A Summary of the Application for the Evaluation of Lyc-O-Mato â lycopene oleoresin from tomatoes under European Regulation (EC) No. 258/97 for extension of food use.

Purpose of Application

Lyc-O-Mato® lycopene oleoresin from tomatoes has had an established use as an ingredient in food supplements and as a food colour (E160d) in the European Union (EU) before 1997 and therefore food supplement usage has been accepted as being exempt from the requirements of the Novel Foods Regulations. This application is being made for the extension of use of Lyc-O-Mato® to include its addition as a source of lycopene in food products other than food supplements. Examples of such foods, showing the levels of use of lycopene from tomato oleoresin, are given in Annex 1 to this summary.

The levels of use in such foods will most likely be about 5mg lycopene per serving. Although there are no official levels of intake for lycopene, the findings from a number of studies indicate that a lycopene intake in the range of 5-10 mg/day has the desired beneficial effects on health. Therefore, there appears to be no physiological need to add larger amounts of lycopene to foods. In addition, technological and organoleptic constraints may, in many instances, preclude the use of high levels of lycopene in foods and beverages.

Investigations have shown that the use of Lyc-O-Mato® as a lycopene source in a product is highly unlikely to be incremental on any existing use of the substance as a colourant in the product.

Scope and Structure of the Application

In this application the safety of Lyc-O-Mato® lycopene oleoresin from tomatoes is discussed within the context of its proposed uses, based upon microbiological, toxicological, analytical-chemical and nutritional data. LycoRed Natural Products Industries Ltd is interested in extending the use of Lyc-O-Mato®, produced from ripe Lycopene Rich Tomato (LRT) varieties, from its current use in food supplements to include use as a food ingredient. The intended dose of lycopene used in dietary supplements is 5 to 15 mg, this amount is equivalent to 83-250 mg Lyc-O-Mato® 6%. In case of food fortification, products will be formulated in such a way that they will

provide ca. 5 mg lycopene suggested (83 mg Lyc-O-Mato® 6%) per daily portion. The use of Lyc-O-Mato® is intended as an additional source of lycopene, aiming at additional health benefits, such as an improved antioxidant function, associated with anticarcinogenic effects (*i.e.* prostate cancer prevention) and UV protection of the skin.
The toxicological studies were performed by an independent laboratory.

The specification of Lyc-O-Mato® is given in Annex 2 to this summary.

The report is structured following the guidelines as given in EC Regulation No 258/97 on Novel Foods and Novel Food Ingredients, and the order of chapters follows the scheme as presented in the Commission Recommendation 97/618/EC, considering that Lyc-O-Mato® is most likely categorised as Category 2.1 'complex novel food from a non-GM source having a history of food use in the Community'. Only the relevant aspects (questions), as mentioned in the Commission Recommendation 97/618/EC, are covered.

More detailed nutritional and toxicological information is presented in appendices A and B. The safety evaluation and conclusions are given in Chapter 11 of the application.

Intake assessment

The total 'maximum' intake of lycopene can only be roughly estimated and will depend on the actual intake and combined use of supplements and fortified products. The estimated daily (background) intake from natural food sources is around 1 mg/day for most European countries, in the Netherlands on average 1.05 mg in men, and 1.33 mg in women. Depending on the actual product use daily lycopene intake can be as high as ca. 6-19 mg from normal daily servings. Lycopene is also used as a food colouring agent (E160d) in the EU. Taking 5-10 ppm as a typical level used in beverages, then the additional intake from these sources would be 5-10 mg per litre consumed.
It is estimated that the total intake due to combined use of supplements and fortified products, on top of 'normal' dietary intake might vary between ca. 6 and maximum 45 mg lycopene per day. This latter estimated higher daily intake level appears however very unlikely and represents an exceptional case.

Nutritional Aspects

Lycopene is fat-soluble and absorbed in parallel with fat digestion and absorption. Bioavailability studies with Lyc-O-Mato® show that it is absorbed and results in a

significant increase in serum lycopene level, at least comparable with that obtained from consumption of equivalent amounts of processed tomatoes (tomato puree), using intake levels of at least 5 mg.

The protective, health beneficial effects of lycopene might be related to its antioxidant potential. Experimental (*in vitro*) studies show that lycopene is an excellent singlet oxygen quencher and has high antioxidant capacity. An increased consumption of tomatoes, a rich source of lycopene, was shown to have a positive effect on biomarkers of oxidative stress, *i.e.* DNA damage and lipid oxidation. In (other) conditions associated with an increased exposure of free radicals (reactive oxygen species), such as smoking, UV (skin) exposure and inflammation, low serum lycopene levels have been reported.
Experimental and observational (epidemiological) studies indicate that consumption of tomato products, containing lycopene, are associated with lower cancer risk, especially in case of prostate cancer. Lycopene, as tomato oleoresin, has also been demonstrated, both *in vitro* as *in vivo,* to inhibit LDL oxidation and have an inhibitory effect on cholesterol synthesis.

In one case-control multicentre study a negative association between lycopene content in adipose tissue (as a marker for long term exposure) and incidence of myocardial infarction in men was observed. This study together with three other epidemiological studies showed odds ratios between 0.39 and 0.81.

It should be noted that the health beneficial effects of fruits and vegetables, especially from tomato products, might be attributed to lycopene, but that a role for other bioactive compounds cannot be excluded. The findings from observational and experimental studies therefore need further extension and confirmation in larger scale, controlled intervention studies.

Safety aspects

The relevant information regarding the nutritional, microbiological, and toxicological aspects of Lyc-O-Mato® is summarised in the respective chapters 8, 9 and 10 of the application and does not indicate any nutritional, microbiological or toxicological concerns associated with the intended use pattern. Yet, a sensitising potential cannot be totally excluded on the basis of the available information. Results of the toxicological evaluation and risk assessment are summarised below.

Toxicological evaluation

The toxicological safety of Lyc-O-Mato® is supported by information from toxicological studies with tomato oleoresin containing 5 or 6% lycopene. The available information allows the following conclusions for Lyc-O-Mato® that are relevant as a starting point for the toxicological risk assessment for the intended application.

No relevant differences in toxicokinetic properties were found between humans and animals. The acute oral and dermal toxicity of Lyc-O-Mato® in rats was low. In rabbits, no eye irritating potential could be detected. A skin sensitising potential was established for a batch of tomato oleoresin containing 5% lycopene. A clarification provided by LycoRed was that there had been a problem with contamination of the fermentation, which might have caused the irritating properties and/or sensitising potential in two irritation and one sensitisation study. However, there is no available data to substantiate this explanation. Therefore, a skin sensitising potential cannot be excluded on the basis of the available information. There is no evidence of significant allergenic potential from oral applications.

A 13-week oral toxicity study (by gavage) was conducted. No relevant effects were observed. The No-Observed-Adverse-Effect-Level (NOAEL) for rats was found to be ca. 4500 mg/kg bw of Lyc-O-Mato®. Lyc-O-Mato® was negative in an Ames study. Despite the fact that only one genotoxicity study was performed, there are no indications for possible genotoxicity of Lyc-O-Mato® 6% or lycopene. There is not a full package of information on reproductive/developmental toxicity and teratogenicity of Lyc-O-Mato® 6%. However, there are no indications for possible reproductive/ developmental toxicity and teratogenicity of Lyc-O-Mato® or lycopene. Furthermore, no adverse effects of lycopene in humans were reported.

Toxicological risk assessment

The available data are considered sufficient for a toxicological risk assessment, as several studies have been conducted with tomato oleoresin 5 or 6% and did not indicate a toxicological concern.

On the basis of the total load of information, summarised above, the NOAEL from the 13-week oral toxicity study in rats of 24500 mg Lyc-O-Mato® 6% per kg bw (highest dose tested) is considered relevant to the intended use of Lyc-O-Mato® and is proposed as the overall NOAEL. Comparison of this overall NOAEL (ca. 4500 mg Lyc-O-Mato®

6% per kg bw) with the anticipated dose of maximally 750 mg Lyc-O-Mato® 6% per day (equivalent to 45 mg Lycopene per day), indicates a margin of safety of 90 or more for individuals weighing 15 kg or more.

Conclusion

A nutritional evaluation and risk assessment was performed and indicated that the evidence provided for a beneficial health effect of Lyc-O-Mato® at the intended dose level is supportive. No adverse nutritional effects are expected.

A toxicological evaluation and risk assessment were performed and did not indicate a toxicological risk associated with the intended use pattern, maximally 750 mg Lyc-O-Mato® 6% per day. Yet, in view of a positive sensitisation study, a sensitising potential cannot be fully excluded. There is no evidence of significant allergenic potential from oral application.

Annex 1

Levels of use of Lycopene from Tomato Oleoresin in Food Products

	Serving Size g	Tomato Lycopene mg/serving	mg/kg (or l) RTE Product
Dairy Products (excepting milk)			
Yoghurts	125	5.0	40
Desserts / Custard	125	5.0	40
Cheese	40	5.0	125
Ice Cream	50 (80ml)	5.0	100 (63mg/l)
Bread and Baked Goods			
Bread	30	0.5	17
Biscuits	20	3.0	150
Fruit Cakes / Cakes	60	5.0	83
Crispbreads	50	5.0	100
Meat Products			
Sausages	120	5.0	42
Pates	33	3.0	91
Meat Substitutes	100	5.0	50
Juices			
Fruit and vegetable Juices	250	5.0	20
Tomato Juice	120	10.0	83
Soups and Sauces			
Soup (other than tomato)	220	5.0	23
Tomato Soup	220	10.0	45
Breakfast Cereals	30	5.0	167
Cereal Bars	25	5.0	200
Snack Foods	25	2.5	100
Pasta Products (not canned)	30	5.0	167
Pizza	200	5.0	25
Fat Spreads			
Margarine	10	3.0	300
Other Spreads	10	3.0	300
Canned Products			
Baked Beans	150	2.5	17
Canned Pasta	200	5.0	25

Annex 2

Specifications of Lyc-O-Mato®

Analysis	Method	Specification	
Physical State	Observation against standard	Red to dark brown viscous liquid	
Clarity	LAB/123/01	Clear solution	
Lycopene identity	LAB/109/01[a]	HPLC retention time	
Total lycopene[b]	LAB/109/01[a]	5.0 to 15.0%	
% trans-Lycopene	LAB/109/01[a]	90 to 95%	
Total carotenoids[c]	LAB/102/02[a]	6.5 to 16.5 %	
Other carotenoids	LAB/118/01[a]	Phytoene: 0.5 to 0.75% Phytofluene: 0.4 to 0.65% ß-carotene: 0.2 to 0.35%	
Total tocopherols	LAB/118/01	1.5 to 3.0%	
Unsaponifiable matter	Study 98/021[a]	13 to 20%	
Total fatty acids[d]	Study 98/021	60 to 75%	
Phytosterols	Study 98/021	0.5 to 2.5%	
Lycopene crystal particle size	Microscopic	90% < 5µ 99% < 10µ	
Water	Karl Fisher	0.5% max	
Sulphated ash	AOAC 34.104	0.5 to 1.5 %	
Residual solvent (ethyl acetate, ethanol)	LAB/114/01[a]	50 mg/kg max	
Pesticides	DFG-S19[e]	Below 3 ppm	
Heavy metals	I.C.P.[e]	Pb < 2 mg/kg Cd, Mo, Ni, Hg all <1 mg/kg	
Arsenic	I.C.P.[e]	As < 2 mg/kg	
Microbiology	USP 24 NF 19/<61>	Total viable count Moulds Yeasts Escherichia coli: Salmonella sp Staphylococcus aureus Pseudomonas aeruginosa Clostridium perfringens	< 1000/g < 100/g < 100/g Not detected in 10g Not detected in 20g Not detected in 10g Not detected in 10g

[a]LycoRed Method, SOP available on request
[b]Combined cis- and trans-lycopenes
[c]Calculated as lycopene
[d]Myristic acid (14:0); palmitic acid (16:0); stearic acid (18:0); oleic acid (18:1); linoleic acid (18:2); linolenic acid (18:3); arachidic acid (20:0); behenic acid (22:0); mono-, di- and tri-glycerides; free fatty acids
[e]Analysis method SOP available on request