

ORIGINAL SUBMISSION



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DIRECT DIAL (202) 737-4291

June 20, 2005

**BY FEDERAL EXPRESS**

Office of Food Additive Safety  
Center for Food Safety and Applied Nutrition  
U.S. Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, Maryland 20740-3835

REC'D JUN 21 2005

Subject: GRAS Notice for Lycopene derived from *Blakeslea trispora*

Dear Sir/Madam:

Pursuant to the proposed rule outlined at 62 Fed. Reg. 18939 (April 17, 1997), Vitatene SAU (Vitatene) hereby submits this notification that the use of its lycopene derived from *Blakeslea trispora*, its 5% and 20% lycopene oil suspensions and its 10% and 20% lycopene cold water dispersible (CWD) products as a nutrient in foods is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Vitatene has determined that such use is generally recognized as safe (GRAS) based on scientific procedures.

To facilitate your review, this notification is submitted in the format suggested under proposed 21 C.F.R. § 170.36 (c) (see 62 Fed. Reg. at 18961). Three copies of the GRAS Exemption Claim and Additional Information documents are enclosed. Also enclosed is an electronic copy (Microsoft Word) of the documents.

Sincerely,

Diane B. McColl  
Counsel to Vitatene SAU

DBM/csd  
Enclosures

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GRAS

EXEMPTION

## GRAS EXEMPTION CLAIM

We hereby claim that the use of Vitatene SAU's lycopene derived from *Blakeslea trispora*, its 5% and 20% lycopene oil suspensions, and its 10% and 20% lycopene cold water dispersible (CWD) products as a source of the nutrient lycopene in foods is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined that such use is generally recognized as safe (GRAS).

### (1) Name and address of the notifier:

Carmelita Rodríguez Otero  
Quality Unit & RRAA Responsible  
Vitatene S.A.  
Avda. Sáenz de Miera, 50  
24009 León  
Spain

### (2) Common or usual names of the substance that is the subject of the GRAS exemption claim:

Lycopene from *Blakeslea trispora*, Lycopene, LICONAT (trade name)

### (3) Product description

Vitatene SAU (Vitatene's) all-*trans* lycopene derived from *Blakeslea trispora* is a red crystalline powder that is identical to the lycopene that occurs naturally in tomatoes. Vitatene formulates its lycopene into 5% and 20% lycopene oil suspensions, and 10% and 20% cold water dispersible (CWD) products prior to marketing.

### (4) Applicable conditions of use of the notified substance:

#### (a) Foods in which the substance is to be used:

Lycopene from *Blakeslea trispora* is intended for use, as a dietary source of the nutrient lycopene, in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, condiments and relishes, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, gravies and sauces, milk products, plant protein products, processed fruits and fruit juices, snack foods, and soups and soup mixes which otherwise contain very little or no lycopene.

**(b) Levels of use in such foods:**

<b>Food Use Levels for Lycopene from <i>Blakeslea trispora</i>.</b>	
<b>Food Category</b>	<b>Maximum Use-Levels (ppm)</b>
Baked Goods and Baking Mixes	50
Beverages and Beverage Bases	25
Breakfast Cereals	50
Cheeses	5.0
Condiments and Relishes	50
Confections and Frostings	25
Fats And Oils	20
Frozen Dairy Desserts and Mixes	25
Gelatins, Puddings, and Fillings	25
Gravies and Sauces	50
Hard Candy	25
Milk Products	50
Plant Protein Products	50
Processed Fruits and Fruit Juices	25
Snack Foods	30
Soft Candy	25
Soups And Soup Mixes	575

**(c) Purposes for which the substance is used:**

Vitatene's products are intended for use as a source of the nutrient lycopene in the fortification of foods. On rare occasion, the addition of Vitatene's lycopene may result in a color change in the finished food, however, the intended use is not for the purpose of imparting color. In accordance with the color additive exemption in 21 C.F.R. § 70.3(g), Vitatene's lycopene products will be used solely for nutrient fortification, and any incidental coloring imparted to the finished food will not contribute to the value, marketability or consumer acceptance of the food, and may in fact be undesirable. Since the cost of Vitatene's lycopene products is high compared to the existing approved food colors, food manufacturers have no incentive to use lycopene as a color. Vitatene will take appropriate measures, e.g., labeling statement, to advise

manufacturers of the non-color use limitation for Vitatene's lycopene products. As similarly indicated by BASF (GRN 000119) for synthetic lycopene and LycoRed (GRN 000156) for tomato-extracted lycopene, Vitatene will submit a color additive petition for FDA premarket approval should manufacturers express a desire to use Vitatene's lycopene as a color additive.

**(d) Description of the population expected to consume the substance:**

Members of the general population who consume at least one of the food categories described above.

**(5) Basis for the GRAS determination:**

The basis of the GRAS determination is through scientific procedures.

**(6) Review and Copying Statement:**

The data and information that are the basis for Vitatene's GRAS determination are available for the Food and Drug Administration's (FDA's) review and copying at reasonable times at the offices of the notifier, or will be sent to FDA upon request.

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Carmelita Rodríguez Otero  
Quality Unit & RRAA Responsible  
Vitatene S.A.

Please address correspondence to:

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ADDITIONAL  
INFORMATION

## ADDITIONAL INFORMATION

### (1) Identity of the notified substance

#### (a) Common or usual name

Lycopene, LICONAT (trade name)

#### (b) Product description

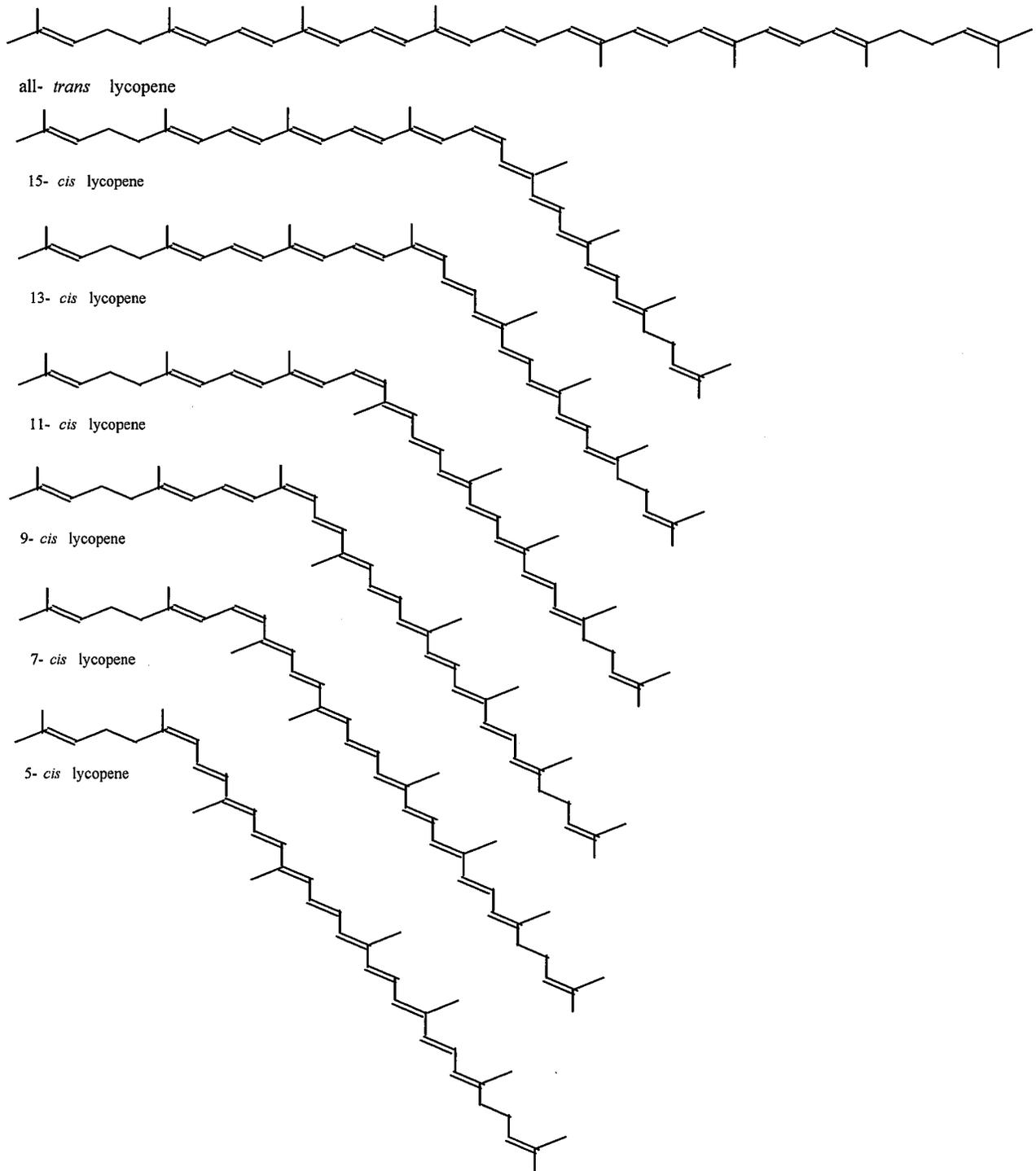
##### (i) Chemical names and CAS numbers for lycopene

all- <i>trans</i> lycopene	[502-65-8]
all- <i>cis</i> lycopene	[4418-71-7]
15- <i>cis</i> lycopene	[59092-07-8]
13- <i>cis</i> lycopene	[13018-46-7]
9- <i>cis</i> lycopene	[64727-64-6]

##### (ii) Structural formulae of lycopene

As depicted in Figure 1, lycopene is an acyclic, nonpolar hydrocarbon chain, consisting of a C<sub>40</sub> isoprenoid skeleton and 2 open-end rings (Rice-Evans *et al.*, 1997). As with all carotenoids, each of the polyene chain double bonds of lycopene could exist in a *cis* or *trans* conformation, resulting in a large number of geometric isomers (see Figure 2.5-1); however, the vast majority of carotenoids exist in the all-*trans* configurations (Furr and Clark, 1997; Rice-Evans *et al.*, 1997; Boileau *et al.*, 1999a; IOM, 2000). Although the all-*trans* form of lycopene predominates in foods, the *cis* isomers, together with *trans* isomers, are common in human blood and tissue (Krinsky *et al.*, 1990; Cronin, 2000; Hadley *et al.*, 2003). Specifically, all-*trans*-lycopene and 5-*cis*-lycopene have been identified as the most abundant lycopene isomers present in human plasma (Hadley *et al.*, 2003).

Figure 1 – Structural Formulae of Lycopene



**(iii) Lycopene chemical and physical characteristics**

Lycopene is an acyclic, nonpolar hydrocarbon chain with molecular formula  $C_{40}H_{56}$  and a molecular weight of 536.87 daltons (Merck, 2001). 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).

**(iv) Vitatene's lycopene product formulations**

All-trans lycopene derived from *Blakeslea trispora* is a red crystalline powder that is identical to lycopene that occurs naturally in tomatoes. Vitatene formulates the lycopene into either oil suspensions or CWD products prior to marketing.

Vitatene's lycopene oil suspensions (5% and 20%) are composed of the following ingredients:

<b>Ingredient</b>	<b>5% Lycopene Oil Suspension</b>	<b>20% Lycopene Oil Suspension</b>	<b>FDA Regulatory Status</b>
Lycopene Crystal	Not less than 5%	Not less than 20%	GRAS
DL- $\alpha$ -Tocopherol*	0.05%	0.2%	GRAS (21 C.F.R. §182.3890)
High Oleic Sunflower Oil	Sufficient quantity for total weight	Sufficient quantity for total weight	GRAS

\*DL- $\alpha$ -Tocopherol percentage may vary

Vitatene's CWD lycopene (10% and 20%) products are composed of the following ingredients:

<b>Ingredient</b>	<b>10% Lycopene CWD Product</b>	<b>20% Lycopene CWD Product</b>	<b>FDA Regulatory Status</b>
Lycopene Crystal	Not less than 10%	Not less than 20%	GRAS
DL- $\alpha$ -Tocopherol*	1%	2%	GRAS (21 C.F.R. §182.3890)
Modified food starch	Sufficient quantity for total weight	Sufficient quantity for total weight	Approved food additive (21 C.F.R. § 172.892)

**(c) Method of manufacture**

Lycopene is produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *Blakeslea trispora*. Both mating types are stable cultures and are preserved under conditions consistent with food GMP. Lycopene produced during fermentation accumulates inside the biomass of the fungus and is recovered *via* solvent extraction using food grade materials. The purified lycopene crystals meet acceptable food grade specifications. Following completion of the extraction process the final crystalline product is then formulated into either an oil suspension containing 5% or 20% lycopene or a CWD product containing 10% or 20% lycopene prior to packaging. The manufacture of the oil suspensions involves mixing and milling the required quantity of lycopene crystals with high oleic sunflower oil and the antioxidant tocopherol. Both the high oleic sunflower oil and tocopherol meet food grade specifications and as noted in Table 1, are permitted for use in foods as GRAS ingredients. The manufacture of the CWD products involves dissolving the lycopene crystals and tocopherol in a food grade solvent. This solution is mixed with an aqueous modified food starch solution until an homogenous emulsion is formed. The solvent is evaporated from the emulsion under vacuum and the emulsion is washed with water to reduce the solvent to trace levels. The resultant liquid is then dried in a fluid bed granulator.

**(d) Characteristic properties**

The lycopene from *Blakeslea trispora* and the lycopene CWD products are red powders. The lycopene oil products are liquid oil suspensions. The specifications for Vitatene's lycopene products are listed in (f) below.

**(e) Any content of potential human toxicants**

None.

**(f) Specifications for Vitatene's lycopene products**

A specially convened Panel of independent experts qualified by training and experience to evaluate the safety of food ingredients reviewed the analytical results of several non-consecutive representative lots of Vitatene's two oil suspensions (5% and 20% lycopene) and two CWD products (10% and 20% lycopene), and concluded the products complied with final product specifications listed below (Tables 1.3 to 1.6).

<b>Table 1.3 Specifications for Lycopene 5% Oil Suspension</b>	
<b>Test</b>	<b>Specification</b>
Identification (spectrometry: max. in hexane)	ca 472
Spectrophotometric Assay (%) (472 nm)	≥ 5
Total lycopene (HPLC) (%)	≥ 5
<i>Trans</i> -lycopene (%)	≥ 90
Lead (ppm)	≤ 1
Mercury (ppm)	≤ 0.5
Cadmium (ppm)	≤ 0.1

<b>Table 1.4 Specifications for Lycopene 20% Oil Suspension</b>	
<b>Test</b>	<b>Specification</b>
Identification (spectrometry: max. in hexane)	ca 472
Spectrophotometric Assay (%) (472 nm)	≥ 20
Total lycopene (HPLC) (%)	≥ 20
<i>Trans</i> -lycopene (%)	≥ 90
Lead (ppm)	≤ 1
Mercury (ppm)	≤ 0.5
Cadmium (ppm)	≤ 0.1

<b>Table 1.5 Specifications for Lycopene 10% CWD Product</b>	
<b>Test</b>	<b>Specification</b>
Color	Dark red powder
Solubility (1% in chloroform)	Clear
Identification (spectrometry: max. in hexane)	ca 472
Spectrophotometric Assay (%) (472 nm)	≥ 10
Loss on drying (%)	≤ 8
Methylene chloride (ppm)	≤ 10
Arsenic (ppm)	≤ 1
Lead (ppm)	≤ 1
Mercury (ppm)	≤ 1
Cadmium (ppm)	≤ 1

<b>Test</b>	<b>Specification</b>
Color	Dark red powder
Solubility (1% in chloroform)	Clear
Identification (spectrometry: max. in hexane)	ca 472
Spectrophotometric Assay (%) (472 nm)	≥ 20
Loss on drying (%)	≤ 8
Methylene chloride (ppm)	≤ 10
Arsenic (ppm)	≤ 1
Lead (ppm)	≤ 1
Mercury (ppm)	≤ 1
Cadmium (ppm)	≤ 1

**(2) Information on any self-limiting levels of use**

There are no self-limiting effects on the use of Vitatene's lycopene products, although excessively high levels may result in undesirable color changes. [Carmelita to confirm]

**(3) Probable Consumption of Lycopene from *Blakeslea trispora***

Lycopene from *Blakeslea trispora* is intended for use, as a dietary source of the nutrient lycopene, in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, condiments and relishes, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, gravies and sauces, milk products, plant protein products, processed fruits and fruit juices, snack foods, and soups and soup mixes which otherwise contain very little or no lycopene. The intended food uses and use levels are listed below in Table 3.

<b>Food Category</b>	<b>Maximum Use-Levels (ppm)</b>
Baked Goods and Baking Mixes	50
Beverages and Beverage Bases	25
Breakfast Cereals	50
Cheeses	5.0
Condiments and Relishes	50
Confections and Frostings	25
Fats And Oils	20
Frozen Dairy Desserts and Mixes	25
Gelatins, Puddings, and Fillings	25
Gravies and Sauces	50
Hard Candy	25
Milk Products	50
Plant Protein Products	50
Processed Fruits and Fruit Juices	25
Snack Foods	30
Soft Candy	25
Soups And Soup Mixes	575

The consumption of lycopene from all proposed food uses was estimated using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996), and the 1998 Supplemental Children's Survey (USDA CSF II 1998) (USDA, 2000). On an all-user basis, the mean intake of lycopene by the total U.S. population from all proposed food-uses was estimated to be 9.3 mg/person/day or 0.19 mg/kg body weight/day. The heavy consumer (90<sup>th</sup> percentile) all-user intake of lycopene by the total U.S. population from all proposed food-uses was estimated to be 18.8 mg/person/day or 0.46 mg/kg body weight/day. The 90<sup>th</sup> percentile intakes of lycopene (18.8 mg/person/day) estimated from all currently proposed food-uses are similar to the levels of lycopene reported *via* the daily consumption of naturally occurring lycopene in foods, with intakes ranging from 1.0 to 25.2 mg/person/day in Europe and North America (Forman *et al.*, 1993; Yong *et al.*, 1994; Scott *et al.*, 1996; Agarwal *et al.*, 2001; Johnson-Down *et al.*, 2002).

**(4) Detailed summary of the basis for the notifier's determination that a particular use of the notified substance is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because such use is GRAS.**

The safety of lycopene from *Blakeslea trispora* was assessed in two separate sub-chronic studies, one of which examined safety of the final product (lycopene 20% oil suspension), and the other examined safety of the biomass (non-viable, disrupted *Blakeslea trispora*). Furthermore, the potential genotoxicity of lycopene from *Blakeslea trispora* [20% cold water dispersible (CWD)] was evaluated using a bacterial mutation test and a chromosome aberration test. The safety of lycopene from sources other than fungal (*i.e.*, corroborative safety data) was assessed in various acute, sub-chronic and chronic, genotoxicity/mutagenicity, and reproductive toxicity studies. In addition, the Food and Drug Administration (FDA) raised no questions in response to GRAS Notices submitted for use of synthetic lycopene (FDA, 2003) and tomato lycopene extract (FDA, 2005), both for use as GRAS ingredients in numerous foods.

The relevancy of the corroborative data is supported by the chemical similarities between lycopene produced from *Blakeslea trispora*, naturally occurring lycopene from tomatoes, and synthetic lycopene, which are outlined in Table 4.

<b>Table 4 Comparison of Lycopene from <i>Blakeslea trispora</i>, Synthetic Lycopene and Lycopene from Tomatoes</b>			
	<b>Synthetic Lycopene*</b>	<b>Lycopene from Tomatoes*</b>	<b>Lycopene from <i>Blakeslea trispora</i></b>
Purity	≥ 96%	≥ 5% of total coloring matters	≥ 95%
Impurities, other pigments	Up to 0.3% of C <sub>25</sub> aldehyde	Other pigments, oils, fats, waxes and natural flavors	Other carotenoids
All- <i>trans</i> isomer	> 70%	94-96%	95-99%
5- <i>cis</i> isomer	< 25%	3-5%	1-5%
9- <i>cis</i> isomer	< 1%	0-1%	
13- <i>cis</i> isomer	< 1%	1%	
Other <i>cis</i> -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 10-20% CWD product

A panel of independent qualified experts (Expert Panel) evaluated the safety of Vitatene's lycopene derived from *Blakeslea trispora*, 5% lycopene oil suspension, 20% lycopene oil suspension, 10% lycopene CWD product, and 20% lycopene CWD product under the conditions of intended use as a nutrient in foods. The Expert Panel found the safety of Vitatene's lycopene products to be supported by experimental animal and genotoxicity studies and analytical specifications and data, which indicate that dietary lycopene does not produce adverse effects on mortality, body weight gain, organ weights, food consumption, clinical observations or genotoxicity. Furthermore, the available experimental data regarding the reproductive toxicity of lycopene are negative, and studies and assessments conducted with Vitatene's lycopene biomass indicate that *Blakeslea trispora* is considered to be non-toxicogenic and non-pathogenic. Prospective clinical trials and intervention studies assessing intakes of lycopene ranging from 15 to 75 mg/person/day indicate that these levels of intake, which are up to 8 and 4 times greater, respectively, than the estimated average and 90<sup>th</sup> percentile all-user intakes by the total population from the intended food uses of lycopene, are generally well tolerated and without reported adverse effects.

Following an independent and collective, critical evaluation of the available pertinent scientific evidence, the Expert Panel concluded that Vitatene's lycopene from *Blakeslea trispora*, 5% lycopene oil suspension, 20% lycopene oil suspension, 10% lycopene cold water dispersible (CWD) product, and 20% lycopene CWD product, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practice, are GRAS based on scientific procedures, under the conditions of intended use in foods. A copy of the Expert Panel's GRAS opinion report is enclosed.

Accordingly, based on the animal studies and human safety data, which reveal no potential for toxicity, and the Expert Panel's GRAS determination, Vitatene concludes that the intended use of its lycopene derived from *Blakeslea trispora*, its 5% and 20% lycopene oil suspensions, and its 10% and 20% lycopene CWD products as a nutrient in foods is GRAS based on scientific procedures.

## 5. References

- 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.
- Boileau, T.W.; Moore, A.C.; Erdman, J.W. (Jr.). 1999. Carotenoids and vitamin A. In: Papas, A.M. (Ed.). *Antioxidant Status, Diet, Nutrition and Health*. CRC Press; Boca Raton, Florida. CRC Series in Contemporary Food Science, pp. 133-158.
- Cronin, J.R. 2000. Lycopene: The powerful antioxidant that makes tomatoes red. *Altern Complement Ther* 6(2):92-94.
- FDA. 2003. Agency Response Letter GRAS Notice No. GRN 000119 [Synthetic Lycopene]. U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition; Rockville, Maryland. [<http://www.cfsan.fda.gov/~rdb/opa-g119.html>].
- FDA. 2005. Agency Response Letter GRAS Notice No. GRN 000156 [Tomato Lycopene Extract and Crystallized Tomato Lycopene Extract]. U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition; Rockville, Maryland. [<http://www.cfsan.fda.gov/~rdb/opa-g156.html>].
- 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* 58:519-524.
- Furr, H.C.; Clark, R.M. 1997. Intestinal absorption and tissue distribution of carotenoids. *J Nutr Biochem* 8:364-377.
- Hadley, C.W.; Clinton, S.K.; Schwartz, S.J. 2003. The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage. *J Nutr* 133:727-732.
- IOM, 2000.  $\beta$ -carotene and other carotenoids. In: IOM. *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.

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.

Krinsky, N.I.; Russett, M.D.; Handelman, G.J.; Snodderly, D.M. 1990. Structural and geometrical isomers of carotenoids in human plasma. *J Nutr* 120:1654-1662.

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

Rice-Evans, C.A.; Sampson, J.; Bramley, P.M.; Holloway, D.E. 1997. Why do we expect carotenoids to be antioxidants *in vivo*? *Free Rad Res* 26: 381-398.

Scott, K.J.; Thurngham, D.I.; Hart, D.J.; Bingham, S.A.; Day, K. 1996. The correlation between the intake of lutein, lycopene and  $\beta$ -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.

USDA. 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. [PB2000-500027 Supercedes PB98-500457].

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.

# APPENDIX A

## EXPERT PANEL OPINION REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF LYCOPENE FROM *BLAKESLEA TRISPORA*

### Introduction

At the request of Vitatene S.A.U. (Vitatene), an Expert Panel (hereinafter referred to as the Panel) of independent scientists, qualified by relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to evaluate the safety and determine the generally recognized as safe (GRAS) status of lycopene derived from the fungus *Blakeslea trispora*, 5% and 20% lycopene oil suspensions prepared with high oleic sunflower oil to provide 5% or 20% crystalline lycopene, and 10% and 20% lycopene cold water dispersible (CWD) products under the conditions of intended use in foods. The Panel included Dr. Ian C. Munro (CANTOX Health Sciences International), Professor Gary Williams (New York Medical College), and Professor John Doull (University Kansas Medical Center). *Curriculae vitae* evidencing the qualifications of the Panel for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel was requested to evaluate the safety of lycopene derived from *Blakeslea trispora*, the 5% and 20% lycopene oil suspensions, and 10% and 20% lycopene CWD products, under the conditions of intended use as a nutrient in foods. The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Vitatene. The data evaluated by the Panel comprised information pertaining to the method of manufacture and product specifications, analytical data, conditions of intended use in foods, consumption estimates for all intended uses, safety studies conducted with lycopene derived from *Blakeslea trispora*, safety studies conducted with *Blakeslea trispora*, and other relevant corroborative data obtained from the literature on the safety of lycopene and the fermentation biomass from which lycopene is extracted.

Following independent critical evaluation of the available data and information, the Panel met on September 11 2003. After review and discussion of the data and information pertinent to safety, the Panel unanimously concluded that Vitatene's lycopene from *Blakeslea trispora*, 5% lycopene oil suspension and 20% lycopene oil suspension, meeting appropriate food grade specifications, and manufactured in accordance with current good manufacturing practices (GMP), are GRAS based on scientific procedures, under the conditions of intended use in foods. Subsequently, the Expert Panel evaluated the available data and information concerning safety of Vitatene's 10% and 20% lycopene CWD products. On April 29, 2005, the Panel unanimously concluded that Vitatene's 10% and 20% lycopene CWD products, also meeting appropriate food grade specifications and manufactured in accordance with food GMPs, are

GRAS, under the conditions of intended use, through scientific procedures. A summary of the basis for the Panel's conclusions is set forth below.

### Composition and Formulations

Vitatene's lycopene oil suspensions (5% and 20%) are composed of the following ingredients:

<b>Table 1.1 Ingredient Composition of Lycopene Oil Suspensions</b>			
<b>Ingredient</b>	<b>5% Lycopene Oil Suspension</b>	<b>20% Lycopene Oil Suspension</b>	<b>FDA Regulatory Status</b>
Lycopene Crystal	Not less than 5%	Not less than 20%	GRAS
DL- $\alpha$ -Tocopherol*	0.05%	0.2%	GRAS (21 C.F.R. §182.3890)
High Oleic Sunflower Oil	Sufficient quantity for total weight	Sufficient quantity for total weight	GRAS

\*DL- $\alpha$ -Tocopherol percentage may vary

Vitatene's CWD lycopene (10% and 20%) products are composed of the following ingredients:

<b>Table 1.2 Ingredient Composition of Lycopene CWD Products</b>			
<b>Ingredient</b>	<b>10% Lycopene CWD Product</b>	<b>20% Lycopene CWD Product</b>	<b>FDA Regulatory Status</b>
Lycopene Crystal	Not less than 10%	Not less than 20%	GRAS
DL- $\alpha$ -Tocopherol*	1%	2%	GRAS (21 C.F.R. §182.3890)
Modified food starch	Sufficient quantity for total weight	Sufficient quantity for total weight	Approved food additive (21 C.F.R.172.892)

Lycopene is an acyclic, nonpolar hydrocarbon chain with molecular formula  $C_{40}H_{56}$ . All-trans lycopene derived from *B. trispora* is a red crystalline powder that is identical to lycopene that occurs naturally in tomatoes.

### Manufacturing and Specifications

Lycopene is produced through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *Blakeslea trispora*. Both mating types are stable cultures and are preserved under conditions consistent with food GMP. Lycopene produced during fermentation accumulates inside the biomass of the fungus and is recovered *via* solvent extraction using food grade materials. The purified lycopene crystals meet acceptable food grade specifications. Following completion of the extraction process the final crystalline product is then formulated into either an oil suspension containing 5% or 20% lycopene or a CWD product containing 10% or 20% lycopene prior to packaging. The manufacture of the oil suspensions involves mixing

and milling the required quantity of lycopene crystals with high oleic sunflower oil and the antioxidant tocopherol. Both the high oleic sunflower oil and tocopherol meet food grade specifications and as noted in Table 1, are permitted for use in foods as GRAS ingredients. The manufacture of the CWD products involves dissolving the lycopene crystals and tocopherol in a food grade solvent. This solution is mixed with an aqueous modified food starch solution until an homogenous emulsion is formed. The solvent is evaporated from the emulsion under vacuum and the emulsion is washed with water to reduce the solvent to trace levels. The resultant liquid is then dried in a fluid bed granulator. The Panel reviewed the analytical results of several non-consecutive representative lots of each of the two formulated oil suspensions (lycopene 5% oil suspension and lycopene 20% oil suspension) and the two CWD products (lycopene 10% CWD and lycopene 20% CWD), and concluded the product complied with final product specifications (Tables 2.1 to 2.4).

**Table 2.1 Specifications for Lycopene 5% Oil Suspension**

Test	Specification	Batch Number		
		LC 052	LC 054	LC 057
Identification (spectrometry: max. in hexane)	ca 472	471.5	471.5	471
Spectrophotometric Assay (%) (472 nm)	≥ 5	5.8	5.8	5.6
Total lycopene (HPLC) (%)	≥ 5	5.7	5.9	5.6
<i>Trans</i> -lycopene (%)	≥ 90	96.0	95.5	93.7
<i>Heavy metals</i>				
Lead (ppm)	≤ 1	< 0.4	< 0.4	< 0.4
Mercury (ppm)	≤ 0.5	< 0.150	< 0.150	< 0.150
Cadmium (ppm)	≤ 0.1	< 0.02	< 0.02	< 0.04

**Table 2.2 Specifications for Lycopene 20% Oil Suspension**

Test	Specification	Batch Number		
		LC 052	LC 054	LC 057
Identification (spectrometry: max. in hexane)	ca 472	471	471.5	471
Spectrophotometric Assay (%) (472 nm)	≥ 20	21.0	20.1	20.0
Total lycopene (HPLC) (%)	≥ 20	20.9	20.5	20.1
<i>Trans</i> -lycopene (%)	≥ 90	95.0	95.6	95.7
<i>Heavy metals</i>				
Lead (ppm)	≤ 1	< 0.4	< 0.4	< 0.4
Mercury (ppm)	≤ 0.5	< 0.150	< 0.150	< 0.150
Cadmium (ppm)	≤ 0.1	< 0.02	< 0.02	< 0.04

Test	Specification	Batch Number		
		152	174	176
Colour	Dark red powder	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	471	471	471
Assay (%) (472 nm)	≥10	10.13	10.91	11.29
Loss on drying (%)	≤8	2.96	2.48	1.69
Methylene chloride (ppm)	≤10	8.9	0.4	1.8
<i>Heavy Metals</i>				
Arsenic (ppm)	≤1	<0.6	<0.6	<0.6
Lead (ppm)	≤1	<0.4	<0.4	<0.4
Mercury (ppm)	≤1	<0.15	<0.15	<0.15
Cadmium (ppm)	≤1	<0.04	<0.04	<0.04

Test	Specification	Batch Number		
		180	182	184
Colour	Dark red powder	Yes	Yes	Yes
Solubility (1% in chloroform)	Clear	Yes	Yes	Yes
Identification (spectrometry: max. in hexane)	ca 472	469	470	471
Assay (%) (472 nm)	≥20	22.6	22.95	23.29
Loss on drying (%)	≤8	2.18	1.43	1.62
Methylene chloride (ppm)	≤10	5.2	8.6	4.0
<i>Heavy Metals</i>				
Arsenic (ppm)	≤1	<0.6	<0.6	<0.6
Lead (ppm)	≤1	<0.4	<0.4	<0.4
Mercury (ppm)	≤1	<0.15	<0.15	<0.15
Cadmium (ppm)	≤1	<0.04	<0.04	<0.04

### **Intended Use and Estimated Intake**

Lycopene, a non-pro-vitamin A carotenoid, is a normal constituent of the human diet due mainly to its presence in red fruits and vegetables (e.g., tomatoes, watermelon, pink grapefruit, apricots and pink guavas), and algae and fungi (Feofilova, 1994; Stahl and Sies, 1996; Boileau *et al.*,

1999; Nguyen and Schwartz, 1999). In two separate studies from the United States conducted with males (Forman *et al.*, 1993) or premenopausal 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. In addition to its presence in foods (*e.g.*, red fruits and vegetables), lycopene is available as a dietary supplement; however, there are no reliable estimates of the amounts of dietary supplements consumed in the United States (IOM, 2000).

Lycopene from *Blakeslea trispora* is intended for use as a food ingredient, as a dietary source of the nutrient lycopene, in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, cheeses, condiments and relishes, confections and frostings, fats and oils, frozen dairy desserts and mixes, gelatins, puddings, and fillings, gravies and sauces, milk products, plant protein products, processed fruits and fruit juices, snack foods, and soups and soup mixes which otherwise contain very little or no lycopene. The intended food uses and use levels are presented in Table 3.

<b>Food Category</b>	<b>Maximum Use-Levels for Lycopene [ppm (%)]</b>
Baked Goods and Baking Mixes	50 (0.005)
Beverages and Beverage Bases	25 (0.0025)
Breakfast Cereals	50 (0.005)
Cheeses	5.0 (0.0005)
Condiments and Relishes	50 (0.005)
Confections and Frostings	25 (0.0025)
Fats And Oils	20 (0.002)
Frozen Dairy Desserts and Mixes	25 (0.0025)
Gelatins, Puddings, and Fillings	25 (0.0025)
Gravies and Sauces	50 (0.005)
Hard Candy	25 (0.0025)
Milk Products	50 (0.0025)
Plant Protein Products	50 (0.005)
Processed Fruits and Fruit Juices	25 (0.0025)
Snack Foods	30 (0.003)
Soft Candy	25 (0.0025)
Soups And Soup Mixes	575 (0.0575)

The consumption of lycopene from all proposed food uses was estimated using the United States Department of Agriculture (USDA) 1994-1996 Continuing Survey of Food Intakes by Individuals (USDA CSFII 1994-1996), and the 1998 Supplemental Children's Survey (USDA

CSF II 1998) (USDA, 2000). On an all-user basis, the mean intake of lycopene by the total U.S. population from all proposed food-uses was estimated to be 9.3 mg/person/day or 0.19 mg/kg body weight/day. The heavy consumer (90<sup>th</sup> percentile) all-user intake of lycopene by the total U.S. population from all proposed food-uses was estimated to be 18.8 mg/person/day or 0.46 mg/kg body weight/day. The 90<sup>th</sup> percentile intakes of lycopene (18.8 mg/person/day) estimated from all currently proposed food-uses are similar to the levels of lycopene reported via the daily consumption of naturally occurring lycopene in foods, with intakes ranging from 1.0 to 25.2 mg/person/day in Europe and North America (Forman *et al.*, 1993; Yong *et al.*, 1994; Scott *et al.*, 1996; Agarwal *et al.*, 2001; Johnson-Down *et al.*, 2002).

### **Data Pertaining to Safety**

The safety of lycopene from *Blakeslea trispora* was assessed in two separate sub-chronic studies, one of which examined the safety of the final product (lycopene 20% oil suspension), and the other examined the safety of the biomass (non-viable, disrupted *Blakeslea trispora*). Furthermore, the potential genotoxicity of lycopene from *Blakeslea trispora* [20% cold water dispersible (CWD)] was evaluated using a bacterial mutation test and a chromosome aberration test. The safety of lycopene from sources other than fungal (*i.e.*, corroborative safety data) was assessed in various acute, sub-chronic and chronic, genotoxicity/mutagenicity, and reproductive toxicity studies. In addition, the FDA raised no questions in response to GRAS Notices submitted for use of synthetic lycopene as a GRAS ingredient (FDA, 2003) and tomato lycopene extract (FDA, 2005), both for use in numerous foods. The relevancy of the corroborative data is supported by the chemical similarities between lycopene produced from *Blakeslea trispora*, naturally occurring lycopene from tomatoes, and synthetic lycopene, which are outlined in Table 4.

	Synthetic Lycopene*	Lycopene from Tomatoes*	Lycopene from <i>B. trispora</i>
Purity	≥ 96%	≥ 5% of total coloring matters	≥ 95%
Impurities, other pigments	Up to 0.3% of C <sub>25</sub> aldehyde	Other pigments, oils, fats, waxes and natural flavors	Other carotenoids
All- <i>trans</i> isomer	> 70%	94-96%	95-99%
5- <i>cis</i> isomer	< 25%	3-5%	1-5%
9- <i>cis</i> isomer	< 1%	0-1%	
13- <i>cis</i> isomer	< 1%	1%	
Other <i>cis</i> -isomers	< 3%	< 1%	
Formulation	10% lycopene with ascorbyl palmitate (5%) and α-tocopherol (1.5%)	Oleo-resin: 2-3% lycopene Powder: 5% lycopene	5-20% oil suspension with α-tocopherol 10-20% CWD product

\*Opinion on Synthetic Lycopene as a coloring matter for use in foodstuffs (SCF, 1999)

As detailed above, lycopene is a normal constituent of the human diet due to its abundant presence in natural products (Nguyen and Schwartz, 1999), with numerous studies in various population groups in Europe and North America reporting mean daily intakes ranging from approximately 1.0 to 25.2 mg/person/day (Forman *et al.*, 1993; Olmedilla *et al.*, 1994; Yong *et al.*, 1994; Järvinen, 1995; Scott *et al.*, 1996; Agarwal *et al.*, 2001; Johnson-Down *et al.*, 2002). Lycopene is also a predominant carotenoid in human plasma (Johnson, 1998; Rao and Agarwal, 1998a,b), contributing between 21 and 43% of total serum carotenoids (Sies and Stahl, 1998). In addition to its presence in human plasma, lycopene is also a constituent of colostrum (104 to 141 µg/L) and mature breast milk (30 to 41 µg/L), with content depending on dietary intake (Giuliano *et al.*, 1994; Sommerburg *et al.*, 2000). In general, consumption of carotenoids in foods has not been shown to cause adverse health effects, even when ingested in large amounts (*e.g.*, >30 mg carotenoid) (Olson, 1996; Omaye *et al.*, 1997). The safety of lycopene was addressed by the Institute of Medicine (IOM, 2000) following an examination of the available data regarding β-carotene and other carotenoids. The IOM concluded that no adverse effects have been reported from the consumption of lycopene in food, and no tolerable upper intake levels were established for lycopene, or for the other carotenoids (β-carotene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin) (IOM, 2000). High atypical intake can result in lycopenodermia, characterized by a deep orange discoloration of the skin, which is reversible (Stahl and Sies, 1996; IOM, 2000).

## **Studies of Absorption, Distribution, Metabolism, and Excretion**

Lycopene, like all carotenoids, is fat-soluble and therefore follows the same digestion and intestinal absorption pathways as dietary fat (Furr and Clark, 1997; Johnson, 1998; van den Berg, 1998). In general, optimal absorption of dietary carotenoids begins with their release from the food matrix and dissolution in the lipid phase, followed by incorporation into lipid micelles in the small intestine, which is required for mucosal uptake, and finally, transport to the lymphatic and/or portal circulation (Erdman *et al.*, 1993). Dietary factors potentially affecting the degree of absorption of lycopene (*i.e.*, the bioavailability of lycopene) following ingestion include the digestibility of the food matrix, the level of fat present and/or absorbed from the diet, interactions with other carotenoids, and lycopene isoforms (Furr and Clark, 1997). Results from intervention studies examining the effects of carotenoid interactions on lycopene bioavailability demonstrate that interactions between both exogenous and endogenous carotenoids occur; however, due to the equivocal nature of the results, definite relationships between specific carotenoids cannot be established.

Absorbed lycopene is primarily transported in blood plasma *via* low-density lipoproteins (LDL), from where it is presumably transferred to tissues through interactions with LDL receptors (Kaplan *et al.*, 1990; Johnson, 1998; Paetau *et al.*, 1998; Mayne *et al.*, 1999; Chopra *et al.*, 2000; Maruyama *et al.*, 2001). The proposed metabolic pathway of lycopene involves its oxidation to lycopene 5,6-epoxide, which subsequently undergoes enzymatic or acidic hydrolysis and forms the epimeric mixture of 2,6-cylcolycopene-1,5-diol I and II (Khachik *et al.*, 1995; 1997a,b). Lycopene appears to be excreted by the sudoral and sebaceous glands, and is in part reabsorbed by the horny layer of the skin; therefore, when ingested in excessive amounts, lycopene causes a yellow discoloration of the skin (La Placa *et al.*, 2000).

Studies reporting post-prandial serum and organ levels of lycopene in rats and humans have been compiled and analyzed in order to compare the uptake and tissue distribution of lycopene between humans and rats. The available data in rats indicate that lycopene is absorbed into the systemic blood supply and is distributed to the tissues in a similar distribution pattern as that seen in humans (Matthews-Roth *et al.*, 1990; Boileau *et al.*, 2000). Tissue levels in rats were comparable to those measured in human tissues, with the highest levels of lycopene occurring in the liver, adrenal glands, and testes. Based on these analyses, it was concluded that the rat can be regarded as a useful and appropriate animal model for the study of lycopene.

## **Toxicological Studies**

The Panel reviewed pivotal toxicological studies conducted with the final product (lycopene 20% oil suspension) (Jonker *et al.*, 2003), and with the non-viable, disrupted *Blakeslea trispora* biomass (Jonker, 2000). In addition, the Panel reviewed corroborative safety studies conducted with lycopene from sources other than *Blakeslea trispora* (*e.g.*, naturally occurring/dietary lycopene and supplemental formulations/synthetic lycopene) (see Table 5).

The oral toxicity of lycopene 20% oil suspension was investigated in male and female Wistar rats in a 90-day oral toxicity study conducted in compliance with Good Laboratory Practice (GLP) (Jonker *et al.*, 2003). 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 hematology, clinical chemistry or urinalysis between the treated and control groups. Similarly, gross necropsy revealed no 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 in Wistar rats corresponded to the highest dose tested; 1.0% in the diet, corresponding to 586 and 616 mg lycopene/kg body weight/day for males and females, respectively (Jonker *et al.*, 2003). The possible sub-chronic toxicity of *Blakeslea trispora* in the fermentative manufacture of lycopene was examined in a 28-day, GLP-compliant study conducted with male and female Wistar albino rats (Jonker, 2000). The administration of lycopene biomass in the diet at 90, 272, and 906 mg/kg body weight in males, and 87, 260 and 868 mg/kg body weight in females was well tolerated and had no effect on appearance, general condition, behavior, body weight, absolute or relative organ weights, histopathology, food consumption or food conversion efficiency (Jonker, 2000). The reported decrease in selected hematological parameters (prothrombin time and mean corpuscular volume) of the high-dose male rats was not considered to be of clinical significance by the Panel, since there were no other changes in hematological measurements and the decrease in prothrombin time was small (6%) and within the historical control range for the laboratory.

Additional experimental animal studies have investigated the potential sub-chronic, genotoxic, and reproductive toxicity of lycopene from sources other than *Blakeslea trispora*. No significant toxicological findings were reported in male and female Wistar rats following 13 (Mellert *et al.*, 2002) and 14 weeks (McClain and Bausch, 2003) of oral exposure to synthetic lycopene formulations and in male and female Sprague-Dawley rats following 13 weeks of oral exposure to natural tomato oleoresin extract (NTOE) (East, 1995). In each study, the no-observable-adverse-effect levels (NOAEL) corresponded to the highest doses tested: 300 mg lycopene/kg body weight/day (Mellert *et al.*, 2002), 500 mg lycopene/kg body weight/day (McClain and Bausch, 2003), and 270 mg lycopene/kg body weight/day (East, 1995). Several additional corroborative experimental studies conducted with mice, rats and dogs, ranging in length from 2 weeks to 10 months, have examined the effects of oral exposure to lycopene (naturally occurring and synthetic formulations) at doses up to 1,000 mg lycopene/kg body weight/day (Zbinden and Studer, 1958; Erdman and Lachance, 1973; Nagasawa *et al.*, 1995; Gradelet *et al.*, 1996; Black, 1998; Zhao *et al.*, 1998; Jewell and O'Brien, 1999; Boileau *et al.*, 2000; Breinholt *et al.*, 2000). The totality of the evidence revealed that exposure to lycopene, regardless of its source, was well tolerated, and there were no adverse effects on mortality, body weight gains, food consumption, or clinical observations. Coat appearance was unaffected by doses of dietary lycopene up to 60 mg/kg body weight/day, with the exception of

discolored 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). In studies investigating the reproductive toxicity of synthetic lycopene formulations, no evidence of maternal, reproductive, or developmental toxicity was reported in rats or rabbits following exposure throughout gestation, at doses up to 1,000 mg lycopene/kg body weight/day (rats) or 200 mg lycopene/kg body weight/day (rabbits) (Zbinden and Studer, 1958; Christian *et al.*, 2003; McClain and Bausch, 2003).

The genotoxic potential of lycopene from *Blakeslea trispora* (20% CWD) was investigated *in vitro* with bacterial (*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2 *uvrA*) and mammalian (human lymphocytes) test systems (CTBR, 2003a,b). The results indicated no increase in the revertant colony counts of any of the strains, either in the presence or absence of S9 mix and likewise showed no statistically significant increase in the incidence of cells with chromosome damage. It was therefore concluded that lycopene 20% CWD showed no evidence of genotoxic activity. Furthermore, naturally occurring and synthetic formulations of lycopene were investigated *in vitro* with bacterial (*Salmonella typhimurium* and *Escherichia coli* strains) and mammalian (mouse lymphoma cells and human lymphocytes) test systems, and *in vivo* with mice, rats and humans. Consistently negative results were reported for the *in vitro* assays (He and Campbell, 1990; Rauscher *et al.*, 1998; Aizawa *et al.*, 2000; McClain and Bausch, 2003, Thompson, 1994). Similarly, oral exposure to naturally occurring lycopene did not induce chromosomal damage in mouse bone marrow cells (Rauscher *et al.*, 1998) or DNA damage in human lymphocytes (Pool-Zobel *et al.*, 1997; Collins *et al.*, 1998; Riso *et al.*, 1999), and there was no evidence of clastogenicity or DNA damage in mouse peripheral blood or rat hepatocytes, respectively, following oral dosing with synthetic lycopene formulations (McClain and Bausch, 2003).

In addition to data obtained from the 28-day oral toxicity study, which concluded that the non-viable, disrupted *Blakeslea trispora* biomass is non-toxic (Jonker, 2000), the Panel reviewed corroborative safety data for the *Blakeslea trispora* biomass. Importantly, *Blakeslea trispora* was classified as a microorganism that presents no risk to humans or vertebrates (risk group 1) by the German "Gentechnik-Sicherheitsverordnung" (Robert Koch Institute, 2001). Also, the Scientific Committee of Food (SCF) has considered  $\beta$ -carotene from *Blakeslea trispora*, produced *via* an identical biosynthetic route and process as lycopene from *Blakeslea trispora*, acceptable for use as a coloring 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,  $\beta$ -carotene, differs from the chemically synthesized  $\beta$ -carotene used as a food colorant" (SCF, 2000). Similarly, following a review of studies on acute toxicity, short-term (28-day) toxicity and genotoxicity, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that "on the basis of the

source organisms, the production process, and its composition characteristics,  $\beta$ -carotene from *Blakeslea trispora* does not raise specific concerns and from a toxicological point of view should be considered equivalent to chemically synthesized  $\beta$ -carotene..." This opinion was supported by the negative results in two tests for genotoxicity (mutagenesis and chromosomal aberration) considered at the present meeting (JECFA, 2002).

Reference	Species	Duration	Dose (mg/kg body weight/day)	Safety-Related Findings
Nagasawa <i>et al.</i> , 1995	SHN/Mei virgin mice (11 control/14 treated)	10 months	0.07 (dietary)	Lycopene <i>versus</i> 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 <i>versus</i> 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) <i>versus</i> control basal diet: $\uparrow$ 'd serum cholesterol levels. Group receiving 9.6 mg lycopene/kg body weight/day <i>versus</i> control hypercholelemic diet: $\uparrow$ 'd liver cholesterol levels.
East, 1995	Male and female Sprague-Dawley rats (20/sex/group)	13 weeks	45, 450, or 4,500 natural tomato oleoresin extract (6% lycopene)	No significant toxicological effects
Gradelet <i>et al.</i> , 1996	Male SPF Wistar rats (5/group)	15 days	45 (dietary)	Lycopene <i>versus</i> control: No difference in food intake, body weights or absolute and relative liver weights. 60% $\downarrow$ 'd activity of liver enzyme nitrosodimethylamine <i>N</i> -demethylase (NDMAD).
Zhao <i>et al.</i> , 1998	Male and female Fischer rats (20 control/10 per treatment)	10 weeks	2.4, 6.0, 12, 24, or 60 (dietary)	Lycopene <i>versus</i> control: No toxic side effects. No adverse effects on weight gain, behavior, 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 <i>versus</i> control: No differences in food intake, body weight changes or organ weights (small intestine, liver, lung, kidney). $\downarrow$ 'd activity of lung enzyme benzyloxyresorufin-O-dearylation (BROD).

Reference	Species	Duration	Dose (mg/kg body weight/day)	Safety-Related Findings
Breinholt <i>et al.</i> , 2000	Female Wistar rats (4/group)	14 days	0, 1, 5, 50, or 100 (gavage)	Lycopene <i>versus</i> 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 <i>et al.</i> , 2000	Male F344 rats (22/group)	8 weeks	0, 0.5, 5.7, 57.5 (dietary)	Lycopene <i>versus</i> control: No difference in food intake.
Jonker, 2000	Male and female Wistar albino rats (20/sex/group)	28 days	0.1, 0.3, or 1.0% (87 to 906 mg/kg bw/d) (dietary)	Lycopene <i>versus</i> control: Decreased prothrombin time and mean corpuscular volume (high-dose male rats). No significant toxicological effects.
Mellert <i>et al.</i> , 2002	Male and female Wistar rats (10/sex/group)	13 weeks	50, 150, or 300 (500, 1,500, or 3,000 mg of 10% lycopene products) (gavage)	Lycopene <i>versus</i> control: Red discoloration of the feces, jejunum and cecum. No significant toxicological effects.
Jonker <i>et al.</i> , 2003	Male and female Wistar rats	90 days	0.25, 0.5, or 1.0% (145 to 616 mg/kg bw/d) (dietary)	No significant toxicological effects.
McClain and Bausch, 2003	Male and female Hanlbm Wistar rats (6/sex/group)	4 weeks	1,000 (lycopene) + <0.01, 0.3, or 2% apo-12'-lycopenal (impurity) (oral)	Lycopene <i>versus</i> control: Red discoloration of the feces, brown-orange discoloration of the liver, brown-yellow fine granulated pigment deposits in the hepatocytes. No significant toxicological effects.
McClain and Bausch, 2003	Male and female Hanlbm Wistar rats (26/sex/group)	14 weeks	50, 150, or 500 (oral)	No significant toxicological effects
Zbinden and Studer, 1958	1 Dog	192 days	100 (capsules)	Pigment deposition in the liver and kidney

### Human Safety Data

In addition to the experimental animal data, the Panel considered clinical data obtained from safety/tolerance studies conducted with lycopene from sources other than *Blakeslea trispora*, to corroborate the safety of lycopene from *Blakeslea trispora*. A total of 12 clinical trials, ranging in length from 1 to 16 weeks, have been conducted with lycopene (natural and synthetic). 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 capsules or lycopene-rich foods (e.g., tomato juice and tomato sauce) 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; Muller *et al.*, 1999; Chopra *et al.*, 2000; Watzl *et al.*,

2000; Kucuk *et al.*, 2001; Olmedilla *et al.*, 2002). The tolerance of lycopene supplementation was assessed in healthy individuals (Micozzi *et al.*, 1992; Agarwal and Rao, 1998; Muller *et al.*, 1999; Hininger *et al.*, 2001) as well as in prostate cancer patients (Chen *et al.*, 2001; Kucuk *et al.*, 2001). Daily doses ranging from 12.0 to 75.0 mg lycopene (*i.e.*, 3 to 20 times normal dietary intake) were generally well tolerated, with no reports of any illnesses or adverse biological effects (Micozzi *et al.*, 1992; Agarwal and Rao, 1998; Muller *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 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 incidences of lycopenodermia (*e.g.*, discoloration of the skin) in 25% of the subjects (healthy Spanish volunteers) supplemented with 15 mg lycopene/day for 16 weeks (compared with 40 and 95% of the subjects supplemented with lutein and carotene, respectively); however, no other adverse effects were reported, and there were no significant changes in the general biochemical or hematological profiles of the subjects. In addition, no other clinical studies have reported symptoms of lycopenodermia. Self-reported occurrences of lycopenodermia have been documented in various case studies reporting the effects of excessive lycopene intakes (*e.g.*, 4 to 5 large tomatoes plus pasta with tomato sauce daily for 3 years) (Reich *et al.*, 1960; La Placa *et al.*, 2000). Lycopenodermia is characterized by orange-yellow discoloration of the skin, which can be accompanied by abdominal pain and hepatic accumulation of lycopene pigments, and is reversible upon termination of lycopene ingestion (Reich *et al.*, 1960; La Placa *et al.*, 2000).

The available clinical data indicate that lycopene, at estimated exposures of 18.8 mg/person/day (90<sup>th</sup> percentile), provided through the intended use of Vitatene's lycopene crystal and oil suspension and CWD products, would not have an adverse effect on body weight, full blood counts, serum lipid profiles, immune function, or liver function. Similarly, the data indicate that daily doses up to 75.0 mg lycopene are generally well tolerated, with no reports of any illnesses or adverse biological effects. Although incidences of lycopenodermia (*e.g.*, discoloration of the skin) have been reported at doses approximating 15 mg lycopene/day, no objective form of skin discoloration measurement was made in this study. Furthermore, lycopenodermia has not been reported in other clinical studies of up to 75.0 mg lycopene. Moreover, intakes of lycopene from natural sources have been reported to range from 1.0 to 25.2 mg/day, thus it is not expected that symptoms of skin pigmentation would result through the intended use of Vitatene's lycopene products in food. Taken together, the scientific evidence indicates that the consumption of Vitatene's lycopene crystal, oil suspension products, and CWD products, under the conditions of intended use, would not be expected to produce adverse effects on human health.

## Summary

The Panel was requested to evaluate the safety of Vitatene's lycopene derived from *Blakeslea trispora*, 5% lycopene oil suspension, 20% lycopene oil suspension, 10% lycopene CWD product, and 20% lycopene CWD product, under the conditions of intended use as a nutrient in foods. The safety of Vitatene's lycopene derived from *Blakeslea trispora*, the lycopene oil formulations (5% and 20%) and the lycopene CWD formulations (10% and 20%) is supported by experimental animal and genotoxicity studies and analytical specifications and data, which indicate that dietary lycopene does not produce adverse effects on mortality, body weight gain, organ weights, food consumption, clinical observations or genotoxicity. Furthermore, the available experimental data regarding the reproductive toxicity of lycopene are negative, and studies and assessments conducted with Vitatene's lycopene biomass indicate that *Blakeslea trispora* is considered to be non-toxicogenic and non-pathogenic. Prospective clinical trials and intervention studies assessing intakes of lycopene ranging from 15 to 75 mg/person/day indicate that these levels of intake, which are up to 8 and 4 times greater, respectively, than the estimated average and 90<sup>th</sup> percentile all-user intakes by the total population from the intended food uses of lycopene, are generally well tolerated and without reported adverse effects.

Overall, animal studies and human safety data reveal no potential for toxicity of the crystalline lycopene derived from *Blakeslea trispora*, the 5% and 20% lycopene oil suspensions, or the 10% and 20% lycopene CWD products, therefore, the scientific data summarized herein support the safety of these products under the conditions of intended use in foods.

## Conclusion

We, the Expert Panel, have, independently and collectively, critically evaluated the available pertinent scientific evidence summarized above, and conclude that Vitatene's lycopene from *Blakeslea trispora*, 5% lycopene oil suspension, 20% lycopene oil suspension, 10% lycopene cold water dispersible (CWD) product, and 20% lycopene CWD product, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practice, are generally recognized as safe (GRAS), based on scientific procedures, under the conditions of intended use in foods, as specified herein.

[Redacted Signature]

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Apr 30, 2005  
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## Conclusion

We, the Expert Panel, have, independently and collectively, critically evaluated the available pertinent scientific evidence summarized above, and conclude that Vitatene's lycopene from *Blakeslea trispora*, 5% lycopene oil suspension, 20% lycopene oil suspension, 10% lycopene cold water dispersible (CWD) product, and 20% lycopene CWD product, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practice, are generally recognized as safe (GRAS), based on scientific procedures, under the conditions of intended use in foods, as specified herein.

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## References

- Agarwal, S.; Rao, A.V. 1998. Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. *Lipids* 33:981-984.
- 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 (J Jpn Soc Biosci Biotechnol Agrochem)* 74(6):679-681.
- Black, H.S. 1998. Radical interception by carotenoids and effects on UV carcinogenesis. *Nutr Cancer* 31:212-217.
- Boileau, T.W.M.; Moore, A.C.; Erdman, J.W. 1999. Carotenoids and vitamin A. *In: Antioxidant Status, Diet, Nutrition, and Health*. Edited by A.M. Papas. CRC Press; Boca Raton, Florida. CRC Series in Contemporary Food Science, pp. 133-151.
- 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.
- 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-fetal toxicity/teratogenicity) toxicity studies of synthetic crystalline lycopene in rats and rabbits. *Food Chem Toxicol* 41(6):773-783.
- 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.
- CTBR. 2003a. Lycopene 20% CWD Bacterial Mutation Test. CTBR Bio Research Inc.; Senneville, Que. CTBR Project No. 960171 unpublished final study report.
- CTBR. 2003b. Lycopene 20% CWD Chromosome Aberration Test. CTBR Bio Research Inc.; Senneville, Que. CTBR Project No. 960172 unpublished final study report.

- East, P.W. 1995. Lycopene: toxicity study by oral (gavage) administration to CD rats for 13 weeks. Pharmaco LSR Ltd., Report No. 94/MAK228/1290. Cited In: Matulka *et al.*, 2004.
- Erdman, J.W. (Jr.); Bierer, T.L.; Gugger, E.T. 1993. Absorption and transport of carotenoids. *Ann N Y Acad Sci* 691:76- 85.
- 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, Prt. 1):Abstract No. 3856.
- FDA. 2003. Agency Response Letter GRAS Notice No. GRN 000119 [Synthetic Lycopene]. U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition; Rockville, Maryland. [<http://www.cfsan.fda.gov/~rdb/opa-g119.html>].
- FDA. 2005. Agency Response Letter GRAS Notice No. GRN 000156 [Tomato Lycopene Extract and Crystallized Tomato Lycopene Extract]. U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition; Rockville, Maryland. [<http://www.cfsan.fda.gov/~rdb/opa-g156.html>].
- 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* 58:519-524.
- Furr, H.C.; Clark, R.M. 1997. Intestinal absorption and tissue distribution of carotenoids. *Nutritional Biochemistry* 8:364-377.
- 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.
- He, Y.; Campbell, T.C. 1990. Effects of carotenoids on aflatoxin B1-induced mutagenesis in *S. typhimurium* TA 100 and TA 98. *Nutr Cancer* 13:243-253.
- 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.

- 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.
- JECFA. 2002. Safety Evaluation of Certain Food Additives and Contaminants – 57<sup>th</sup> 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 Organization of the United States (FAO) WHO Food Additives Series, No. 48
- 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 *Blakeslea trispora* in rats. *Regul Toxicol Pharmacol* 37(3):396-406.
- Kaplan, L.A.; Lau, J.M.; Stein, E.A. 1990. Carotenoid composition, concentrations, and relationships in various human organs. *Clin Physiol Biochem* 8:1-10.
- Khachik, F.; Beecher, G.R.; Smith, J.C. (Jr.). 1995. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J Cellul Biochem Suppl* 22:236-246.
- Khachik, F.; Spengler, C.J.; Smith, J.C. (Jr.), Canfield, L.M.; Steck, A.; Pfander, H. 1997a. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal Chem* 69(10):1873-1881.
- Khachik, F.; Steck, A.; Pfander, H. 1997b. Bioavailability, metabolism, and possible mechanism of chemoprevention by lutein and lycopene in humans. In: Ohigashi, H.; Osawa, T.; Terao, J.; Watanabe, S.; Yoshikawa, T. (Eds.). *Food Factors for Cancer Prevention*. Springer-Verlag; Tokyo, pp. 542-547.
- 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.

- Maruyama, C.; Imamura, K.; Oshima, S.; Suzukawa, M.; Egami, S.; Tonomoto, M.; Baba, N.; Harada, M.; Ayaori, M.; Inakuma, T.; Ishikawa, T. 2001. Effects of tomato juice consumption on plasma and lipoprotein carotenoid concentrations and the susceptibility of low density lipoprotein to oxidative modification. *J Nutr Sci Vitaminol* 47(3):213-221.
- Mathews-Roth, M.M.; Welankiwar, S.; Sehgal, P.K.; Lausen, N.C.; Russett, M.; Krinsky, N.I. 1990. Distribution of [<sup>14</sup>C]canthaxanthin and [<sup>14</sup>C]lycopene in rats and monkeys. *J Nutr* 120(10):1205-1213.
- Matulka, R.A.; Hood, A.M.; Griffiths, J.C. 2004. Safety evaluation of a natural tomato oleoresin extract derived from food-processing tomatoes. *Regul Toxicol Pharmacol* 39:390-402.
- Mayne, S.T.; Cartmel, B.; Silva, F.; Kim, C.S.; Fallon, B.G.; Briskin, K.; Zheng, T.; Baum, M.; Shor-Posner, G.; Goodwin, W.J. (Jr.). 1999. Plasma lycopene concentrations in humans are determined by lycopene intake, plasma cholesterol concentrations and selected demographic factors. *J Nutr* 129(4):849-854.
- McClain, R.M.; Bausch, J. 2003. Summary of safety studies conducted with synthetic lycopene. *Regulatory Toxicology and Pharmacology* 37: 274-285.
- 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.
- 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.
- Müller, H., Bub, A., Watzl, B.; Rechkemmer, G. 1999. Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. *Z Ernährungswiss* 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.
- Nguyen, M.L.; Schwartz, S.J. 1999. Lycopene: Chemical and biological properties. *Food Technol* 53(2):38-45.
- 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.
- Olson, J.A. 1996. Vitamin A. In: *Present Knowledge in Nutrition*. 7<sup>th</sup> Edition. E.E. Ziegler and L.J. Filer, Jr., eds. International Life Sciences Institute, Washington, D.C. pp 109-119.
- Omaye, S.T.; Krinsky, N.I.; Kagan, V.E.; Mayne, S.T.; Liebler, D.C.; Bidlack, W.R. 1997. Symposium overview.  $\beta$ -Carotene: friend or foe? *Fundamental and Applied Toxicology* 40:163-174.

- Paetau, I.; Khachik, F.; Brown, E.D.; Beecher, G.R.; Kramer, T.R.; Chittams, J.; Clevidence, B.A. 1998. Chronic ingestion of lycopene- rich tomato juice or lycopene supplements significantly increases plasma concentrations of lycopene and related tomato carotenoids in humans. *Am J Clin Nutr* 68(6):1187-1195.
- 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.
- 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* 31:199-203.
- 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. Lycopopenia. 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* 69:712-718.
- Robert Koch Institute. 2001. Liste Risikobewerteter Spender- und Empfängerorganismen für Gentechnische Arbeiten (Volltext der Organismenliste (PDF) Fassung vom April 2001). Robert Koch Institut; Berlin [<http://www.rki.de/GENTEC/ZKBS/ZKBS.HTM>].
- SCF. 1999. Opinion of the Scientific Committee on Food on Synthetic Lycopene as a Coloring 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, Belgium. SCF/CS/ADD/COL/160 Final.
- SCF. 2000. Opinion of the Scientific Committee on Food on  $\beta$ -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  $\beta$ -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.
- Sies, H.; Stahl, W. 1998. Lycopene and  $\beta$ -carotene bioavailability and biological effects. *Free Radicals, Oxidative Stress, and Antioxidants*.

- Sommerburg, O.; Meissner, K.; Nelle, M.; Lenhartz, H.; Leichsenring, M. 2000. Carotenoid supply in breast-fed and formula-fed neonates. *Eur J Pediatr* 159(1&2):86-90.
- Stahl, W.; Sies, H. 1996. Lycopene: A biologically important carotenoid for humans? *Arch Biochem Biophys* 336(1):1-9.
- USDA. 2000. 1994-1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII) and Diet and Health Knowledge Survey (DHKS) (On CD-ROM). U.S. Department of Agriculture (USDA); Riverdale, Maryland. [PB2000-500027 Supercedes PB98-500457].
- Thompson, P.W. 1994. Lycopene: six strain reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*. Safepharm Laboratories Limited, Project No. 306/208. Cited In: Matulka *et al.*, 2004.
- van den Berg, H. 1998. Effect of lutein on beta-carotene absorption and cleavage. *Int J Vitam Nutr Res* 68(6):360-365.
- Watzl, B., Bub, A., Blockhaus, M., Herbert, B.M., Lührmann, P.M., Neuhäuser-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.
- 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  $\beta$ -carotin, lycopin, 7,7-dihydro- $\beta$ -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.

# REFERENCES

Page NA has been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Submission End

## *Reference List for Industry Submission, GRN 000173*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
NA	Yong, Lee-Chen; Forman, Michele R.; Beecher, Gary R.; Graubard, Barry I.; Campbell, William S.; Reichman, Marsha E.; Taylor, Philip R.; Lanza, Elaine; Holden, Joanne M.; Judd, Joseph T.	Relationship between dietary intake and plasma concentrations of carotenoids in premenopausal women: application of the USDA- NCI carotenoid food- composition database	1994	American Journal of Clinical Nutrition	Volume 60, pgs 223-230
NA	Johnson-Down, Louise; Saudny- Unterberger, Helga; Gray-Donald, Katherine	Food habits of Canadians: Lutein and lycopene intake in the Canadian populaton	July 2002	Journal of The American Dietetic Asssocation	Volume 102, Number 7, pgs 988-991
NA	Krinsky, Norman I.; Russett, Mark D.; Handelman, Garry J.; Snodderly, D. Max	Vitamins: Structural and Geometrical Isomers of Carotenoids in Human Plasma	1990	Journal of Nutrition	Volume 120, pgs 1654-1662
NA	NA	Lycophyll	2001	The Merck Index, Thirteenth Edition	pgs 1007
NA	Forman, Michele R.; Lanza, Elaine; Yong, Lee-Chen; Holden, Joanne M.; Graubard, Barry I.; Beecher, Gary R.; Melitz, Marc; Brown, Ellen D.; Smith, J. Cecil	The correlation between two dietary assessments of carotenoid intake and plasma carotenoid concentrations: application of a carotenoid food- composition database	1993	American Journal of Clinical Nutrition	Volume 58, pgs 519-524
NA	Scott, K. John; Thurnham, Daivd I.; Hart, David J.; Bingham, Shelia A.; Day, Ken	The correlation between the intake of lutein, lycopene and Beta- carotene from vegetables and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK	1996	British Journal of Nutrition	Volume 75, pgs 409-418
NA	Agarwal, Anita; Shen, Honglei; Agarwal, Sanjiv; Rao, A.V.	Lycopene Content of Tomato Products: Its Stability, Bioavailability and In Vivo Antioxidant Properties	2001	Journal of Medicinal Food	Volume 4, Number 1, pgs 9-15

*NA- Not applicable*

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
NA	Hadley, Craig, W.; Clinton, Steven K.; Schwartz, Steven J.	Human Nutrition and Metabolism: The Consumption of Processed Tomato Products Enhances Plasma Lycopene Concentrations in Association with a Reduced Lipoprotein Sensitivity to Oxidative Damage	2003	Journal of Nutrition	Volume 133, pgs 727 - 732
NA	Rice-Evans, Catherine; Sampson, Julia; Bramley, Peter M. Holloway, Daniel E.	Why Do We Expect Carotenoids to be Antioxidants in vivo?	1997	Free Radical Research	Volume 26, pgs 381-398
NA	Furr, Harold C.; Clark, Richard M.	Intestinal absorption and tissue distribution of carotenoids	1997	Journal of Nutritional Biochemistry	Volume 8 , pgs 364-377
NA	Cronin, Joseph R.	Lycopene - The Powerful Antioxidant That Makes Tomatoes Red	April 2000	The Biochemistry of Alternative Medicine	pgs 92-94
NA	Boileau, Thomas W.M.; Moore, Amy C.; Erdman, John W. Jr.	Carotenoids and Vitamin A	1999	NA	pgs 133-158
NA	Cronin, Joseph R.	The Biochemistry of Alternative Medicine: Lycopene: The Powerful Antioxidant That Makes Tomatoes Red	April 2000	Antioxidant Status, Diet, Nutrition, and Health	pgs 92-94
NA	NA	Beta-Carotene and Other Carotenoids	2000	Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids	pgs 325 - 382

*NA- Not applicable*

**Garcia, Edmundo**

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**From:** Diane B. McColl [DBM@hpm.com]  
**Sent:** Wednesday, November 16, 2005 12:45 PM  
**To:** edmundo.garcia@fda.hhs.gov  
**Subject:** Lycopene from B. trispora GRAS Notice



Use level table  
om GRAS doss.

Dear Edmundo,

As requested, attached is a table showing the different use levels for soup mixes versus condensed soups and prepared soups. Vitatene's lycopene from B. trispora is intended for use in soup mixes at levels up to 575 ppm, and in condensed soups and prepared soups at levels up to 7 ppm.

If you have any additional questions, please do not hesitate to ask.

Sincerely,  
Diane McColl  
Counsel to Vitatene SA

I. Table from GRAS dossier:

Table 4-1 Summary of the Individual Proposed Food Uses and Use-Levels for Lycopene in the U.S.		
Food Category	Proposed Food-Use	Use-Levels for Lycopene (ppm)
Baked Goods and Baking Mixes	Nutrient Bars	50
	Crackers	30
Beverages and Beverage Bases	Meal Replacements	25
Breakfast Cereals	Ready-to-Eat Cereals	50
Cheeses	Processed Cheese Spread	5
Condiments and Relishes	Sauces, Seasonings, Relishes, and Pickles	50
Confections and Frostings	Decorations, Fillings, and Icings	25
Fats and Oils	Table Fat Spreads	5
	Low Fat Salad Dressings	20
Frozen Dairy Desserts and Mixes	Frozen Dairy Desserts	25
Gelatins, Puddings, and Fillings	Gelatin Desserts, Puddings, and Custards	25
Gravies and Sauces	Tomato-Based, Gravies, and Specialty Sauces	50
Hard Candy	Hard Candy	25
Milk Products	Dairy-Based Fruit Drinks	50
	Milk-Based Meal Replacements	25
	Cultured Dairy Drinks	20
Plant Protein Products	Meat Substitutes	50
Processed Fruits and Fruit Juices	Energy, Sport, and Isotonic Drinks	25
	Fruit-Flavored Drinks	25
	Fruit Juice	25
	Nectars	25
Snack Foods	Salty Snacks	30
Soft Candy	Jelly Products	25
Soups and Soup Mixes	Prepared and Condensed Soups	7
	Dry Soup Mixes	575