APPLICATION FOR THE APPROVAL OF LYCOPENE 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

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APPLICATION FOR THE APPROVAL OF LYCOPENE 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

ADMINISTRATIVE DATA

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GENERAL INTRODUCTION

Vitatene proposes to market lycopene, derived from the fungus *Blakeslea trispora*, for use as a nutritional food ingredient and dietary supplement 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 ingredients* (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).

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 from *B. trispora* is thus considered a novel food/food ingredient due to its 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 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 (an oil suspension prepared with high oleic sunflower oil to provide 5 or 20% crystalline lycopene) have a history of food use in the Community, the source organism does not. Accordingly, lycopene 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.

[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.

As detailed herein, the safety of lycopene from *B. trispora* is supported by 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 use, safety data provided by Vitatene for the final lycopene suspension 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*).

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I. SPECIFICATIONS OF LYCOPENE 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

Vitatene's lycopene is derived from the fungus *Blakeslea trispora*. The proposed trade name for lycopene derived from *B. trispora* is LICONAT.

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:

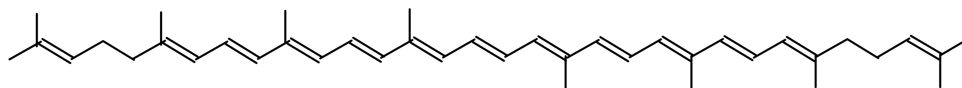
all-*trans* lycopene [502-65-8]

I.c Empirical Formula

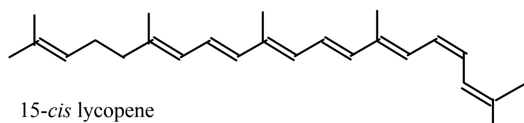
Lycopene is a nonpolar hydrocarbon chain with two open-end rings, a molecular weight of 536.87 daltons, and empirical formula $C_{40}H_{56}$ (Merck, 2001).

I.d Structural Formulae

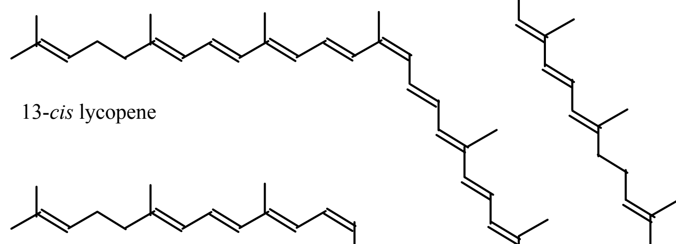
Lycopene occurs in an all-*trans* form (predominant form in foods), and has various *cis* isomers (common in human blood and tissue) (Cronin, 2000). All-*trans* lycopene is a red crystalline powder with a melting point of 173°C that is soluble in fats and certain organic solvents but virtually insoluble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



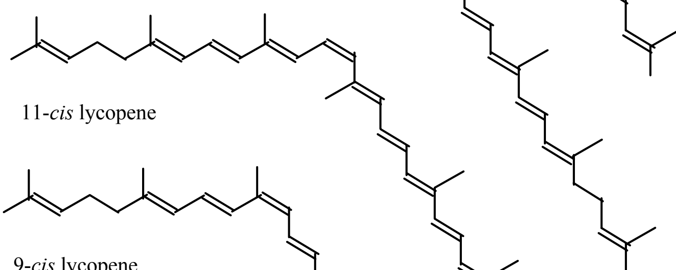
all-*trans* lycopene



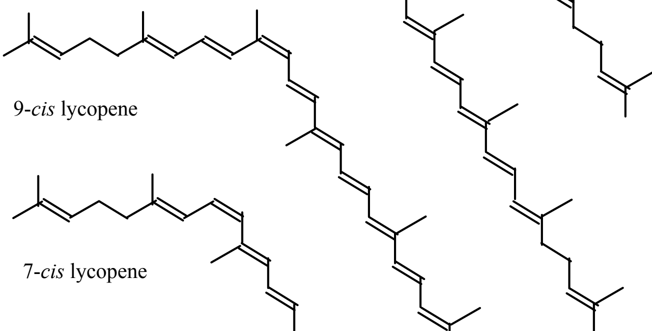
15-*cis* lycopene



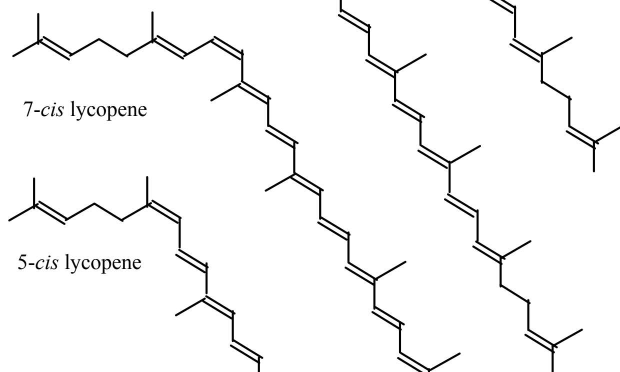
13-*cis* lycopene



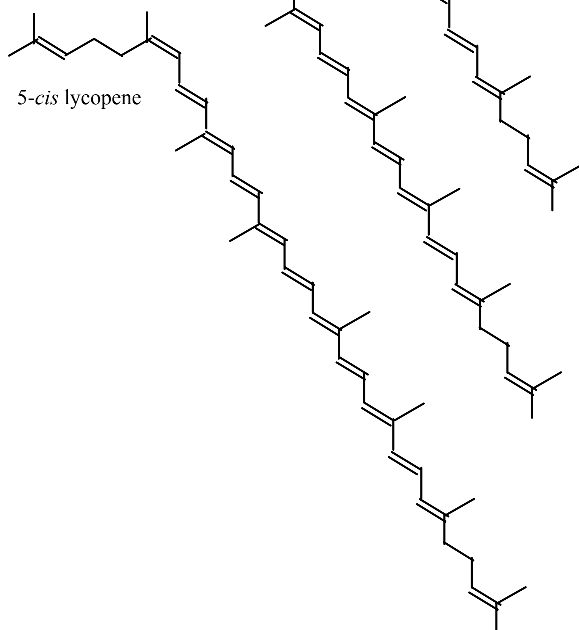
11-*cis* lycopene



9-*cis* lycopene



7-*cis* lycopene



5-*cis* lycopene

I.e Product Specifications and Analyses for Lycopene Crystals and Lycopene Oil Suspensions (5% and 20%)

Lycopene oil suspension is described as a dark red viscous liquid intended to be used as a nutritional food ingredient. Lycopene produced following a fermentation process is extracted from the biomass, purified and crystallised. The crystals are then added to high oleic sunflower oil containing the antioxidant tocopherol, which is added in accordance with Directive 95/2/EC. The product specifications and the analyses of 3 non-consecutive, representative lots of lycopene crystals, 20% lycopene oil suspension, and 5% lycopene oil suspension 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.

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 Product Specifications and Analyses of Lycopene 20% Oil Suspension

Test	Specification*	Batch Number		
		LC 052	LC 054	LC 057
Colour	Dark red viscous liquid	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	471	471.5	471
Assay (%) (472 nm)	≥20	21.0	20.1	20.0
Total lycopene (%)	≥20	20.9	20.5	20.1
Trans-lycopene (%)	≥90	95.0	95.6	95.7
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	<0.150	<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-3 Product Specifications and Analyses of Lycopene 5% Oil Suspension

Test	Specification*	Batch Number		
		LC 052	LC 054	LC 057
Colour	Dark red viscous liquid	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	471.5	471.5	471
Assay (%) (472 nm)	≥5	5.8	5.8	5.6
Total lycopene (%)	≥5	5.7	5.9	5.6
Trans-lycopene (%)	≥90	96.0	95.5	93.7
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	<0.150	<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

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 5 and 20% lycopene oil suspension formulations, 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 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.a Raw Materials Used in the Manufacturing Process

II.a.1 *Blakeslea Trispora*

Lycopene is produced by co-fermentation of two strains of the fungus *B. trispora*. The 2 sexual mating types (*plus and minus*) were selected by classical strain techniques so as to increase the efficiency (*i.e*., increase production of lycopene) of naturally occurring strains of *B. trispora*. Both strains are stable cultures and are preserved under conditions consistent with Good Manufacturing Practices (Appendix A). 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).

B. trispora is a Phycomycete of the order *Mucorales*, and it is a heterothallic species of plus and minus strains (sexes). Studies have shown that while individual *plus* and *minus* strains are able to grow alone, produce asexual spores and synthesize small amounts of carotenoids (namely β–carotene), the production of these are notably enhanced by growing *plus* and *minus* cultures together. The evidence indicates that mated, but not unmated cultures produce a family of acidic compounds, which stimulate synthesis. These compounds are referred to as trisporic acids or β-factor because they are proven to stimulate β-carotene synthesis, mainly in the minus strain (Sutter and Rafelson, 1968; Sutter *et al.*, 1973).

II.a.2 High Oleic Sunflower Oil

High oleic sunflower oil, meeting food grade specifications, is used during the lycopene oil suspension manufacture method. Specifications for high oleic sunflower oil are listed in Appendix A.

II.a.3 Tocopherol

Tocopherol is added as an antioxidant to the lycopene oil suspension formulation (1% of the final lycopene level). Tocopherol, meeting food grade specifications is used in accordance with EU Directive 95/2/EC (see Appendix A).

II.b Manufacturing Process

The manufacturing of lycopene is carried out in two phases. The fermentation phase refers to the production of lycopene by the fungus *B. trispora* under adequate conditions, and the extraction and recovery phase involves solvent extraction of lycopene from the biomass of the fermentation broth, and recovery of the product as high purity lycopene crystals. The final lycopene product is formulated into an oil suspension prior to packaging. All the recovery, formulation, and packaging processes are carried out under nitrogen atmosphere to avoid degradation of the product.

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

The chemical similarities between lycopene produced from *B. trispora,* naturally occurring lycopene from tomatoes and synthetic lycopene are outlined in Table II.c-1. In addition, 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.

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	5-20% oil suspension with α-tocopherol (1% of lycopene level)

[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. Synthetic lycopene however, 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.

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 isomerization and degradation when exposed to light and heat (Lee and Chen, 2002).

II.d.1 Stability of Lycopene Oil Suspension

According to an internal stability protocol written following ICH guidelines (Q1A: "Stability testing of new drug substances and products", and Q1E: "Evaluation of stability data"), the stability of lycopene oil suspensions (average values for batch numbers LC 052, LC 054, and LC 057 for each of the 5% and 20% oil suspensions), 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% relative humidity (RH) for 3, 6, and 12 months (Table II.d.1-1), at 40°C ± 2°C and 75% ± 5% RH for 0.5, 1, 3, and 6 months (Table II.d.1-2), and under conditions of intended use at 5°C ± 3°C for 3, 6, and 12 months (Table II.d.1-3). In addition, the stability of lycopene 20% oil suspension (batch number 029), used during the sub chronic toxicity study and stored in an aluminium bottle filled to the top without air displacement by nitrogen, has been evaluated under conditions of intended use at 5°C ± 3°C over a 2.5-year period (Table II.d.1-4).

Table II.d.1-1 Stability of 5% and 20% Lycopene Oil Suspension Following Storage at 25°C ± 2°C and 60% ± 5% RH for 6 Months**

Test	5% Lycopene Oil Suspension				20% Lycopene Oil Suspension			
	Duration (months)				Duration (months)			
	Initial	3	6	12	Initial	3	6	12
Assay (%)	5.71	5.63	5.66	5.68	20.37	20.42	20.29	20.45
Total lycopene (HPLC) (%)	5.75	5.42	5.60	5.65	20.50	20.61	19.74	20.51

**See Appendix C

Table II.d.1-2 Stability of 5% and 20% Lycopene Oil Suspension Following Storage at 40°C ± 2°C and 75% ± 5% RH for 6 Months**

Test	5% Lycopene Oil Suspension					20% Lycopene Oil Suspension				
	Duration (months)					Duration (months)				
	Initial	0.5	1	3	6	Initial	0.5	1	3	6
Assay (%)	5.71	5.44	5.57	5.59	5.71	20.37	19.97	20.49	20.17	20.14
Total lycopene (HPLC) (%)	5.75	5.48	5.35	5.60	5.62	20.50	20.65	20.14	20.34	19.98

**See Appendix C

Table II.d.1-3 Stability of 5% and 20% Lycopene Oil Suspension Following Storage at 5°C ± 3°C for 6 Months**

Test	5% Lycopene Oil Suspension				20% Lycopene Oil Suspension			
	Duration (months)				Duration (months)			
	Initial	3	6	12	Initial	3	6	12
Assay (%)	5.71	5.59	5.65	5.64	20.37	20.34	20.53	20.53
Total lycopene (HPLC) (%)	5.75	5.53	5.58	5.62	20.50	20.08	19.94	21.14

**See Appendix C

Table II.d.1-4 Stability of 20% Lycopene Oil Suspension Following Storage at 5°C ± 3°C for 2.5 Years**

Test	Duration (months)					
	Initial	7	14.5	18.5	25	30
Assay (%)	20.0	20.6	19.85	19.59	19.94	19.06
Total lycopene (HPLC (%)	20.22	21.4	20.2		20.21	19.19

**See Appendix C

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

II.d.2 Stability of Lycopene in Food

Since the lycopene delivered in the oil suspension 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 in suspension are similar to those that would occur naturally; however, it has been suggested that the stability of lycopene is enhanced when delivered in sunflower oil due to the presence of antioxidants (Lee and Chen, 2002). Furthermore, the presence of macromolecules in a food system have been suggested to offer additional protection for lycopene (Lee and Chen, 2002); therefore, the lycopene suspension stability data presented above are expected to be a conservative representation of the stability of lycopene under the proposed conditions of intended use (*i.e.*, lycopene oil suspension in food).

The stability of the lycopene oil suspension in food is supported by a stability experiment conducted with rat feed containing lycopene at 0, 0.25, 0.50, and 1.0% (Jonker *et al*., 2003). For each concentration level, diets were sampled immediately after preparation, following storage 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).

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).

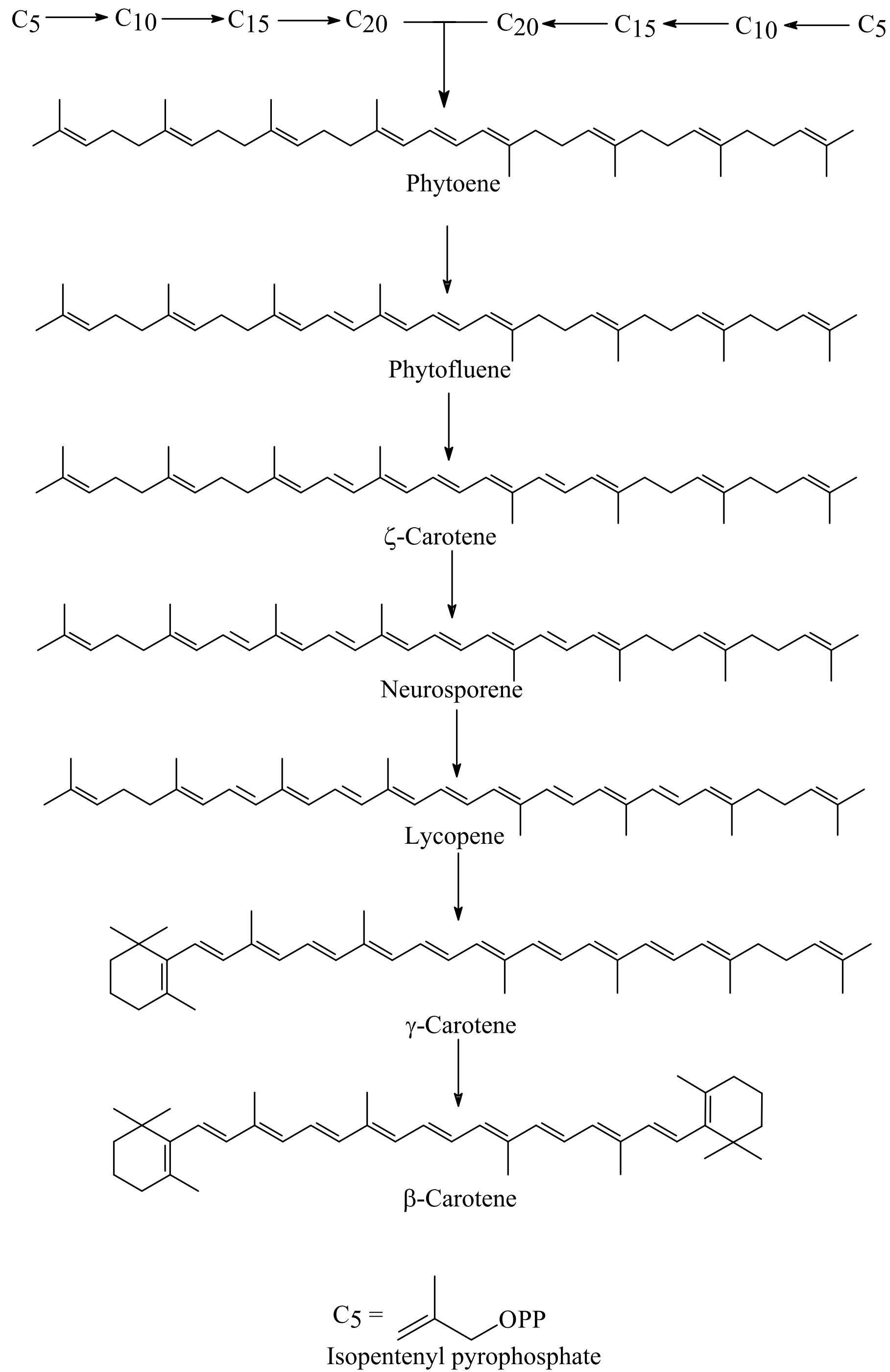
A similar position regarding β-carotene has recently been taken by the Joint Expert Committee on Food Additives (JECFA, 2001), who concluded that β-carotene isolated from different sources, including that from *Blakeslea trispora* is acceptable for food additive use, as long as it is of sufficient purity to meet the specifications for synthetic β-carotene. Therefore neither the SCF or 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 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 H).

Figure III.c-1 Lycopene Biosynthetic Route and Process are Identical to β–Carotene from *B. Trispora*



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), the final formulated product (5% and 20% oil suspension) (Table III.d-2 and III.d-3, respectively) and a sample of the biomass of *B. trispora*. Aflotoxin B2, Aflotoxin G1 and Aflotoxin G2 were also analysed for the biomass (Table III.d-4).

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 Lycopene 20% Oil Suspension

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-3 Product Specifications and Analyses of Lycopene 5% Oil Suspension

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-4 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 or the oil suspension formulations. 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, which 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 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.b.

IX.a Conditions of Intended Food Use

Lycopene oil suspension is intended for use as a nutritional food ingredient and dietary supplement. The individual proposed food-uses for lycopene from *B. trispora* in the E.U. are summarized in Table IX.a-1.

Table IX.a-1 Summary of the Individual Proposed Food Uses and Use-Levels for Lycopene in the E.U.

Food Category	Proposed Food-Use	Use-Level (ppm)
Fat Spreads	Butter	2.0
	Margarine and Margarine-Like Spreads	5.0
Milk and Milk Products	Desserts, Puddings, and Custards	4.0
	Ice Cream, Ice Milk, Frozen Yogurt, Sherbet, and Novelties	6.0
	Processed Cheese	3.0
	Ripened Orange, Yellow, and White Cheese	3.0
Miscellaneous	Condiments, Seasonings, Relishes, and Pickles	6.0
	Mustard	5.0
	Savoury Sauces and Gravies	7.0
	Soups and Soup Mixes	6.0
Sugar, Preserves, and Confectionery	Sweet Spreads, Fillings, and Icings	5.0

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 from *Blakeslea Trispora* from Proposed Food Uses

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

Estimates for the intake of lycopene in the E.U. were based on the proposed use-levels for lycopene summarized in Table IX.a-1 and food consumption data collected as part of the United Kingdom (U.K.) Food Standards Agency’s, Dietary Survey Programme (DSP). The main component of the DSP is the U.K. National Diet and Nutrition Survey (NDNS) programme commissioned jointly in 1992 by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health, and transferred to the Food Standards Agency on its inception in April

2000. The NDNS programme consists of four different surveys for specific age groups, conducted approximately every 3 years in succession. In 2001, an NDNS for adults aged 19 to 64 was completed, but the raw data is not yet available for public use. 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 Dietary and Nutritional Survey of British Adults, aged 16 to 64 (DNSBA, 1986-1987) (UKDA, 1991), were used to generate estimates in the current intake analysis.

Combined, these surveys provide the most up-to-date data for evaluating food-use, food-consumption patterns, and nutritional status in the U.K., containing 4- or 7-day weighed food records for individuals selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001) or local authority wards (UKDA, 1991) as the primary sampling unit.

NDNS data were collected from individuals and households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighed dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviour due to seasonality. Dietary data were recorded by survey respondents, or in the case of the children's survey, by parents or guardians, for the duration of the survey period. DNSBA 1986-1987 contains 7-day weighed dietary records for more than 2,190 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, 1991, 1995, 2001). The overall response rate (assessed as completion of a full dietary record) for individuals selected for participation in the child, youth, and adult surveys, were 81%, 64%, and 70%, respectively (Gregory *et al.,* 1990, 1995; UKDA, 2001).

In addition to collecting information on the types and quantities of foods being consumed, 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. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples. Sample weights were developed and incorporated with the youth survey (NDNS, 1997) to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to differential sampling probabilities and differential non-response rates, particularly the lower response obtained from males, aged 15 to 18 years (UKDA, 2001).

To facilitate comparison with the adult and youth 7-day dietary survey data, dietary data from the children's survey (4-day data) was weighted to seven days, based on the assumption that

intake patterns on non-recording weekdays are similar to dietary intakes on recorded weekdays; the two weekend days were not reweighted. Accordingly, all food and drink consumed on the two-recorded weekdays were averaged to give a daily intake value, which was multiplied by 5 to approximate intakes for all weekdays. These values were then combined with consumption data from weekend dietary records. Full details of the weighting method applied are provided in Appendix J of the report on the children's diet and nutrition survey (Gregory *et al*., 1995).

Consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, were collated by computer and used to generate estimates for the intake of lycopene by the U.K. population. Estimates for the daily intake of lycopene represent projected 7-day averages for each individual from days 1 to 7 of NDNS data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. All-person intake refers to the estimated intake of lycopene averaged over all individuals surveyed regardless of whether they consumed food products in which lycopene is currently proposed for use, while all-user intake refers to the estimated intake of lycopene by those individuals consuming food products in which the use of lycopene is under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which lycopene is proposed for use on one of the 7 survey days.

Calculations for the mean and high-level (97.5th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for lycopene. Similar calculations were used to determine the estimated total intake of lycopene from all proposed food-uses combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

children, ages 1½ to 4½ ;
young people, ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64.

The estimated total consumption of lycopene from all proposed food uses is summarized in Tables IX.b-1 and IX.b-2 on a mg/person/day and µg/kg body weight/day basis, respectively. A complete intake report is provided in Appendix E.

As would be expected for a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment; greater than 98% of the population groups consisted of users of those food products in which lycopene is currently proposed for use (Table IX.b-1). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results

will be discussed in detail. Of the individual population groups, male adults were determined to have the greatest mean and 97.5th percentile all-user intakes of lycopene on an absolute basis, at 0.60 mg/person/day and 1.68 mg/person/day, respectively, while children had the lowest intakes of 0.22 and 0.65 mg/person/day, respectively (Table IX.b-1). Estimated daily lycopene intakes increased with age in all groups, but were lower in females relative to males.

Table IX.b-1 Summary of the Estimated Daily Intake of Lycopene 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	97.5		90	95	97.5
Children	1½ - 4½	98.8	1,573	0.21	0.43	0.53	0.65	0.22	0.43	0.54	0.65
Young People	4-10	99.8	835	0.37	0.69	0.80	0.93	0.37	0.69	0.81	0.93
Female Teenager	11-18	99.3	445	0.39	0.78	0.91	1.02	0.40	0.79	0.91	1.02
Male Teenager	11-18	99.8	415	0.42	0.84	0.99	1.18	0.42	0.84	0.99	1.18
Female Adults	16-64	99.6	1100	0.46	0.88	1.04	1.23	0.46	0.88	1.04	1.23
Male Adults	16-64	99.5	1082	0.60	1.15	1.37	1.68	0.60	1.15	1.37	1.68

Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 97.5th percentile all-user lycopene intakes of 15.1 and 44.9 μg/kg body weight/day, respectively, while female adults and female teenagers had the lowest intakes mean and 97.5th percentile intakes, respectively, at 7.4 and 20.6 μg/kg body weight/day (Table IX.b-2).

Table IX.b-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Lycopene 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 (μg/kg)	Percentile (μg/kg)			Mean (μg/kg)	Percentile (μg/kg)		
					90	95	97.5		90	95	97.5
Children	1½ - 4½	98.8	1,573	14.9	30.5	37.6	44.9	15.1	30.6	37.7	44.9
Young People	4-10	99.8	835	14.5	27.6	31.5	36.0	14.6	27.9	31.5	36.0
Female Teenager	11-18	99.3	445	7.3	14.8	17.7	20.6	7.6	15.1	17.7	20.6
Male Teenager	11-18	99.8	415	7.8	16.2	19.3	23.8	7.9	16.2	19.3	23.8
Female Adult	16-64	99.6	1100	7.4	14.1	17.4	21.0	7.4	14.2	17.6	21.0
Male Adult	16-64	99.5	1082	8.0	15.4	18.5	22.4	8.1	15.4	18.7	22.6

IX.b.2 Estimated Daily Lycopene Intake from Individual Proposed Food-Uses

Estimates for the mean all-user intakes of lycopene from each of the individual food-uses demonstrated that (Appendices A and B of Appendix E), similar to the case observed for the total intakes of lycopene in the U.K., male adults consuming soups and soup mixes experienced the highest mean and 97.5th percentile all-user intakes of lycopene, of 468.8 and 1,736.0 µg/person/day, respectively, while children consuming soups and soup mixes were identified as having the highest mean and 97.5th percentile all-user intakes of lycopene on a body weight basis, 23.3 and 64.2 µg/kg body weight/day, respectively.

IX.c Dietary Supplement Use

In addition to its proposed use in food, lycopene from *B. trispora* will be used as a dietary ingredient intended for incorporation into dietary supplements. Lycopene in a diluted oil formulation will be incorporated into tablets or capsules at a level providing up to 20 mg purified lycopene per day. Dietary supplements containing lycopene are already widely marketed throughout the European Union although to date there are no reliable estimates of the amounts consumed. Since lycopene dietary supplements from sources other than *B. trispora* are already in the European marketplace, it is considered unlikely that the proposed usage as a dietary supplement will increase the level of intake; rather it is anticipated that this product will replace those already being consumed. The only additional intake levels would therefore be related to those incorporated into food.

IX.d Conclusions

Consumption data and information pertaining to the individual proposed food-uses for lycopene were used to estimate the all-person and all-user lycopene 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 97.5th percentile intakes of lycopene by the U.K. population from all proposed food-uses in the E.U., as observed in male adults, were estimated to be 0.60 mg/person/day (8.1 µg/kg body weight/day) and 1.68 mg/person/day (22.6 µg/kg body weight/day). On a body weight basis, children consumed the greatest amount of lycopene, with mean and 97.5th percentile all-user intakes of 15.1 and 44.9 µg/kg body weight/day, respectively. These consumption estimates are equivalent to dietary lycopene

intakes reported in Europe (see Section X.a.1) and significantly less than those reported in Canada and the U.S. (see Section X.a.2).

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 mitochondrial, toxicological and/or allergenicity problems?"

These questions have been addressed collectively in Section X.a.

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).

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) (USDA, 1996), 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).

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

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; 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 E.U. (SCF/CS/ADD/COL/160 Final 6/12/99) but is considered Generally Recognised as Safe (GRAS) for use as a food ingredient in the U.S. (GRAS Notice No. GRN 000119).

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 oil suspension (α-tocopherol and high oleic sunflower oil) are similarly present in food, it is expected that the lycopene oil suspension derived from *B. trispora* is nutritionally equivalent to naturally occurring lycopene.

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 G, 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.b-3.

XII.a Microbiological Specifications and Analyses for Lycopene Crystals and Lycopene 5% and 20% Oil Suspensions

As outlined in Tables XII.a-1, XII.a-2, and XII.a-3, typical food borne microbes (*e.g.*, moulds, yeasts, *Salmonella*, *Escherichia coli*) do not appear in the final lycopene crystals, lycopene 20% oil suspension, and lycopene 5% oil suspension products, respectively.

Table XII.a-1 Product 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 B

Table XII.a-2 Product Specifications and Analyses of Lycopene 20% Oil Suspension

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 B

Table XII.a-3 Product Specifications and Analyses of Lycopene 5% Oil Suspension

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 B

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.c.

XIII.a 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 F 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. Based on these data, it was concluded that the rat can be regarded as a useful and appropriate animal model for the study of lycopene.

XIII.a.1 Biomass

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, neurobehavioral 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.a.2 Final Product

The long-term safety of lycopene 5 and 20% oil suspension 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 guidelines (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 weigh/day for males, and 0, 156, 312, and 616 mg/kg body weigh/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).

Two further 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* (draft summary reports currently available, 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.a.3 Margin of Safety

Based on the average NOEL of 601 mg lycopene/kg body weight per day from the rat sub-chronic study, and the maximum level of intake from proposed food uses and from dietary supplement intake (see Section IX), it is possible to calculate the human safety margin following the consumption of lycopene in these forms. The difference between the NOEL and the food use intake level of between 1 and 2 mg/day provides an approximate 20,000-fold safety margin whereas a dietary supplement intake level of 20 mg/day provides an approximate 2,000-fold safety margin.

XIII.b 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.a.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, 1992), 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 H).

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.c Toxicological Assessment of Lycopene from Sources Other than *Blakeslea Trispora*

A number of different sources of lycopene have been evaluated in both pre-clinical and clinical investigation studies. The different sources include natural tomato extracts, tomato paste and synthetically produced lycopene. In addition a number of different formulations have been tested including cold-water dispersible formulations (CWD), water soluble (WS) beadlet formulations, and dietary supplements.

XIII.c.1 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. Table XIII.c.1-1 identifies the LD_{50} values for each route of administration.

Table XIII.c.1-1 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

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.

XIII.c.2 Sub-Chronic and Chronic Toxicity Studies

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 cold water dispersible (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. Opthalmoscopic 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, hematocrit, 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 ceacum 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 HanIbm 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 one 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).

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.c.2-1, 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.c.3 and Table XIII.c.3-1).

Table XIII.c.2-1 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 (Jewell and O'Brien, 1999; Gradelet *et al*., 1996). 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.c.3 Carcinogenicity Studies

To date, no experimental animal carcinogenicity studies have been conducted with lycopene; however, several studies examining the potential chemopreventive effects of lycopene on the incidence of experimentally-induced tumours have been performed. Chemoprevention studies conducted with mice and rats that include reports on safety-related endpoints of lycopene are summarized in Table XIII.c.3-1.

Table XIII.c.3-1 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.
Rats				
Wang *et al*., 1989	Tumour cell (C-6 glioma cells) inoculation (subcutaneous injection)	10 mgl/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.

Table XIII.c.3-1 Summary of Anti-Cancer Studies Examining Safety-Related Endpoints of Lycopene				
Reference	**Tumour Induction**	**Lycopene Dose**	**Duration of Lycopene Treatment**	**Safety-Related Findings**
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.

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). 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).

XIII.c.4 Mutagenicity/Genotoxicity Studies

Data pertaining to the mutagenicity and genotoxicity of lycopene are summarized in Table XIII.c.4-1.

Table XIII.c.4-1 Summary of Studies Evaluation the Genetic Toxicity of Lycopene				
Reference	**Assay/End Point**	**Strain/Cell Type**	**Lycopene Dose**	**Result**
In Vitro				
He and Campbell, 1990	Ames: *Salmonella Typhimurium*	TA98, TA100	100 μg lycopene from tomato paste/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

Table XIII.c.4-1 Summary of Studies Evaluation the Genetic Toxicity of Lycopene				
Reference	**Assay/End Point**	**Strain/Cell Type**	**Lycopene Dose**	**Result**
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
Aizawa *et al*., 2000	*Eschericha coli*	WP2 UVR A	0.05 to 5000 µg lycopene from tomato paste /plate (with and without metabolic activation)	Negative
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				
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.

Table XIII.c.4-1 Summary of Studies Evaluation the Genetic Toxicity of Lycopene				
Reference	**Assay/End Point**	**Strain/Cell Type**	**Lycopene Dose**	**Result**
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.

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). 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 two of the three 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.c.5 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 Cold Water Dispersion (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.c.6 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.c.6.2) 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 June 2002. Section XIII.c.6.1 discusses case studies reporting the effects of excessive lycopene intakes.

XIII.c.6.1 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.c.6.2 Clinical Studies

In a critical review of the scientific literature related to the safety of lycopene, 11 clinical studies were identified that evaluated safety-related endpoints, including tolerance (see Table XIII.c.6.2-1). 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;). 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) as well as in prostate cancer patients (Chen *et al.*, 2001; Kucuk *et al.*, 2001). 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). Gastrointestinal intolerances (not specified) were reported in prostate cancer patients 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). 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) occurred 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.c.6.2-1). 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.c.6.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
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		
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)		
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	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).
					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		

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Table XIII.c.6.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
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)	Verbal communication regarding tolerance of supplemented doses assumed.	No adverse biological effects were reported.
					Lutein (15 mg/day *via* capsules)		
					Lycopene (15 mg/day *via* capsules)		
					Placebo (15 mg/day *via* capsules)		
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.
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.

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Table XIII.c.6.2-1 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.
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.
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.

Table XIII.c.6.2-1 Summary of Clinical Studies Evaluating Safety-Related Endpoints of Lycopene

Study	Population	Age	Duration	Study Type	Treatment	Safety-related Endpoints	Results
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 I	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 Bitsh, 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		

XIII.c.6.3 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 G 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.c.7 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.a, 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.c.8 Potential Allergenicity Concerns

It is unlikely that the microorganism (*B. trispora*) would be present in the final crystal or oil 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 I).

EVALUATION AND CONCLUSION

As demonstrated in Sections II.c and XI.a, lycopene from *B. trispora* 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 the extracted lycopene formulation and two mutagenicity studies 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.

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 oil suspension produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *B. trispora* as a nutritional ingredient in foods. The safety of lycopene 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 the final lycopene suspension 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, 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 from *B. trispora* is safe for human consumption.

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REFERENCES

Agarwal, S.; Rao, A.V. 1998. Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. Lipids 33(10):981-984.

Agarwal, S.; Rao, A.V. 2000. Tomato lycopene and its role in human health and chronic diseases. CMAJ 163(6):739-744.

Agarwal, A.; Shen, H.; Agarwal, S.; Rao, A.V. 2001. Lycopene content of tomato products: Its stability, bioavailability and in vivo antioxidant properties. J Med Food 4(1):9-15.

Aizawa, K.; Inakuma, T.; Oshima, S. 2000. Assessment of the mutagenicity of lycopene by the Ames test. Nippon Nogeikagaku Kaishi 74(6):679-681.

Arab, L.; Steck, S. 2000. Lycopene and cardiovascular disease. Am J Clin Nutr 71(6 Suppl.):1691S-1695S [& Discussion, pp. 1696S-1697S].

Black, H.S. 1998. Radical interception by carotenoids and effects on UV carcinogenesis. Nutr Cancer 31(3):212-217.

Böhm, V.; Bitsch, R. 1999. Intestinal absorption of lycopene from different matrices and interactions or other carotenoids, the lipid status, and the antioxidant capacity of human plasma. Eur J Nutr 38(3):118-125.

Boileau, T.W.; Clinton, S.K.; Erdman, J.W. 2000. Tissue lycopene concentrations and isomer patterns are affected by androgen status and dietary lycopene concentration in male F344 rats. J Nutr 130(6):1613-1618.

Bonnetblanc, J.M.; Bonafe, J.L.; Vidal, E. 1987. Carotenodermies dietetiques = [Dietary carotenodermias]. Ann Dermatol Venereol 114(9):1093-1096.

Breinholt, V.; Lauridsen, S.T.; Daneshvar, B.; Jakobsen, J. 2000. Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rat. Cancer Lett 154(2):201-210.

Carughi, A.; Hooper, F.G. 1994. Plasma carotenoid concentrations before and after supplementation with a carotenoid mixture. Am J Clin Nutr 59(9):896-899.

Chen, L.; Stacewicz-Sapuntzakis, M.; Duncan, C.; Sharifi, R.; Ghosh, L.; van Breemen, R.; Ashton, D.; Bowen, P.E. 2001. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 93(24):1872-1879.

Chopra, M.; O'Neill, M.E.; Keogh, N.; Wortley, G.; Southon, S.; Thurnham, D.I. 2000. Influence of increased fruit and vegetable intake on plasma and lipoprotein carotenoids and LDL oxidation in smokers and nonsmokers. Clin Chem 46(11):1818-1829.

Christian, M.S.; Schulte, S.; Hellwig, J. 2003. Developmental (embryo-foetal toxicity/teratogenicity) toxicity studies of synthetic crystalline lycopene in rats and rabbits. Food Chem Toxicol 41(6):773-783.

Clinton, S.K. 1998. Lycopene: Chemistry, biology, and implications for human health and disease. Nutr Rev 56(2, Part 1):35-51.

Collins, A.R.; Olmedilla, B.; Southon, S.; Granado, F.; Duthie, S.J. 1998. Serum carotenoids and oxidative DNA damage in human lymphocytes. Carcinogenesis 19(12):2159-2162.

Cronin, J.R. 2000. Lycopene: The powerful antioxidant that makes tomatoes red. Altern Complement Ther 6(2):92-94.

CTBR. 2003a. Lycopene 20% CWD Bacterial Mutation Test. CTBR Bio Research Inc.; Senneville, Que. [Draft Summary Report].

CTBR. 2003b. Lycopene 20% CWD Chromosome Aberration Test. CTBR Bio Research Inc.; Senneville, Que. [Draft Summary Report].

Di Mascio, P.; Kaiser, S.; Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274(2):532-538.

Erdman, J.W. (Jr.); Lachance, P.A. 1973. The effect of lycopene upon serum, liver and intestinal cholesterol in hypercholesterolemic rats. Fed Proc 32(3, Part 1) [Abstract No. 3856].

Feofilova, E.P. 1994. Fungal carotenoids: Their biological functions and practical use (Review). Appl Biochem Microbiol 30(2):143-154.

Forman, M.R.; Lanza, E.; Yong, L.C.; Holden, J.M.; Graubard, B.I.; Beecher, G.R.; Meltiz, M.; Brown, E.D.; Smith, J.C. 1993. The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: Application of a carotenoid food- composition database. Am J Clin Nutr 58(4):519-524.

Gandhi, M.; Walton, S.; Wyatt, E.H. 1988. Hypercarotenaemia in a tomato soup faddist. BMJ 297(6664):1635.

Gerster, H. 1997. The potential role of lycopene for human health. J Am Coll Nutr 16(2):109-126.

Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemologic literature. J Natl Cancer Inst 91(4):317-331.

Giuliano, A.R.; Neilson, E.M.; Yap, H.-H.; Baier, M.; Canfield, L.M. 1994. Quantitation of and inter/intra-individual variability in major carotenoids of mature human milk. J Nutr Biochem 5(11):551-556.

Gradelet, S.; Astorg, P.; Leclerc, J.; Chevalier, J.; Vernevaut, M.-F.; Siess, M.-H. 1996. Effects of canthaxanthin, astaxanthin, lycopene and lutein on liver xenobiotic-metabolizing enzymes in the rat. Xenobiotica 26(1):49-63.

Gregory, J., Foster, K., Tyler, H.; Wiseman, M. 1990. The Dietary and Nutritional Survey of British Adults. U.K. Office of Population Censuses and Surveys, Social Survey Division / U.K. Ministry of Agriculture, Fisheries and Food (MAFF) / U.K. Department of Health / Her Majesty's Stationary Office (HMSO), London, Engl., p 75.

Gregory, J.R.; Collin, D.L.; Davies, P.S.W.; Hughes, J.M.; Clarke, P.C. 1995. National Diet and Nutrition Survey: Children Aged 1 ½ to 4 ½ Years. Vol. 1: Report of the Diet and Nutrition Survey. Her Majesty's Stationary Office (HMSO); London, Engl.

Guttenplan, J.B.; Chen, M.; Kosinska, W.; Thompson, S.; Zhao, Z.; Cohen, L.A. 2001. Effects of a lycopene-rich diet on spontaneous and benzo[a]pyrene-induced mutagenesis in prostate, colon and lungs of the lacZ mouse. Cancer Lett 164(1):1-6.

He, Y.; Campbell, T.C. 1990. Effects of carotenoids on aflatoxin B1-induced mutagenesis in *S. typhimurium* TA 100 and TA 98. Nutr Cancer 13(4):243-253.

Herbert, V. 1996. Prooxidant effects of antioxidant vitamins: Introduction. J Nutr 126(4, Suppl.):1197S-2008S & Erratum, 126(7):1902.

Higazi, A.M.; El-Ebrashy, N.; El-Ahmady, L.; El-Ashmawy, S.; Khalafallah, A.; Gaber, A. 1971. Changes in serum triglycerides and blood coagulation in response to acute fat loads in atherosclerotics. J Egypt Med Assoc 54(10):659-670.

Hininger, I.A.; Meyer-Wenger, A.; Moser, U.; Wright, A.; Southon, S.; Thurnham, D.; Chopra, M.; Van Den Berg, H.; Olmedilla, B.; Favier, A.E.; Roussel, A.M. 2001. No significant effects of lutein, lycopene or beta-carotene supplementation on biological markers of oxidative stress and LDL oxidizability in healthy adult subjects. J Am Coll Nutr 20(3):232-238.

ICH Steering Committee. 1997. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals (ICH Harmonised Tripartite Guideline). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Recommended for Adoption at Step 4 of the ICH Process on 16July 1997 by the ICH Steering Committee).

ILSI. 1999. Safety assessment and potential health benefits of food components based on selected scientific criteria (North American Technical Committee on Food Components for Health Promotion). Crit Rev Food Sci Nutr 39(3):203-206.

IOM. 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). National Academy Press (NAP); Washington, DC, pp. 325-382.

Jain, M.G.; Hislop, G.T.; Howe, G.R.; Ghadirian, P. 1999. Plant foods, antioxidants, and prostate cancer risk: Findings from case-control studies in Canada. Nutr Cancer 34(2):173-184.

Järvinen, R. 1995. Carotenoids, retinoids, tocopherols and tocotrienols in the diet: The Finnish Mobile Clinic Health Examination survey. Int J Vitam Nutr Res 65(1):24-30.

JECFA. 1999. Evaulation of Certain Food Additives and Contaminants. Forty-Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives, 1997, Rome. World Health Organization (WHO); Geneva. WHO Technical Report Series, No. 884, pp. 56-64,

JECFA. 2001. Compendium of Food Additives Specification (Addendum 9). 57th Joint FAO/WHO Expert Committee on Food Additives Session, June 5-14, 2001, Rome. Food and Agriculture Organization of the United Nations (FAO); Rome. FAO Food and Nutrition Paper, No. 52(Add. 9), pp. 9-11.

JECFA. 2002. ß-carotene derived from Blakeslea trispora. In: JECFA. Safety Evaluation of Certain Food Additives and Contaminants – 57th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 5-14, 2001, Rome. World Health Organization (WHO); Geneva, Switz. International Programme on Chemical Safety (IPCS), Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food and Agriculture Oganization of the United States (FAO) WHO Food Additives Series, No. 48, pp. 33-35.

Jewell, C.; O'Brien, N.M. 1999. Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. Br J Nutr 81(3):235-242.

Johnson, E.J. 1998. Human studies on bioavailability and plasma response of lycopene. Proc Soc Exp Biol Med 218(2):115-120.

Johnson-Down, L.; Saudny-Unterberger, H.; Gray-Donald, K. 2002. Food habits of Canadians: Lutein and lycopene intake in the Canadian population. J Am Diet Assoc 102: 988-991.

Jonker, I.D. 2000. Repeated Dose (28-day) Oral Toxicity Study with Carotene Biomass and Lycopene Biomass in Rats. TNO Nutrition and Food Research Institute.

Jonker, D.; Kuper, C.F.; Fraile, N.; Estrella, A.; Otero, C.R. 2003. Ninety-day oral toxicity study of lycopene from *Blakesla trispora* in rats. Regul Toxicol Pharmacol 37(3):396-406.

Khachik, F.; Beecher, G.R.; Smith, J.C. (Jr.). 1995. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J Cell Biochem Suppl 22:236-246.

Kim, W.M.; Merskey, C.; Deming, Q.B.; Adel, H.N.; Wolinsky, H.; Clarkson, T.B.; Lofland, H.B. 1976. Hyperlipidemia, hypercoagulability, and accelerated thrombosis: Studies in congeniality hyperlipidemic rats and in rats and monkeys with induced hyperlipidemia. Blood 47(2):275-286.

Kim, D.J.; Takasuka, N.; Kim, J.M.; Sekine, K.; Ota, T.; Asamoto, M.; Murakoshi, M.; Nishino, H.; Nir, Z.; Tsuda, H. 1997. Chemoprevention by lycopene of mouse lung neoplasia after combined initiation treatment with DEN, MNU and DMH. Cancer Lett 120(1):15-22.

Kim, J.M.; Araki, S.; Kim, D.J.; Park, C.B.; Takasuka, N.; Baba-Toriyama, H.; Ota, T.; Nir, Z.; Khachik, F.; Shimidzu, N.; Tanaka, Y.; Osawa, T.; Uraji, T.; Murakoshi, M.; Nishino, H.; Tsuda, H. 1998. Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. Carcinogenesis 19(1):81-85.

Klebanov, G.I.; Kapitanov, A.B.; Teselkin, Y.U.O.; Babenkova, I.V.; Zhambalova, B.A.; Lyubitsky, O.B.; Nesterova, O.A.; Vasil'eva, O.V.; Popov, I.N.; Lewin, G.; Vladimirovy, U.A. 1998. The antioxidant properties of lycopene. Membr Cell Biol 12(2):287-300.

Kohlmeier, L.; Kark, J.D.; Gomez-Gracia, E.; Martin, B.C.; Steck, S.E.; Kardinaal, A.F.; Ringstad, J.; Thamm, M.; Masaev, V.; Riemersma, R.; Martin-Moreno, J.M.; Huttunen, J.K.; Kok, F.J. 1997. Lycopene and myocardial infarction risk in the EURAMIC study. Am J Epidemiol 146(8):618-626.

Kucuk, O.; Sarkar, F.H.; Sakr, W.; Djuric, Z.; Pollak, M.N.; Khachik, F.; Li, Y.W.; Banerjee, M.; Grignon, D.; Bertram, J.S.; Crissman, J.D.; Pontes, E.J.; Wood, D.P. (Jr.). 2001. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomarkers Prev 10(8):861-868.

La Placa, M.; Pazzaglia, M.; Tosti, A. 2000. Lycopenaemia. J Eur Acad Dermatol Venereol 14(4):311-312.

Larsen, L.F.; Bladbjerg, E.M.; Jespersen, J.; Marckmann, P. 1997. Effects of dietary fat quality and quantity on postprandial activation of blood coagulation factor XII. Arterioscler Thromb Vasc Biol 17(11):2904-2909.

Lee, M.T.; Chen, B.H. 2002. Stability of lycopene during heating and illumination in a model system. Food Chem 78(4):425-432.

López-Nieto, M.J.; Pérez, J.C.; Cezón, E.P.; Méndez, E.; Rodríguez-Sáiz, M.; de la Fuente, J.L.; Cabri, W.; Barredo, J.L. 2003. Scaling-up lycopene production by *Blakeslea trispora* fermentation. Appl Environ Microbiol [Submitted for Publication, Feb., 2003].

Mares-Perlman, J.A.; Brady, W.E.; Klein, B.E.K.; Klein, R.; Haus, G.J.; Palta, M.; Ritter, L.L.; Shoff, S.M. 1995. Diet and nuclear lens opacities. Am J Epidemiol 141:322-334.

McClain, R.M.; Bausch, J. 2003. Summary of safety studies conducted with synthetic lycopene. Regul Toxicol Pharmacol 37(2):274-285.

McGirr, L.G.; Copeland, T.D. 2000. Consumption of the Carotenoid, Lycopene, by Free Living Individuals in the United States. Presented to the Institute of Food Technologists (IFT), 2000, Dallas, Texas. CanTox Health Sciences International; Mississauga, Ont.

Mellert, W.; Deckardt, K.; Gembardt, C.; Schulte, S.; Ravenzwaay, B.; Slesinski, R. 2002. Thirteen-week oral toxicity study of synthetic lycopene products in rats. Food Chem Toxicol 40(11):1581-1588.

Merck. 2001. Lycopene. In: The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals (13th Ed.). Merck & Co., Inc.; Whitehouse Station, New Jersey, p.1007 [No. 5640].

Micozzi, M.S.; Brown, E.D.; Edwards, B.K.; Bieri, J.G.; Taylor, P.R.; Khachik, F.; Beecher, G.R.; Smith, J.C. (Jr.) 1992. Plasma carotenoid response to chronic intake of selected foods and beta-carotene supplements in men. Am J Clin Nutr 55(6):1120-1125.

Milani, C.; Maccari, M.; Mosconi, P. 1970. Action of lycopene in the experimental gastric ulcer. Pharmacology 4(6):334-340.

Miller, G.J. 1997. Dietary fatty acids and blood coagulation. Prostaglandins Leukot Essent Fatty Acids 57(4&5):389-394.

Müller, H., Bub, A., Watzl, B.; Rechkemmer, G. 1999. Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. Z Ernahrungswiss 38(1):35-44.

Nagasawa, H.; Mitamura, T.; Sakamoto, S.; Yamamoto, K. 1995. Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res 15(4):1173-1178.

Narisawa, T.; Fukaura, Y.; Hasebe, M.; Ito, M.; Aizawa, R.; Murakoshi, M.; Uemura, S.; Khachik, F.; Nishino, H. 1996. Inhbitory effects of natural carotenoids, a-carotene, ß-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. Cancer Lett 107 (1):137-142.

Nguyen, M.L.; Schwartz, S.J. 1999. Lycopene: Chemical and biological properties. Food Technol 53(2):38-45.

Okajima, E.; Ozono, S.; Endo, T.; Majima, T.; Tsutsumi, M.; Fukuda, T.; Akai, H.; Denda, A.; Hirao, Y.; Okajima, E.; Nishino, H.; Nir, Z.; Konishi, Y. 1997. Chemopreventive efficacy of piroxicam administered alone or in combination with lycopene and ß-carotene on the development of rat urinary bladder carcinoma after n-butyl-n-(4-hydroxybutyl)nitrosamine treatment. Jpn J Cancer Res 88(6):543-552.

Olmedilla, B.; Grandado, F.; Blanco, I.; Rojas-Hidalgo, E. 1994. Seasonal and sex-related variations in six serum carotenoids, retinol, and alpha-tocopherol. Am J Clin Nutr 60(1):106-110.

Olmedilla, B.; Granado, F.; Southon, S.; Wright, A.J.A.; Blanco, I.; Gil-Martinez, E.; van Den Berg, H.; Thurnham, D.; Corridan, B.; Chopra, M.; Hininger, I. 2002. A European multicentre, placebo-controlled supplementation study with alpha-tocopherol, carotene-rich palm oil, lutein or lycopene: Analysis of serum responses. Clin Sci 102(4):447-456.

Palozza, P. 1998. Prooxidant actions of carotenoids in biologic systems. Nutr Rev 56(9):257-265.

Pool-Zobel, B.L.; Bub, A.; Muller, H.; Wollowski, I.; Rechkemmer, G. 1997. Consumption of vegetables reduces genetic damage in humans: First results of a human intervention trial with carotenoid-rich foods. Carcinogenesis 18(9):1847-1850.

Postaire, E.; Jungmann, H.; Bejot, M.; Heinrich, U.; Tronnier, H. 1997. Evidence for antioxidant nutrients-induced pigmentation in skin: Results of a clinical trial. Biochem Mol Biol Int 42(5):1023-1033.

Rao, A.V.; Agarwal, S. 1998a. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutr Res 18(4):713-721.

Rao, A.V.; Agarwal, S. 1998b. Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer 31(3):199-203.

Rao, A.V.; Agarwal, S. 2000. Role of antioxidant lycopene in cancer and heart disease. J Am Coll Nutr 19(5):563-569.

Rao, M.; Rao, A.V.; Fleshner, N.; Khachik, F.; Kucuk, O. 2002. Prostate cancer prevention 2000: The role of nutrition. Lycopene Prev Chronic Dis 1(1):15-18.

Rauscher, R.; Edenharder, R.; Platt, K.L. 1998. In vitro antimutagenic and in vivo anticlastogenic effects of carotenoids and solvent extracts from fruits and vegetables rich in carotenoids. Mutat Res 413(2):129-142.

Reich, P.; Shwachman, H.; Craig, J.M. 1960. Lycopenemia. A variant of carotenemia. N Engl J Med 262:263-269.

Riso, P.; Pinder, A.; Santangelo, A.; Porrini, M. 1999. Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? Am J Clin Nutr 69(4):712-718.

Rissanen, T. 2002. Lycopene, atherosclerosis and coronary heart disease. Lycopene Prev Chronic Dis 1(1):35-37.

Robert Koch Institute. 1992. Dritter Bericht nach Inkrafttreten des Gentechnikgesetzes (GenTG) für den Zeitraum 1.1. 1992 bis 31.12.1992. Tätigkeitsbericht der Zentralen Kommission für die Biologische Sicherheit (ZKBS) [http://www.rki.de/GENTEC/ZKBS/ZKBS.HTM?ORGLISTE/LISTE_VOR.HTM&1].

Robert Koch Institute. 2002. Zwölfter Bericht nach Inkraft-treten des Gentechnikgestetzes (GenTG) für den Zeitraum vom 1.1.2001 bis 31.12.2001. Die Arbeit der Zentralen Kommission für die Biologische Sicherheit (ZKBS) im Jahr 2001. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz 45(8):643-651. [http://www.rki.de/GENTEC/ZKBS/ZKBS.HTM?ORGLISTE/LISTE_VOR.HTM&1].

Rodríguez-Sáiz, M.; Paz, B.; de la Fuente, J.L.; López-Nieto, M.J.; Cabri, W.; Barredo, J.L. 2003. The *carB* and *caRP* genes of *Blakeslea trispora* involved in ß-carotene biosynthesis are functional in Mucor circinelloides. Appl Environ Microbiol [Submitted for Publication, Feb., 2003].

SCF. 1999. Opinion of the Scientific Committee on Food on Synthetic Lycopene as a Colouring Matter for Use in Foodstuffs (Opinion expressed by the SCF on 2 December 1999). European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food; Brussels, SCF/CS/ADD/COL/160 Final.

SCF. 2000. Opinion of the Scientific Committee on Food on β-Carotene from Blakeslea trispora. Correction. (Opinion Adopted by the SCF on 22 June 2000, and corrected on 7 September 2000). European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food; Brussels, Belgium. SCF/CS/ADD/COL/158 Final-correction.

Scott, K.J.; Thurngham, D.I.; Hart, D.J.; Bingham, S.A.; Day, K. 1996. The correlation between the intake of lutein, lycopene and ß- carotene from vegetables and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK. Br J Nutr 75(3):409-418.

Sharoni, Y.; Giron, E.; Rise, M.; Levi, J. 1997. Effect of lycopene-enriched tomato oleoresin on 7,12-dimethyl-benz[a] anthracene- induced rat mammary tumors. Cancer Detect Prev 21(2):118-123.

Sies, H.; Stahl, W. 1998. Lycopene and ß-carotene bioavailability and biological effects. In: Özben, T. (Ed.). Free Radicals, Oxidative Stress, and Antioxidants: Pathological and Physiological Significance. Plenum Press; New York. NATO ASI Series Series A, Life Sciences, Vol. 296, pp. 315-322.

Stahl, W.; Junghans, A.; de Boer, B.; Driomina, E.; Briviba, K.; Sies, H. 1998. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: Synergistic effects of lycopene and lutein. FEBS Lett 427(2):305-308.

Sutter, R.P.; Rafelson, M.E. 1968. Separation of ß-factor synthesis from stimulated ß-carotene synthesis in mated cultures of *Blakeslea trispora*. J Bacteriol 95(2): 426-432.

Sutter, R.P.; Capage, D.A.; Harrison, T.L.; Keen, W.A. 1973. Trisporic acid biosynthesis in separate plus and minus cultures of *Blakeslea trispora*: Identification by Mucor assay of two mating-type-specific components. J Bacteriol 114(3):1074-1082.

UKDA. 1991. Dietary and Nutritional Survey of British Adults, 1986-1987 [Computer File]. Office of Population Censuses and Surveys, Social Survey Division, Ministry of Agriculture, Fisheries and Food (MAFF) / U.K. Department of Health / UK Data Archive (UKDA); Colchester, Engl. [Distributor, 18 September 1991. SN: 2836].

UKDA. 1995. National Diet, Nutrition and Dental Survey of Children Aged 1 ½ to 4 ½ Years, 1992-1993 [Computer File]. Office of Population Censuses and Surveys, Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF) / U.K. Department of Health / UK Data Archive (UKDA); Colchester, Engl. [Distributor, 13 December 1995. SN: 3481].

UKDA. 2001. National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, 1997 [Computer File]. Office for National Statistics Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF) / U.K. Department of Health / UK Data Archive (UKDA); Colchester, Engl. [Distributor, 25 January 2001. SN: 4243].

USDA. 1996. Continuing Survey of Food Intakes by Individuals (CSFII) / Diet and Health Knowledge Survey (DHKS) 1989-91. U.S. Department of Agriculture (USDA); Washington, DC [PB96-501747, CD-ROM].

Wang, C.H.; Chou, M.Y.; Lin, J.K. 1989. Inhibition of growth and development of the transplantable C-6 glioma cells inoculated in rats by retinoids and carotenoids. Cancer Lett 48(2):135-142.

Watzl, B., Bub, A., Blockhaus, M., Herbert, B.M., Lührmann, P.M., Neuhä user-Berthold, M.; Rechkemmer, G. 2000. Prolonged tomato juice consumption has no effect on cell-mediated immunity of well-nourished elderly men and women. J Nutr 130(7):1719-1723.

Weisburger, H.J. 1998. Evaluation of the evidence on the role of tomato products in disease prevention. Proc Soc Exp Biol Med 218(2):140-143.

Wright, A.J.; Hughes, D.A.; Bailey, A.L.; Southon, S. 1999. Beta-carotene and lycopene, but not lutein, supplementation changes the plasma fatty acid profile of healthy male non-smokers. J Lab Clin Med 134 (6):592-598.

Yong, L.-C.; Forman, M.R.; Beecher, G.R.; Graubard, B.I.; Campbell, W.S.; Reichman, M.E.; Taylor, P.R.; Lanza, E.; Holden, J.M.; Judd, J.T. 1994. Relationship between dietary intake and plasma concentrations of carotenoids in premenopausal women: Application of the USDA-NCI carotenoid food-composition database. Am J Clin Nutr 60(2):223-230.

Zbinden, G.; Studer, A. 1958. Tierexperimentelle untersuchungen über die chronische verträglichkeit von ß-carotin, lycopin, 7,7-dichydro-ß-carotin und bixin. Z Lebensm Unters Forsch 108(2):113-134.

Zhao, Z.; Khachik, F.; Rihie, J.P. (Jr.).; Cohen, L.A. 1998. Lycopene uptake and tissue disposition in male and female rats. Proc Soc Exp Biol Med 218(2):109-114.

Zwaal, R.F.; Comfurius, P.; Bevers, E.M. 1998. Lipid-protein interactions in blood coagulation. Biochim Biophys Acta 1376(3):433-453.