed singlet-oxygen-quenching ability (Di Mascio *et al.*, 1989; Khachik *et al.*, 1995; Agarwal and Rao, 2000; Rao and Agarwal, 2000), and it has been demonstrated both *in vitro* (Klebanov *et al.*, 1998; Stahl *et al.*, 1998) and *in vivo* (Rao and Agarwal, 1998b) that lycopene possesses antioxidant activity.

It has been stated that all antioxidants are redox (reduction-oxidation) agents, which can protect against free radicals in some circumstances (antioxidant activity), and promote free radical generation in others (prooxidant activity) (Herbert, 1996). As reviewed by Palozza (1998), increasing evidence suggests that the antioxidant activity of carotenoids may shift into prooxidant activity depending on the redox potential of the carotenoid molecule, as well as on the biologic environment in which it acts. Factors implicated in the possible prooxidant activity of carotenoids (*i.e.*, factors favouring a prooxidant over antioxidant behaviour of

carotenoids) include increased carotenoid concentration, increased oxygen tension of tissues, and/or inadequate pre-existing antioxidant defences (Palozza, 1998). The development of excessive prooxidant activity in normal cells may pose a safety concern due to the potential generation of oxidative damage; however, the majority of the data in the literature pertaining to a prooxidative role of carotenoids has been inconclusive and limited to β-carotene (Palozza, 1998).

XIII.b.10 Potential Allergenicity Concerns

It is unlikely that the microorganism (*B. trispora*) would be present in the final crystal or CWD formulations given the purification process. This is supported through the high level of purity of the final crystal and the fact that analytical procedures failed to detect the presence of yeasts, moulds, *Salmonella* or *E. coli*. Furthermore, no proteins were detected in samples of the high oleic sunflower oil, the 5%, or the 20% lycopene oil suspension, as determined by the Bradford assay (detection limit 1 µg protein/mL or 1 µg protein in 400 mg of lycopene oil suspension) indicating a lack of allergenic potential (see Appendix H).

EVALUATION AND CONCLUSION

As demonstrated in Sections II.c and XI.a, lycopene from *B. trispora* [strains VKPM F-744 (-) and VKPM F-816 (+)] is chemically and nutritionally equivalent to naturally occurring lycopene. The safety of lycopene from *B. trispora* is therefore largely supported by an extensive knowledge of lycopene metabolism, a history of use due to the natural presence of lycopene in food, and published literature on the safety (acute toxicity, sub-chronic toxicity, reproductive toxicity, carcinogenicity, mutagenicity/genotoxicity, and clinical trials) of lycopene derived from sources other than fungal (*i.e.*, dietary lycopene). Furthermore, minimal exposure to the fungal-derived lycopene product is expected based on the conditions of intended food use and the estimated intakes derived there from.

Safety of the fungal source of lycopene has been confirmed in two toxicological studies including a 28-day oral feeding study conducted with the *B. trispora* biomass in rats (Jonker, 2000) and a 90 day study with lycopene oil suspensions, as well as in two mutagenicity studies conducted with the lycopene CWD products, including a bacterial mutation test and a chromosome aberration test (CTBR, 2003a,b). The safety of *B. trispora* is further supported by expert reviews of the literature, which concluded that the *B. trispora* strains are considered to be non-toxigenic and non-pathogenic, and by the SCF and JECFA who have verified the safety of the micro organism and the resultant production of β-carotene (SCF, 2000; JECFA, 2001). In addition, stability tests, microbiological tests and protein presence analysis have been performed on the final lycopene product, which demonstrate that it is free of protein, mycotoxins and other toxic metabolites.

The mean estimated intakes of lycopene (as CWD products) from the proposed food-uses vary from 1.35 to 3.54 mg/person/day, and the 95th percentile intakes range from 4.19 to 8.16 mg/person/day. These are within the range of the dietary intakes of lycopene from natural dietary sources estimated by EFSA in its evaluation of a lycopene oil suspension from *B. trispora* (average intake between 0.5 and 5 mg/day, and high intakes up to approximately 8 g/day) (EFSA, 2005b).

SUMMARY

Approval is sought under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredie*nts, for the approval of lycopene CWD products produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *B. trispora* (strains VKPM F-744 (-) and VKPM F-816 (+)) as a nutritional ingredient in foods (European Parliament and the Council of the European Union, 1997). The safety of lycopene CWD products from *B. trispora* is based on the purity of lycopene from *B. trispora* (> 95%), the conformity between biosynthetically-derived lycopene in nature and chemically-derived lycopene from *B. trispora*, the historical consumption of lycopene as a normal component of the diet (*e.g.*, red fruits and vegetables including tomatoes, watermelon, pink grapefruit, apricots), minimal exposure

under the conditions of intended food use, safety data provided by Vitatene for lycopene from *B. trispora* and for the biomass, additional safety data for the biomass, and published toxicological and clinical data conducted with lycopene (from sources other than *B. trispora*). Lycopene from *B. trispora*, in an oil suspension formulation, is approved as a novel food in the EU and is considered to be nutritionally identical to natural dietary lycopene. Furthermore, the SCF and JECFA have previously considered that β-carotene from *B. trispora*, produced *via* an identical biosynthetic route and process, as lycopene from *B. trispora* (see Figure III.b-1), to be acceptable for use as a colouring agent for foodstuffs, thereby providing additional support that lycopene CWD products from *B. trispora* are safe for human consumption. Finally, the estimated intakes of lycopene (as CWD products) from the proposed food-uses are within the range of background intakes of lycopene from natural dietary sources estimated by EFSA in its evaluation of a lycopene oil suspension from *B. trispora*.

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