Lycopeen

Tweede beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten

Lycopene

Second opinion regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel ingredients

Gezondheidsraad:
Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (VNV)

Health Council of the Netherlands
Committee on the Safety Assessment of Novel Foods

aan/to

de minister van Volksgezondheid, Welzijn en Sport/
the Minister of Health, Welfare and Sport

de minister van Landbouw, Natuur en Voedselkwaliteit/
the Minister of Agriculture, Nature and Food Quality

Nr 2004/02VNV, Den Haag, 24 juni 2004
No. 2004/02VNV, The Hague, June 24, 2004

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De adviezen van de Gezondheidsraad zijn openbaar en worden in bijna alle gevallen opgesteld door multidisciplinaire commissies van – op persoonlijke titel benoemde – Nederlandse en soms buitenlandse deskundigen.

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Datum : 24 juni 2004

Mijnheer de minister,

Dit schrijven dient ter beantwoording van de adviesaanvraag over de veiligheid van nieuwe voedingsmiddelen en nieuwe voedselingrediënten, die door u mede namens de Minister van Landbouw, Natuurbeheer en Voedselkwaliteit aan de Gezondheidsraad is voorgelegd. Aan de orde is een zogenoemde tweede beoordeling, conform de Europese verordening 258/97, van het gebruik van Lycopeen uit *Blakeslea trispora*. De lycopeen is bedoeld als voedingssupplement en als ingrediënt in boter en margarine, melk en melkproducten, sauzen, soepen en smaakmakers, en in gebak in concentraties van 2 tot 7 mg/kg. Het product zal vermarkt worden in een oliesuspensie van 5% en een van 20% lycopeen. De aanvrager die dit product op de markt wil brengen is de firma Vitatene. De beoordeling is verricht door de Commissie 'Veiligheidsbeoordeling nieuwe voedingsmiddelen' van de Gezondheidsraad (Commissie VNV).

De eerste beoordeling van de aanvraag voor markttoelating is verricht door de Engelse deskundigencommissie *'Advisory Committee on Novel Foods and Processes'* (ACNFP). De ACNFP heeft enkele aanvullende vragen gesteld bij het ingediende dossier. De ACNFP heeft lycopeen uit *Blakeslea trispora* beoordeeld als veilig voor de gevraagde toepassingen. De bevoegde autoriteit, de Engelse Food Standards Agency, heeft dit advies overgenomen.

De Commissie VNV baseert haar oordeel op het rapport van de eerste beoordeling door de ACNFP en op de informatie in het dossier. De Commissie VNV stemt grotendeels in met de Engelse beoordeling. De lycopeen uit de nieuwe bron, de schimmel *Blakeslea trispora,* is vergelijkbaar met de lycopeen in groenten en fruit. Lycopeen is een carotenoïd waaraan anti-oxidatieve eigenschappen worden toegeschreven. De schimmel *Blakeslea trispora* is al eerder door

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internationale deskundigencommissies goedgekeurd als bron voor β-caroteen. De aanvrager heeft aanvullend een 28-dagen toxiciteitsonderzoek gedaan met de lycopeenrijke biomassa. Dit leverde geen aanwijzingen op voor nadelige effecten.

Met geconcentreerd lycopeen uit verschillende bronnen zijn door derden uitgebreide toxicologische onderzoeken gedaan zoals acuut, subchronisch en chronisch proefdieronderzoek. Ook resultaten van carcinogeniteits-, mutageniteits- en reproductietoxiciteitsonderzoek door derden zijn beschikbaar en leveren geen aanwijzingen voor nadelige invloeden van consumptie van lycopeen. Gegevens over hoge lycopeeninname van mensen duiden niet op gezondheidsrisico's. De aanvrager heeft met het eigen lycopeenpreparaat van 20% lycopeen in olie gesuspendeerd een 90-dagen onderzoek in proefdieren laten uitvoeren. Daaruit is een *No Observed Adverse Effect Level* (NOAEL) afgeleid van 601 mg per kg lichaamsgewicht per dag. Deze waarde wordt vervolgens vergeleken met de gemiddelde inname van lycopeen door de Engelse consument als deze uitsluitend de voorgestelde voedingsmiddelen met de nieuwe lycopeen zou nuttigen in het huidige eetpatroon. Dit leidt tot consumptieniveaus van 44,9 μg per kg lichaamsgewicht voor kinderen en 22,6 μg per kg lichaamsgewicht voor volwassen mannen (97 percentiel). Tussen de geschatte toekomstige inname en NOAEL is de veiligheidsmarge meer dan 10.000. Als de NOAEL wordt vergeleken met de inname door het gebruik van reeds op de markt zijnde voedingssupplementen met 20 mg lycopeen is er nog altijd een veiligheidsmarge van 2000.

De huidige inname van lycopeen uit groenten en fruit varieert zeer sterk van persoon tot persoon en van dag tot dag. Indicaties van gemiddelde innames zijn: 0,8 mg per persoon per dag in Finland, 1 mg per persoon per dag in het Verenigd Koninkrijk (lycopeenrijk eetpatroon) en 5 mg tot uitschieters van 25 mg per persoon per dag in de Verenigde Staten. De inname van lycopeen door de Engelse consument als deze uitsluitend de voorgestelde voedingsmiddelen met de nieuwe lycopeen zou nuttigen in het huidige eetpatroon wordt 0,65 mg per persoon per dag voor jonge kinderen en 1,5 mg per persoon per dag voor volwassenen (97 percentiel). Het lycopeenpreparaat bevat geen meetbare hoeveelheden eiwit waardoor de kans op allergene eigenschappen verwaarloosbaar is.

De Commissie VNV is het grotendeels eens met het oordeel van de ACNFP. Zij heeft de volgende aanvullingen en kanttekeningen bij het oordeel. Er zijn de commissie twee onderzoeken bekend die een indicatie geven van de consumptie van lycopeen door de Nederlandse bevolking. Het ene onderzoek is een cohortonderzoek bij ouderen en betreft een berekening van de inname

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Onderwerp : Tweede beoordeling veiligheid Lycopeen uit *Blakeslea trispora*
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van carotenoïden. De inneming van lycopeen bedroeg bij mannen 1,0 mg per persoon per dag (SD 1,56) en bij vrouwen 1,3 mg per persoon per dag (SD 1,88) (Gol98). Gegevens uit het andere onderzoek, bij personen van 25-45 jaar, geven een mediane inname van 4,9 mg lycopeen per persoon per dag (spreiding 2,8 – 7,5 mg) (O'Ne01). Nederlanders consumeren dus al relatief veel lycopeen en de procentuele toename door het op de markt komen van het nieuwe preparaat is naar verwachting klein. De Commissie VNV is van mening dat de door de aanvrager voorgestelde toepassingen en gehalten de suggestie wekken van gebruik als kleurstof. Ten opzichte van het pakket aan toxicologisch onderzoek dat standaard geëist wordt voor toelating van een voedseladditief (SCF01) schiet het dossier op enkele punten tekort. Daardoor kan er ook geen *acceptable daily intake* (ADI) worden vastgesteld en slechts een *no observed adverse effect level* (NOAEL). Voor stoffen die toepassing vinden als voedselingrediënt én als voedseladditief verdient een volledig dossier en afleiding van een ADI de voorkeur. Omdat het productenassortiment beperkt blijft en de gehaltes laag, vindt de Commissie VNV dit lycopeendossier vooralsnog acceptabel.

De Commissie VNV maakt geen bezwaar tegen toelating van de nieuwe lycopeen als voedselingrediënt op de Europese markt op de voorgestelde wijze.

Ik onderschrijf de conclusies en aanbevelingen van de Commissie VNV.

Hoogachtend,

prof. dr JGAJ Hautvast

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Letter to the Dutch Minister of Health, Welfare and Sport

On June 15, professor JGAJ Hautvast, Vice-president of the Health Council of the Netherlands wrote as follows to the Minister of Health, Welfare and Sport:

This letter had been prepared in reply to your request for advice regarding the safety of novel foods and food ingredients, also made on behalf of the Minister of Agriculture, Nature and Food Quality. The subject in question is a so-called second opinion, in accordance with European Regulation 258/97, concerning Lycopene from *Blakeslea trispora*. The lycopene is intended for use as a dietary supplement and as an ingredient in butter and margarine, milk and milk products, sauces, soups and seasonings, and in confectionery in concentrations of 2–7 mg/kg. The product will be marketed in an oil suspension of 5% and of 20% lycopene. The applicant wishing to place this product on the market is Vitatene. This assessment was carried out by the Health Council's Committee on Safety Assessment of Novel Foods (VNV Committee).

The initial assessment of the application for marketing authorisation was carried out by the *Advisory Committee on Novel Foods and Processes* (ACNFP), in the United Kingdom. The ACNFP raised several supplementary questions in connection with the dossier that was submitted. The ACNFP judged lycopene from *Blakeslea trispora* to be safe for the proposed uses. The Competent Authority, the UK Food Standards Agency, has adopted this advice.

The VNV Committee bases its opinion on the report from the initial assessment by the ACNFP and on the information in the dossier. The VNV Committee largely agrees with the UK assessment. The lycopene from the novel source, the fungus *Blakeslea tris-*

pora, is comparable to the lycopene in vegetables and fruit. Lycopene is a carotenoid to which anti-oxidative properties are ascribed. The *Blakeslea trispora* fungus has already been approved by international expert committees as a source of β-carotene. The applicant has additionally carried out a 28-day toxicity study with the lycopene-rich biomass. This did not produce any indications of adverse effects.

Extensive toxicological studies (e.g. acute, sub-chronic and chronic animal studies) have been performed by third parties using concentrated lycopene from different sources. Results are also available from carcinogenicity, mutagenicity and reproductive toxicity studies conducted by third parties and these show no evidence of adverse effects from consumption of lycopene. Data on high-level human intake of lycopene do not suggest any health risks. The applicant has had a 90-day study performed in experimental animals using its own lycopene preparation containing 20% lycopene suspended in oil. From this a No Observed Adverse Effect Level (NOAEL) of 601 mg per kg body weight per day has been derived. This value is then compared with the average intake of lycopene by the UK consumer if the proposed foods with the novel lycopene is consumed in accordance with current food consumption patterns. This results in consumption levels of 44.9 µg per kg body weight for children and 22.6 µg per kg body weight for adult males (97 percentile). There is a safety margin of more than 10,000 between estimated future intake and the NOAEL. If a comparison is made between the NOAEL and the intake through the use of dietary supplements containing 20 mg lycopene already on the market, there is still a 2,000-fold safety margin.

Current intake of lycopene from vegetables and fruit varies massively from person to person and from day to day. The following are indications of average intakes: 0.8 mg per person per day in Finland, 1 mg per person per day in the United Kingdom (lycopene-rich eating patterns), and a range of between 5 mg and 25 mg per person per day (in outliers) in the United States. The intake of lycopene by the UK consumers if they were to consume only the proposed foods with the novel lycopene in accordance with current eating patterns is 0.65 mg per person per day for young children and 1.5 mg per person per day for adults (97 percentile). As the lycopene preparation contains no measurable amounts of protein, the risk of allergenic properties is negligible.

While the VNV Committee is largely in agreement with the ACNFP opinion, it has the following additions and comments to make in connection with it. The Committee knows of two studies that give an indication of the consumption of lycopene by the Dutch population. One is a cohort study with elderly people which estimates the intake of carotenoids. In the case of men, intake of lycopene was 1,0 mg per person per day (SD 1,6) and for women it was 1.3 mg per person per day (SD 1,9) (Goldbohm *et al*, 1998). Data from the other study with people of 25-45 years of age give a median intake of 4.9 mg lycopene per person per day (variation 2.8 – 7.5 mg) (O'Neill *et al*, 2001). Thus the Dutch consume relatively large amounts of lycopene and the percentage

increase resulting from the market introduction of the novel preparation is expected to be small. The VNV Committee believes that the applications and levels proposed by the applicant closely resemble use as a colourant. As far as the package of toxicological studies that is usually required for authorisation of a food additive (SCF 2001) is concerned, the dossier is lacking in some areas. It is therefore not possible to establish an acceptable daily intake (ADI), only a no observed adverse effect level (NOAEL). For substances that are used as a food ingredient *and* as a food additive, it is preferable to have a complete dossier and derivation of an ADI. Because the product assortment is still limited and the levels are low, the VNV Committee finds this acceptable for the time being.

The VNV Committee does not object to the authorisation of the novel lycopene as a food ingredient on the European market in the proposed way.

I endorse the conclusions and recommendations of the VNV Committee,

(signed) professor JGAJ Hautvast

Literatuur/Literature

EG97 Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad van 27 januari 1997 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Publikatieblad van de Europese Gemeenschappen 1997; L43: 1-6
(Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities 1997; L43: 1-6).

EG97a Aanbeveling (EG) nr. 97/618/EG van de Commissie van 29 juli 1997 betreffende de wetenschappelijke aspecten en de presentatie van de informatie die nodig is om aanvragen voor het in de handel brengen van nieuwe voedingsmiddelen en nieuwe voedselingrediënten te ondersteunen alsmede het opstellen van de verslagen van de eerste beoordeling uit hoofde van Verordening (EG) nr. 258/97 van het Europees Parlement en de Raad. Publikatieblad van de Europese Gemeenschappen 1997; L253: 1-36
(97/618/EC: 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 and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament of the Council. Official Journal of the European Communities 1997; L253: 1-36).

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GR02 Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen. Veiligheidsbeoordeling van nieuwe voedingsmiddelen. Den Haag, Gezondheidsraad 2002, publicatienummer 2002/05VNV.
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O'Ne01 O'Neill ME, Caroll Y, Corridan B., e.a. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. Br. J. Nutr. 2001; 85: 499-507.

SCF01 Guidance on submissions for food additive evaluations by the Scientific Committee on Food. Brussels, Scientific Committee on Food, 2001, documentcode SCF/CS/ADD/GEN/26Final.

WHO91 Strategies for assessing the safety of foods produced by biotechnology. Report of a joint FAO/WHO Consultation. Geneva, WHO 1991.

A	De adviesaanvraag/Request for advice
B	De commissie/The committee
C	EU-procedure/EU-procedure
D	Samenvatting van het dossier/Executive summary of the dossier
E	Eerste beoordeling/First assessment

Bijlagen/Annexes

Bijlage **A**

De Adviesaanvraag/Request for advice

Op 18 augustus 1999 schreef de Minister van Volksgezondheid, Welzijn en Sport aan de Voorzitter van de Gezondheidsraad (brief kenmerk GZB/VVB 993428):

Sinds mei 1997 is in de Europese Unie de Verordening (EG) 258/97 van kracht inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Daarmee werd de veiligheidsbeoordeling onderdeel van een communautaire procedure.

Met u is reeds de mogelijkheid besproken de beoordeling door de Gezondheidsraad te laten uitvoeren. Ik verzoek u dan ook mede namens de Staatssecretaris van Landbouw, Natuurbeheer en Visserij, in deze eerste fase van uitvoering van de Europese Verordening (EG) 258/97 gedurende een aantal jaren, de veiligheidsbeoordeling gestalte te geven. Voor het onderbrengen bij de Gezondheidsraad pleit het experimentele karakter dat de beoordeling de eerste jaren zal hebben. Dit experimentele karakter komt voort uit het feit dat het een nieuw soort beoordeling betreft van deels nieuwe categorieën van voedingsmiddelen of voedselingrediënten. Het is namelijk een veiligheidsbeoordeling vóór het op de markt brengen van met name voedingsmiddelen van een genetisch gemodificeerde oorsprong en zogenaamd functional foods (nutriceutica). Daarnaast ga ik ervan uit dat de onafhankelijke wetenschappelijke advisering door de Gezondheidsraad het vertrouwen van de Europese Commissie en de andere lidstaten in het Nederlandse oordeel nog versterkt.

Mijn beleid is erop gericht een zo groot mogelijke openheid en transparantie te realiseren van de gevolgde procedure en de beoordeling om de consument vertrouwen te geven in de veiligheid van de

nieuwe voedingsmiddelen. Ik verzoek de Gezondheidsraad hieraan bij te dragen door bijvoorbeeld inzage te geven in de dossiers waarvoor een aanvraag wordt ingediend, waarbij uiteraard bedrijfsvertrouwelijke gegevens worden beschermd en door de criteria, waarop de veiligheid zal worden beoordeeld, te publiceren.

De Minister van Volksgezondheid, Welzijn en Sport,
w.g. dr E Borst-Eilers

English translation

On 18 August 1999, the Minister of Health, Welfare and Sport wrote as follows to the President of the Health Council of the Netherlands (under reference GZB/VVB 993428):

Since May 1997, Regulation (EC) 258/97 concerning novel foods and novel food ingredients has been in force in the European Union. Under the Regulation, the safety of novel foods has to be assessed as part of a community procedure.

Following discussions regarding the possibility of the Health Council making such assessments, the State Secretary for Agriculture, Nature Management and Fisheries and I wish the Council to take responsibility for safety assessment for a period of several years during the fist phase of implementation of European Regulation (EC) 258/97. It is considered appropriate that the Health Council should initially take on this role because the assessment activities will be of an experimental nature, involving both a new form of assessment (i.e. pre-marketing assessment) and, in many cases, new categories of foodstuff (primarily foodstuffs with a genetically modified basis and functional foods or nutraceuticals). We also feel that if assessments are made by a body with the Council's independent scientific status, this will support the validity of the Netherlands' opinion in the eyes of the European Committee and other member states.

My wish is to make the procedure and the assessment as open and transparent as possible, so as to enhance consumer trust in the safety of novel foods. I would like the Health Council to support this objective by, for example, allowing perusal of the application dossier (insofar as consistent with the need to protect the confidentiality of commercially sensitive information) and publishing the criteria upon which safety assessments are made.

The Minister of Health, Welfare and Sport,
(signed) dr E. Borst-Eilers

Bijlage **B**

De commissie/The committee

- Prof. dr EG Schouten, *voorzitter/chairman*
  hoogleraar epidemiologie; Wageningen Universiteit and Researchcentrum/ professor of epidemiology; Wageningen University and Research centre
- Prof. dr CAFM Bruijnzeel-Koomen
  hoogleraar dermatologie/allergologie; Academisch Ziekenhuis Utrecht/professor of dermatology/allergology; Academic Hospital Utrecht
- Ir EJ Kok
  toxicoloog; RIKILT-DLO Wageningen/toxicologist; State Institute for Quality Control of Agricultural Products, Wageningen
- Dr CF van Kreijl
  moleculair-bioloog; RIVM Bilthoven/molecular biologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr P van der Laan
  emeritus hoogleraar statistiek; Technische Universiteit Eindhoven/professor of statistics; Technical University Eindhoven
- Dr FM Nagengast
  gastro-enteroloog; Academisch Ziekenhuis Nijmegen/gastro-enterologist; Academic Hospital Nijmegen
- Dr ir JMA van Raaij
  voedingsfysioloog; Wageningen Universiteit and Researchcentrum/ food physiologist; Wageningen University and Research centre

- Prof. dr ir G Schaafsma
  hoogleraar voeding; TNO Voeding, Zeist/professor of nutrition; TNO Nutrition and Food Research, Zeist
- Dr GJA Speijers
  toxicoloog; RIVM Bilthoven/toxicologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr WJ Stiekema
  hoogleraar bioinformatica; Wageningen Universiteit en Researchcentrum/ professor of bioinformatics; Wageningen University and Research centre
- Ir R Top, *adviseur/advisor*
  Ministerie van VWS; Den Haag/Ministry of Health, Welfare and Sport; The Hague
- Prof. dr WM de Vos
  hoogleraar microbiologie; Wageningen Universiteit en Researchcentrum/ professor of microbiology; Wageningen University and Research centre
- Dr RA Woutersen
  toxicoloog, toxicologisch patholoog; TNO Voeding, Zeist/toxicologist, toxicologic pathologist; TNO Nutrition and Food Research, Zeist
- Dr ir F van der Wilk, *adviseur/advisor*
  COGEM, Bilthoven/Committee on Genetic Modification, Bilthoven
- Dr JAG van de Wiel, *senior secretaris/senior scientific staff member*
  Gezondheidsraad, Den Haag/Health Council of the Netherlands, The Hague

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Bijlage **C**

EU-procedure/EU-procedure

Als een fabrikant een nieuw voedingsmiddel op de markt brengt, dient de veiligheid voor de consument gewaarborgd te zijn. In 1997 werd de Europese verordening van kracht waarin de procedure is geregeld voor de goedkeuring voor marktintroductie van een nieuw voedingsmiddel (EG97). Bij deze procedure zijn verschillende actoren betrokken. De aanvrager moet beoordelen of het product werkelijk 'nieuw' is, dat wil zeggen dat het nog niet eerder in de Europese Unie in substantiële mate voor menselijke voeding is gebruikt en ook niet wezenlijk gelijkwaardig is aan een bestaand product. (Voor een wezenlijk gelijkwaardig product kan worden volstaan met een kennisgeving van de marktintroductie.) Ook moet het niet gaan om een levensmiddelenadditief, aroma of extractiemiddel, omdat deze producten op een andere wijze worden beoordeeld. Voor een nieuw voedingsmiddel in de zin van de Europese verordening moet de aanvrager een veiligheidsdossier overleggen volgens aanbevelingen van de Europese Commissie (EG97a). Deze aanbevelingen zijn gebaseerd op rapporten van verschillende instanties die zich met het onderwerp nieuwe voedingsmiddelen bezighouden, te weten de OECD (OECD93, OECD96) en de WHO/FAO (FAO96, WHO91). Ook de Gezondheidsraad heeft zich al eerder over dit onderwerp gebogen (GR92). Sinds het verschijnen van de aanbevelingen van de EU wordt in internationaal verband gewerkt aan explicitering en aanpassing aan de stand van de wetenschap (GR02).

De fabrikant levert het volgens de richtlijnen samengestelde dossier in bij het land waar het product het eerst op de markt zal komen. Daarop komt de nationale veiligheids-beoordelingsautoriteit in actie. In Nederland is dat de Minister van Volksgezondheid, Welzijn en Sport. Zij heeft de Gezondheidsraad verzocht haar van advies te dienen. De

Voorzitter van de Gezondheidsraad heeft hiertoe de commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (commissie VNV) ingesteld.

De commissie beoordeelt op basis van de huidige stand van de wetenschap of de door de fabrikant geleverde gegevens juist en volledig zijn en of zij het eens is met diens conclusies. Zij maakt een verslag van haar bevindingen — ook volgens de Europese aanbevelingen (EG97a, deel III) — en biedt dat de minister aan. De minister formuleert het Nederlandse oordeel over een voedingsmiddel en brengt dat in bij het Europese overleg in het Permanent Comité voor de voedselketen en de diergezondheid. Alle Europese lidstaten worden uitgenodigd hun oordeel (de zogeheten tweede beoordeling) te geven over het dossier en over de eerste beoordeling alvorens genoemd Comité een eindoordeel velt. Als een dossier veel vragen oproept, gaat er een adviesvraag van de Europese Autoriteit voor voedselveiligheid (EAV). Komt men dan nog niet tot overeenstemming dan beslist de Europese Ministerraad. Sinds 18 april 2004 moeten veiligheidsdossiers van voedingsmiddelen van genetisch gemodificeerde oorsprong rechtstreeks ingediend worden bij de EAV (EG03).

English translation

When manufacturers bring novel foodstuffs onto the market, consumer safety has to be ensured. In 1997, a European Regulation (EG97) came into force, laying down the procedure for approving the market introduction of novel foodstuffs. The procedure recognizes various actors. The applicant must decide whether a product is a novel foodstuff, i.e. a substance that has not previously been available for human consumption to any substantial extent within the European Union and is not substantially equivalent to any existing product. (If a foodstuff is substantially equivalent to any existing product, it is sufficient to inform the authorities of its market introduction). Food additives, aromas and extracts are excluded from the provisions of the directive, since they fall within the scope of an established assessment regime. Before marketing a novel foodstuff, the applicant must compile a safety dossier that complies with the Recommendations of the European Commission (EG97a). These Recommendations are based on reports by a number of bodies that have studied the issue of novel foodstuffs, in particular the OECD (OECD93, OECD96) and the WHO/FAO (FAO96, WHO91). The Health Council of the Netherlands has also considered the question earlier (GR92). Since publication of the EU recommendations, international efforts have been made to clarify and adapt the latest scientific knowledge in the field (GR02).

Having compiled a dossier in line with the guidelines, the manufacturer has to submit it to the competent authority in the country where the product is to be marketed first. This dossier is assessed by the national safety assessment authority. In the Netherlands, this is the Minister of Health, Welfare and Sport, who is advised by the Health Council.

The President of the Health Council has created a Committee on the Safety Assessment of Novel Foods (VNV Committee) to advise the minister on behalf of the Council.

On the basis of the scientific state of the art, the committee has to decide whether the information provided by the manufacturer is accurate and complete and whether the manufacturer's conclusions are sound. The committee then draws up a report on its findings for the minister; this report must also comply with the European Recommendation (EG97a, part III). After considering the report, the minister formulates the Netherlands' opinion regarding the foodstuff in question, which is discussed at European level in the Standing Committee on the Food Chain and Animal Health. All other European member states are invited to express a 'second opinion' regarding the dossier and the first opinion. The Standing Committee then arrives at a final judgement. If a dossier is particularly contentious, the European Commission calls upon the European Food Safety Authority (EFSA) for advice. If consensus still cannot be reached, the issue is referred to the European Council of Ministers. From April 18th 2004 safety dossiers of food from genetically modified origin have to be submitted to EFSA directly (EC03).

Bijlage **D**

Samenvatting van het dossier/ Executive summary of the dossier

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

SUMMARY

Applicant: Vitatene
Antibioticos S.A.U
Avda. de Antibioticos, 59-61
24080 León,
Spain

October 22, 2003

APPLICATION FOR THE APPROVAL OF LYCOPENE FROM *BLAKESLEA TRISPORA*: USE AS A NOVEL FOOD INGREDIENT IN EUROPE

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October 22, 2003

APPLICATION FOR THE APPROVAL OF LYCOPENE FROM *BLAKESLEA TRISPORA*: USE AS A NOVEL FOOD INGREDIENT IN EUROPE

Applicant

The application is submitted by:

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Novel Food Classification

Under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredient*s (hereafter referred to as EC 258/97), Vitatene seeks approval to market lycopene, derived from the fungus *Blakeslea trispora*, for use as a novel food ingredient in Europe. Vitatene therefore submit following an application dossier ("Application for the approval of Lycopene from *Blakeslea trispora*") 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), concerning the scientific aspects of information necessary for the placing on the market of novel foods and novel food ingredients.

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.

Application

This application seeks approval for the use of lycopene in the form of an oil suspension as a nutritional food ingredient. The individual proposed food-uses and their maximum usage levels for lycopene from *B. trispora* in the E.U. are summarized in the following Table.

Table 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

Information Requirements

Section 4 of the Commission Recommendation of 1997 outlines recommendations 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. Lycopene from *B. trispora* is 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. 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". Pursuant to Table II, Part I under the Commission Recommendation of 1997, the essential information requirements corresponding with this classification are outlined as follows, expanded upon in the dossier ("Application for the approval of Lycopene from *B. trispora*"), and summarized herein.

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 form previous human exposure to the Novel Food or its source
XI. Nutritional information on the Novel Food
XII. Microbiological information on the Novel Food
XIII. Toxicological information on the Novel Food

Executive Summary

Manufacturing/specifications

The manufacturing of lycopene is carried out in two phases. The fermentation phase refers to the production of lycopene through a co-fermentation process using the 2 sexual mating types (*plus* and *minus*) of the fungus *B. trispora*. 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 (5 or 20% lycopene) prior to packaging. Results of analysis for representative lots of 5 and 20% lycopene oil suspensions demonstrated compliance with final product physical, chemical, and microbiological specifications.

History/Safety of Lycopene and the Source Organism

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, and a 90-day study with the extracted lycopene formulation and two mutagenicity studies including a bacterial mutation test and a chromosome aberration test. 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 microorganism and the resultant production of β-carotene. 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.

Previous Human Exposure

Lycopene from *B. trispora* is chemically and nutritionally equivalent to naturally occurring lycopene, since the biosynthetic routes from the fungal source are identical to those occurring in nature. 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. A number of studies have been completed where intakes of dietary lycopene have been assessed in various populations. These have indicated intakes as high as 25 mg/day in Canadian males while data from an elderly female study in the United

Kingdom (U.K.) has indicated levels of intake around 1 mg/day. The consumption of lycopene containing fruits and vegetables in the human diet has also been found to coincide with lycopene being found as a constituent of mature breast milk and a predominant carotenoid in human plasma.

Estimated Intake of the Novel Food

Anticipated lycopene intakes in the E.U. were estimated using the U.K. food consumption data collected as part of the U.K. Food Standards Agency's, Dietary Survey Programme (DSP). The results indicated minimal exposure to the fungal-derived lycopene product in the all-person and all-user specific demographic groups in the U.K. population using consumption data and information pertaining to the individual proposed food-uses for lycopene. 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.

Safety of the Novel Food

The safety of lycopene from *B. trispora* is supported by a 90-day toxicity study and two genetox studies as well as 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). Based on the average no-observed-effect level (NOEL) of 601 mg lycopene/kg body weight per day from the rat sub-chronic study, and the maximum level of human intake from proposed food uses and dietary supplement intake, it can be concluded that there is a large margin of safety.

Conclusion

In conclusion, this application demonstrates the safety of lycopene from *B. trispora*, which 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*).

Bijlage **E**

Eerste beoordeling/First assessment

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR LYCOPENE FROM *BLAKESLEA TRISPORA*

Applicant **Vitatene**

Responsible person **Dr Rodríguez-Otero**

EC Classification **2.2**

INTRODUCTION

1. An application was submitted by Vitatene to the UK Competent Authority for authorisation of lycopene derived from the fungus *Blakeslea trispora* for use as a novel food ingredient.

2. Lycopene ($C_{40}H_{56}$) is an aliphatic branched hydrocarbon with a molecular weight of 536.9 Daltons. It exists predominantly in the trans- form and is a red crystalline powder soluble in fats and organic solvents, but virtually insoluble in water, methanol or ethanol.

3. Solvent extracted lycopene from tomatoes is approved for use as an additive (E160d) and is used in dietary supplements and as an ingredient (food colour) in a range of foods. Synthetic lycopene is also used as a dietary supplement outside the EU, but is not permitted for use as a colour additive. *Blakeslea trispora* is a fungus found on a number of tropical plants, and strains of *B. trispora* are able to synthesise large quantities of carotenoids. Following the publication of a positive opinion from the SCF in 2001 β-carotene from *B. trispora* was approved for food additive use. Although lycopene *per se* has a history of consumption, and is produced using the same biosynthetic pathway as β- carotene, the organism has not hitherto been used for production of lycopene sold in

the EU and the product requires authorisation under regulation (EC) 258/97 before it can be marketed.

I. Specification of the novel food

Information on this aspect is provided on pages 1 – 6 of the Application dossier

4. The applicant intends to market lycopene from *B. trispora* as a nutritional food ingredient. The purified, crystalline lycopene is dissolved in high oleic sunflower oil, supplemented with tocopherol to minimise oxidation. Tocopherol is added at levels consistent with those specified in the relevant food additives directive 95/2/EC. The Novel Food (NF) will be available in this oil suspension form (5% and 20%) only.

5. Detailed compositional analyses of the NF are given in the Application dossier For these analyses the company has tested both crystalline lycopene and oil suspensions. The Applicant's specification of the novel food states that it should be not less than 95% lycopene of which at least 90% is trans-lycopene. The remainder comprises of a number of low level contaminants, such as the extraction solvent, isobutyl acetate (not greater than 1%), sulphated ash (not greater than 1%) and subsidiary colouring matters (not greater than 5%). This company's specification was exceeded in each of three non-consecutive, representative lots described in the application.

Discussion. *The Committee was satisfied with the specification of the novel food.*

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

Information on this aspect is provided on pages 5, 7-14 of the Application dossier

6. Lycopene from *B. trispora* is obtained by the co-fermentation of 2 sexual mating types of the fungus, obtained using classical strain selection techniques to increase the efficiency of lycopene production. The strains used are the same as those approved for the production of β-carotene. The mating types are stable and are preserved and maintained using GLP methods and are deposited in a culture collection.

7. Fermentation of the fungi to produce lycopene is a two-stage process. Flasks are inoculated with each of the mating types, and grown under controlled conditions. Once vegetative growth is established, the contents of the flasks are individually transferred asceptically to larger growth tanks containing sterile medium. Once sufficient cell mass has accumulated the strains are transferred asceptically into another tank where co-fermentation commences. It is at this point that the fungi start to produce lycopene. The

process is further controlled by the addition of imidazole which inhibits the formation of carotene.

8. After completion of the fermentation process, lycopene rich biomass is subject to an initial purification process using isopropyl alcohol, which removes any oils and other lipophilic substances. The residue is evaporated to dryness, milled and extracted with isobutyl acetate. The resulting enriched solvent is separated and concentrated by vacuum distillation. The lycopene is then crystallised. Due to its susceptibility to oxidation the lycopene is crystallised under nitrogen. The crystalline lycopene is dissolved in high oleic acid sunflower oil containing tocopherol (1%) and diluted in accordance with the desired specification. The purification and extraction processes are identical to those used in the production of beta-carotene from *B. trispora*, which have been examined and cleared by the SCF. Each batch of the final product is assayed to check compliance with the specification Application dossier Section 1.e.

9. The applicant has supplied data indicating that in comparison with lycopene from other sources, lycopene from *B. trispora* is predominantly present in the trans- form (at least 90%). The data also indicates that the purity of the fungal lycopene is comparable with synthetic lycopene (Application dossier Table II c-1).

10. The applicant has demonstrated that the NF (20% oil suspension) is stable for a period of at least two years when stored at 5°C. Other studies demonstrate that lycopene (5% and 20% oil suspension) can be stored in sealed containers for at least 6 months at a range of temperatures (3°C, 25°C and 40°C) with no appreciable deterioration in product quality. In all cases the tests took place in conditions conducive to oxidation as, although the NF was sealed in bottles, the applicant did not sparge with nitrogen.

***Discussion**. The Committee was satisfied that the production process is controlled and that the in-process monitoring steps are appropriate to ensure a safe and consistent product, that does not deteriorate during storage. The Committee accepted clarification from the applicant that consumption of the novel food in a dietary supplement form did not raise levels of exposure to the extraction solvent, isobutyl acetate to levels that would be toxicologically significant.*

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

Information on this aspect is provided on pages 15 – 17 of the Application dossier

11. The applicant has based previous dietary exposure to *B. trispora* on its use as a source of β-carotene, noting that the safety of the organism was assessed by the SCF (2000)

and the Joint Expert Committee on Food Additives (JECFA) (2001). The SCF concluded that, based on the information supplied, the organism is non-pathogenic and non-toxigenic. A subsequent 28-day oral feeding study using Wistar rats, Jonker (2000) (see also Section XIII) also demonstrated that the organism was both non-toxigenic and non-pathogenic.

12. JECFA concluded that β-carotene from *B. trispora* is acceptable for food additive use, providing that it met the specification of its synthetic counterpart. The applicant is of the view that this finding is consistent with their view that the source organism is safe.

13. The applicant also carried out mycotoxin assays on each of three non-consecutive batches to determine whether aflatoxin B1, Mycotoxin T2, ochratoxin and zearalenone were present. The results, for both crystalline and oil suspended lycopene, were all negative.

Discussion *The Committee was reassured that the SCF assessment of the use of the source organism in the production of beta-carotene provided reassurance that there was a history of safe food use. The Committee also noted the similarity of the production process for production of the novel food would not give raise to any additional concerns.*

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

Information on this aspect is provided on pages 21-27 of the Application dossier

14. The applicant intends to use the NF as a nutritional food ingredient. In addition to its use in dietary supplements, the ingredient will be used in a range of foodstuffs, including fat spreads, milk products and confectionery. A full list of the proposed uses is given in the Application dossier (Table IX a-1).

15. In order to predict the intake of the NF the applicant has used the most up to date information available from UK dietary surveys. The applicant has used proposed maximum use levels for **all** foods described above to predict potential intake. In order to compare the data over a 7-day period across a number of different surveys that target different sub-groups of the UK population, the applicant has applied a weighting factor. The UK CA sought the views of experts in the Food Standards Agency who were satisfied with the validity of the methodology.

16. The applicant has used dietary intake data for children (1.5–4.5), young people (4-10), male and female teenagers and male and female adults. Given that the proposed range of foodstuffs is wide, the applicant notes that the percentage of potential users was high amongst all age groups (>98%).

17. The intake estimates are summarised below. The largest consumers of the NF on an absolute basis are predicted to be male adults, whereas children have the highest predicted intakes on a body weight basis. These figures are likely to overestimate actual consumption, as they are based on the assumption that consumers always select foods that are fortified at the maximum level.

	ESTIMATED DAILY INTAKE			
Population Group (age)	Mean (mg)	97th %tile (mg)	Mean (µg/kg bw)	97th %tile (µg/kg bw)
Children (1½-4½)	0.22	0.65	15.1	44.9
Young People (6-11)	0.37	0.93	14.6	36.0
Teenager (F) (11-18)	0.40	1.02	7.6	20.6
Teenager (M) (11-18)	0.42	1.18	7.9	23.8
Adult (F) (16-64)	0.46	1.23	7.4	21.0
Adult (M) (16-64)	0.60	1.68	8.1	22.6

18. Based on the available intake data the applicant notes that the highest amount of lycopene from a food source would be obtained by consumption of fortified soups and soup mixes.

19. The applicant also intends to market the NF in supplement form at levels up to 20mg per day. Supplements containing lycopene from other sources are currently on the market in the EU, and it is likely that the NF would replace those already being consumed and overall consumption levels would not increase. In contrast, incorporation of lycopene into foods would result in additional intake.

20. The applicant has used the most recent adult dietary survey data available, however the Food Standards Agency is able to make estimates of intake based on a 2001 survey of British adults, which is not currently in the public domain in a form that the applicant could use to assess consumption of their product. Analyses of these data that confirm the applicant's consumption estimated are similar to those obtained with the newer survey data..

Discussion. *As the proposed levels of incorporation were low the Committee was content that the intended use of the product did not give any cause for concern, based on scientific information currently available.*

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

Information on this aspect is provided on pages 28-30 of the Application dossier

21. Lycopene is a normal constituent of the diet in a number of red fruits and vegetables such as tomatoes and watermelon. Levels of lycopene in tomato are dependent both on the species of tomato and the degree of ripening but are generally in the range 3.1-7.7 mg / 100g.

22. The applicant highlighted a 1996 UK study that indicated that consumption of a lycopene-rich diet would lead to consumption of 1.03mg/person/day lycopene. These results are similar to levels seen in Finland (0.70 and 0.87 mg/day for females and males respectively).

23. However the applicant also highlighted other studies that show that intake of lycopene outside the EU shows markedly varied levels of consumption. The applicant has summarised a number of North American dietary surveys that reinforce the European findings that consumption of lycopene is intrinsically varied and dependent on dietary preference. Consumption of lycopene in North America indicates a large variation dependent upon method of data collection, however in all cases mean levels were significantly higher than those seen for UK subjects. A USDA study showed that mean lycopene intake for the general US population was 4.7mg/day however a number of other dietary surveys indicate that consumption could be as high as 25.2mg/person/day.

24. The Applicant also notes that there are no reliable consumption figures available for the current consumption of lycopene in dietary supplement form despite such products being freely available in Europe and the North America.

Discussion *The Committee was reassured that lycopene has a history of consumption in the EU, albeit from a different source. The Committee noted that, in addition to its presence in fresh fruit and vegetables, dietary supplements containing lycopene extracted from tomatoes at levels in excess of 20mg were widely available in the UK.*

XI. Nutritional information on the novel food

Information on this aspect is provided on pages 31-33 of the Application dossier

25. The applicant is of the view that, although the source of lycopene is novel, the nutritional value of the novel food is unchanged when compared to existing lycopene. Other constituents of the novel food (high oleic acid sunflower oil and tocopherol) will have a negligible impact on the nutritional value of the lycopene oil suspension as they are relatively common in the diet.

26. Lycopene is an effective antioxidant, and these antioxidant properties are perceived to be primarily responsible for the potential health benefits of dietary carotenes.

Discussion *The Committee was reassured that altering the source of the novel food would not affect its nutritional value.*

XII. Microbiological information on the novel food

Information on this aspect is provided on pages 34-35 of the Application dossier

27. Microbiological information supplied by the applicant indicates that, three non-consecutive batches had no detectable moulds, yeast, *Salmonella* or *Escherichia coli.* These findings applied to both the crystalline lycopene, and oil suspension (5% and 20% forms).

Discussion *The Committee was content with the microbiological data supplied, but requested further information from the applicant to demonstrate the absence of the anaerobic spore forming pathogen Clostridium botulinum. The applicant was able to supply this information, and the Committee was satisfied that the absence of this organism from the final product could be demonstrated.*

XIII Toxicological Information on the Novel Food

Information on this aspect is provided on pages 36-57 of the Application dossier

28. The applicant presented a number of toxicological studies on both the novel food and the source organism. The applicant has noted that the NF is chemically comparable to others on the market (Application dossier Table 2.c-1) and has therefore included toxicological studies on lycopene products from other manufacturers as supporting data.

Summary of studies

29. The applicant assessed the sub-chronic toxicity of the source of the novel food by testing the lycopene-rich biomass extracted from *B. trispora*. Supplementary information to demonstrate the safety of the source organism has been supplied from an independent

scientist, the SCF and JECFA. A 90-day oral toxicity study has been carried out on the NF (20% oil suspension).

30. The applicant also highlighted details of acute, sub-chronic and chronic, carcinogenicity, mutagenicity and genotoxicity, reproductive toxicity trials and human safety data for lycopene from other sources. Developmental toxicity investigations were carried out on two US lycopene products, whilst human safety data were mostly based on high levels of consumption of commonly available lycopene-rich foods.

Lycopene biomass (Application dossier p37)

31. Lycopene-rich biomass obtained under the fermentation conditions described in section II was used in a sub-chronic toxicity study. Four groups of 40 rats (20/sex) were assigned. The first formed a control group whilst the other three received lycopene biomass at levels of 0.1, 0.3 or 1% of the total diet. These percentages corresponded to daily doses of 90, 272 and 906 mg/kg body weight in males and 87, 260 and 868 mg/kg bodyweight in females respectively. The lycopene-enriched diet was administered for a period of 28 days following which the animals were sacrificed.

32. Clinical observations, neurobehavioural observations, growth, food consumption and food conversion efficiency were assessed throughout the study and haematology, clinical chemistry, organ weights and macroscopic and microscopic examinations were carried out at necropsy.

33. No treatment related differences were found in mean body weights and relative/absolute organ weights between the control and treatment groups. Food consumption and food conversion efficiency were also not adversely affected by the treatment. No treatment related clinical signs or neurotoxic indications were found as a result of the lycopene biomass administration. These were assessed using neurobehavioural observations and motor activity assessments.

34. Haematological measurements showed a statistically significant decrease in mean corpuscular volume and prothrombin time in the high dose male group only. However no significant changes were noted for other red blood cell groups, coagulation variables, white blood cell counts, packed cell volume or haemoglobin concentrations and the authors considered the decrease in mean corpuscular volume as an incidental finding and of no toxicological significance. The decrease in prothrombin times was found to be small (6%) and within the limits of historical controls.

35. No adverse effects were noted in the clinical chemistry variables and macroscopic and microscopic examinations at necropsy revealed no treatment related changes except a statistically significant decreased incidence of increased hyaline droplet nephropathy in the high dose male group. Again, the authors of the study attached no toxicological significance to this finding.

Toxicological assessment of *B. trispora* (Application dossier p40)

36. The two mating strains of *B. trispora* are stable cultures that are preserved under conditions that adhere to good manufacturing practices. The strains are considered to be non-toxigenic and non-pathogenic on the basis of 28-day oral feeding study described above. The applicant also notes that *B. trispora* is formally classified in Germany as “risk group 1”, organisms that pose no risk for humans and vertebrates

37. The production of lycopene by *B. trispora* is an intermediary of the beta-carotene synthetic pathway and the SCF considered the use of *B. trispora* as a source of beta-carotene as acceptable. The Committee concluded that the “source organisms and the production process yielded no grounds to suppose that the final crystalline product, beta-carotene, differs from the chemically synthesised beta-carotene used as a food colourant” (SCF, 2000)

Final Product (Application dossier p38)

38. A 90-day oral toxicity study was carried out to assess the toxicity of the 20% lycopene oil suspension in male and female Wistar rats. Groups of 20 rats received a diet containing 0, 0.25, 0.5, or 1.0 % lycopene in the form of a sunflower oil suspension. These percentages corresponded to daily doses of 0, 145 291 and 586 mg/kg bodyweight for males and 0, 156, 312 and 616 mg/kg bodyweight for females.

39. The animals were monitored for viability, clinical signs of toxicity, body weights and food consumption. Prior to necropsy, neurobehavioural testing and ophthalmoscopic examinations were performed and blood and urine analyses were obtained. Following necropsy, gross and histopathological examinations of various tissues were performed and organ weights recorded.

40. A pink discolouration of the fur was noted in all animals in the high dose group and many in the mid-dose group. This was attributed to the direct contact of the animals to the red staining lycopene mixture in the diet. No adverse effects were noted from the examinations described above and as a result the no observed effect level (NOAEL) was set at 1% in the diet. This was equivalent to a dose of 601mg / bodyweight per day, averaging the doses received by the male and female groups.

41. The genotoxicity of a 20% cold water dispersal of lycopene from *B. trispora* was assessed using a bacterial mutation test and an *in vitro* chromosome aberration test. As a result of these studies the investigator concluded that lycopene is not genotoxic.

Margin of safety (Application dossier p39)

42. Comparing the NOEL of 601mg lycopene/kg bodyweight/day from the sub-chronic rat study with the anticipated maximum intake from food use of between 1 and 2 mg/day gives a 20000-fold safety margin. Likely intake from food supplement at a level of 20mg/ day is associated with a 2000-fold safety margin.

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

43. The applicant has supplied details of additional toxicological studies with lycopene derived from natural tomato extracts, tomato paste and synthetically produced lycopene in a number of forms including cold water dispersible (CWD) and water-soluble (WS) beadlet formulations and dietary supplements.

- Acute toxicity studies (Application dossier p40).
- Sub-chronic and chronic toxicity studies (Application dossier p41).
- Carcinogenicity studies (Application dossier p45).
- Mutagenicity / Genotoxicity studies (Application dossier p46).
- Reproductive toxicity studies (Application dossier p49).
- Human safety data (Application dossier p45).

***Discussion**. The Committee was satisfied with the toxicological data supplied by the applicant. However the Committee requested further information on the relevance of a significant change in the incidence of hyaline droplets in the sub-chronic toxicity study (Application dossier p38). The Committee also requested confirmation that the sub-chronic toxicity study parallel tests done using beta carotene biomass (Application dossier p38) did not raise any additional concerns. The applicant has responded to these comments highlighting that the increase in hyaline droplet nephropathy seen in male rats is not a toxicologically significant finding, noting that the mechanism of action, is of no relevance to humans. The applicant also confirmed that the parallel test with the beta carotene biomass revealed no additional toxicological findings. The Committee was content with the applicant's responses.*

Allergenicity

Information on this aspect is provided on page 58 of the Application dossier

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

Discussion *The Committee was content that the final product did not give rise to any allergenic potential.*

Labelling

Information on this aspect is provided on page 22 of the Application dossier

45. The applicant proposes that the ingredient would be described on food labels as "lycopene" without identifying the source to the consumer. The applicant confirms that labelling of products containing the NF will comply with current EU regulations and may include the statement ‘contains an additional source of lycopene’.

Discussion *The Committee was of the view that the proposed labelling should be expanded to indicate the source of the lycopene, in order that individuals who do not wish to consume products derived from, or containing fungi are adequately informed.*

OVERALL DISCUSSION

46. The applicant has provided a clear specification of the proposed novel food and indicated, on the basis of analysis from a number of non-consecutive batches, that the specification is achievable. The process is similar to the production of beta-carotene from *Blakeslea trispora,* which was given a positive evaluation by the SCF in 2001.

47. Given that lycopene is present in a large range of fresh fruits and vegetables, and lycopene extracted from tomatoes is widely available no additional nutritional concerns or benefits associated with consumption of the novel food have been identified. Based on scientific information currently available to the applicant there is sufficient reassurance that consumption of the novel food does not give rise to any toxicological concerns.

48. The applicant has demonstrated that the novel food is stable under normal conditions and when subject to mild temperature abuse. The applicant has also demonstrated that the novel food is microbiologically safe.

49. Although the proposed labelling of the product is adequate, the applicant should comply with general food labelling legislation and ensure that the labelling of the products and the source does not mislead the consumer.

Conclusion

50. The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Vitatene that the range of uses for lycopene from *Blakeslea trispora* is acceptable subject to the applicant's adherence to the proposed specification, and the production parameters described above.

April 2004