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

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Submitted to:

The Advisory Committee on Novel Foods and Processes

Food Standards Agency

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Contents

1. Purpose of Application

Lyc-O-Mato® 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 recommended levels of use of lycopene from tomato oleoresin, are:

	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
Non-alcoholic flavoured drinks	220	5.0	23
Soups and Sauces			
Soup (other than tomato)	220	5.0	23
Tomato Soup	220	10.0	45

(cont)

	Serving Size g	Tomato Lycopene mg/serving	mg/kg (or l) RTE Product
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

In practice, the levels of use in such foods will most likely be about 5mg lycopene per serving or less. 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.

The EU Directive on food colours (Directive 94/36/EC) permits the use of E160d Lycopene (which is the same substance as the subject of this application) in a wide range of food categories with maximum levels as a colourant ranging from 50 – 500 mg/kg (and mg/l). For example, non-alcoholic flavoured drinks are permitted to contain a maximum level of 100mg lycopene/l; desserts and flavoured milk products 150mg/kg; edible ices 150mg/kg and savoury snacks 100mg/kg. In many of these foods the maximum permitted level for colouring purposes, such as 20mg/serving for soft drinks, is considerably higher than the intended use of the substance as a source of lycopene.

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.

2. Introduction

In this application the safety of Lyc-O-Mato® is discussed within the context of its uses, based upon microbiological, toxicological, analytical-chemical and nutritional data as provided by LycoRed Natural Products Industries Ltd (address: P.O. Box 320, BEER-SHEVA 84102, Israel; further referred to as 'LycoRed'), or by external independent laboratories and as found in the scientific literature. Lyc-O-Mato® is produced from red ripe, Lycopene Rich Tomato (LRT) varieties (non-genetically modified organism, conventional breeding only), and does not contain any carriers or additives.

LycoRed is interested in using Lyc-O-Mato® as a food ingredient in addition to its current uses in food supplements and as a colour. The dose of lycopene used in dietary supplements is 5 to 15 mg, this amount is equivalent to 83-250 mg Lyc-O-Mato® 6% or less when a more concentrated extract is used. In the case of food fortification, products will be formulated in such a way that they will provide ca. 5 mg lycopene (83 mg Lyc-O-Mato® 6%, or less in the case of higher concentration) per recommended 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 report is structured following the guidelines as given in European Commission Recommendation 97/618/EC with respect to EC Regulation No 258/97 on Novel Foods and Novel Food Ingredients.

The order of chapters follows the scheme as presented in the Commission Recommendation 97/618/EC, and it is considered that Lyc-O-Mato® is most likely categorised as a 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), covered 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.

The safety assessment was based on the information made available by LycoRed. The toxicological studies were performed by an independent laboratory.

3. Background information

The scheme presented in Table II, Part II under Commission Recommendation 97/618/EC has been followed to determine which of the schemes I-XIII are essential to provide data permitting a safety and nutritional evaluation of Lyc-O-Mato® (see Annex I A.2). In this respect it was considered that Lyc-O-Mato® should be evaluated as a food ingredient falling into Class 2.1 'complex novel food from a non-GM source having a history of food use in the Community' on the basis of the Novel Food Recommendation 97/618/EC and Regulation (EC) No 258/97, the following information is required:

I Specification of Lyc-O-Mato®

II Effect of the production process applied to Lyc-O-Mato®

III History of the organism used as the source of Lyc-O-Mato®

IX Anticipated intake/extent of use of Lyc-O-Mato®

X Information from previous human exposure to Lyc-O-Mato® or its source

XI Nutritional information on Lyc-O-Mato®

XII Microbiological information on Lyc-O-Mato®

XIII Toxicological information on Lyc-O-Mato®

The issues mentioned above will be discussed in Chapters 4-11 in the respective sequence.

4. (I) Specification of Lyc-O-Mato®

- ***Depending on the derivation and composition of Lyc-O-Mato®, is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?***

Yes, there is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients. The proposed specifications of Lyc-O-Mato® are as follows:

Table 1. Chemical composition of Lyc-O-Mato®

	Compound	Content [%] Min	Max.
1	Fatty acids and acylglycerols	69	74
	of which*		
	Myristic acid (14:0)	0.50	0.55
	Palmitic acid (16:0)	22.37	23.01
	Stearic acid (18:0)	5.12	5.36
	Oleic acid (18:1)	12.42	13.45
	Linoleic acid (18:2)	46.72	48.69
	Linolenic acid (18:3)	8.78	10.85
	Arachidic acid (20:0)	0.94	1.07
	Behenic acid (22:0)	0.52	—
	Free fatty acids	5	
2	**Unsaponifiable matter**	14	19
	Lycopene	4.9	15
	Phytoene	0.8	1.4
	Phytofluene	0.4	1.1
	Tocopherols	1.0	3.0
	Sterols	1.5	2.5
	Others (i.e. waxes)	5.0	8.4
3	**Water soluble matter**	2.7	4.7
	Lactic acid	0.45	0.69
	Other organic acids		0.1
	Others	2.2	4.0
4	**Water**	0.48	0.86
5	**Total Phosphorus**	0.35	0.52
	Organic phosphorus	0.32	0.45
6	**Phospholipids**	8.9	14
	(estimated from phosphorus determined by ICP		
7	**Nitrogen**	0.16	0.31
8	**Ash**	0.7	0.8
	Total:	96	114

* % of total peak area

(LycoRed Natural Products Industries Ltd)

Table 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

- ***Is the information representative of Lyc-O-Mato® when produced on a commercial scale?***

Yes, the ranges specified on pages 6 and 7 are representative of a number of different commercial batches of Lyc-O-Mato®. The carotenoid content of production batches has been monitored over a period of eight years.

The Lyc-O-Mato® oleoresin is produced only from conventionally bred tomato varieties without any genetic manipulation.

As is the case with other fruits and vegetables, the composition of the tomato is subjected to natural fluctuations with some tomatoes containing more lycopene than others. The range of lycopene content in commercial tomato varieties varies from 70 to 130 ppm (Table 3). The lycopene content is influenced by the variety, the geographic location, technique of cultivation, climatic variations, degree of ripeness *etc.* The tomato oleoresin is the ethyl acetate extract of ripe tomato fruits and therefore has similar natural fluctuations. Its lycopene concentration ranges from 6% to 15%, according to the nature of the fruit from which it was extracted and the amount of tomato seed oil that is included in the oleoresin. Tomatoes with lycopene content at the lower end of the range will result in an oleoresin containing around 5% lycopene and those at the higher end can produce an oleoresin containing about 15% lycopene. It is standardised to the desired concentration by mixing various lots of the extract. The standardisation process is such that the tolerances above and below the target levels are low. For example, the tolerances on Lyc-O-Mato® 6% are ± 0.2%.

The Lyc-O-Mato® production process has not changed since it was introduced by LycoRed. Therefore, the toxicological evaluations that were conducted on the tomato oleoresin in the mid –1990s are valid for the current production of Lyc-O-Mato®.

It is already stated above that Lyc-O-Mato® oleoresins are produced only from ripe tomato pulp. The process is based on conventional, physical unit operations, chemical reactions are not involved and no additives are used. Throughout the process great care is taken to protect lycopene and the other tomato phytonutrients from oxidation and therefore their original composition is preserved in the oleoresin. Thus the various oleoresins may differ in their lycopene concentration, whether standardised or non-standardised, but have similar content of other tomato lipids as can be seen in Table 3.

Table 3: Carotenoids in Tomato Oleoresin Determined by HPLC

No.	Year	Lot#	Lycopene, %	Phytoene, %	Phytofluene, %	b-carotene, %	Total Tocopherol %	Total carotenoids, %
1	1995	**511008**	6.09%	0.55%	0.51%	0.11%	1.25%	7.26%
2	1996	**408056**	6.33%	0.57%	0.49%	0.13%	1.23%	7.52%
3	1996	**408058**	6.14%	0.67%	0.54%	0.11%	1.18%	7.46%
4	1997	**303039**	7.66%	0.55%	0.44%	0.11%	1.65%	8.75%
5	1997	**306139**	6.05%	0.60%	0.54%	0.23%	1.54%	7.42%
6	1998	**207078**	8.98%	0.67%	0.56%	0.10%	1.76%	10.31%
7	1998	**207063**	5.91%	0.52%	0.38%	0.17%	1.94%	6.98%
8	1999	**101008**	6.07%	0.61%	0.61%	0.27%	1.79%	7.56%
9	1999	**112070**	6.94%	0.51%	0.51%	0.26%	1.75%	8.22%
10	2000	**#001072**	6.00%	0.59%	0.54%	0.27%	1.58%	7.40%
11	2000	**#002105**	5.84%	0.50%	0.45%	0.27%	1.76%	7.07%
12	2000	**#003120**	10.21%	0.82%	0.58%	0.35%	2.29%	11.96%
13	2001	**902172**	10.10%	0.69%	0.59%	0.32%	1.85%	11.70%
14	2001	**902161**	6.10%	0.73%	0.66%	0.36%	2.10%	7.85%
15	2001	**903166**	10.60%	0.69%	0.68%	0.33%	1.55%	12.30%
16	2001	**911070**	6.98%	0.59%	0.55%	0.39%	2.50%	8.51%
17	2002	**803125**	6.10%	0.56%	0.53%	0.41%	1.99%	7.60%
18	2002	**803126**	7.12%	0.61%	0.51%	0.42%	1.86%	8.66%
19	2002	**810048 G**	10.50%	0.54%	0.53%	0.44%	1.99%	14.00%
20	2003	**709015 G**	6.16%	0.57%	0.50%	0.18%	1.94%	9.35%
21	2003	**709019 G**	10.97%	0.63%	0.55%	0.17%	1.86%	14.18%
22	2003	**703134**	7.15%	0.62%	0.59%	0.30%	2.14%	8.66%
23	2003	**710023**	10.64%	0.96%	0.86%	0.24%	2.12%	12.70%
24	2003	**705151**	15.6%	0.64%	0.58%	0.21%	2.03%	16.53%
25	2003	**703127**	14.91%	0.72%	0.64%	0.23%	2.1%	16.49%

- ***Is there an appropriate specification to ensure that Lyc-O-Mato® marketed is Lyc-O-Mato® as that evaluated?***

Yes, the specifications of Lyc-O-Mato® to be marketed are the same as those given in Table 2. Further detailed information is given in the following sections.

4.1 Identity of Lyc-O-Mato®

Primary name
Oleoresin from lycopene-rich tomatoes (Lyc-O-Mato®)

Chemical names
Lyc-O-Mato® does not have a chemical name as it is a tomato oleoresin containing lycopene.

The active ingredient Lycopene is specified as: (all-trans)-lycopene.

CAS-number
Lyc-O-Mato® does not have a CAS number.
The CAS number of the active ingredient lycopene is [502-65-8].

Other common names
Lyc-O-Mato® is also referred to as:

Lycopene oleoresin

Natural tomato extract containing [x %] lycopene.

Lycopene is also referred to as ØØ-carotene.

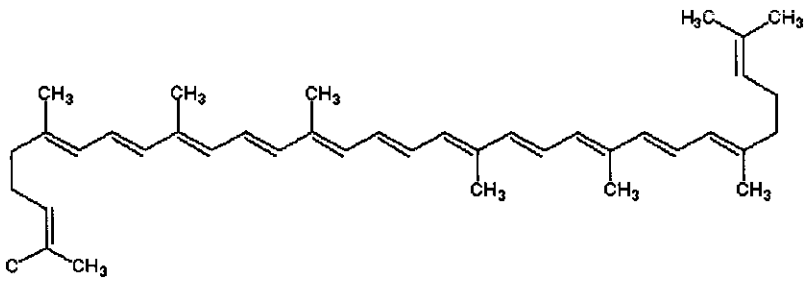
Molecular formula and weight
Molecular formula and weight of lycopene is as follows:

Molecular formula : $C_{40}H_{56}$

Molecular weight : 537

Structural formula

The structural formula of the active ingredient lycopene is presented below:



Isomerism

Regular tomatoes and tomato products mainly contain the all-E (trans-)isomers of lycopene (between 35-96% of total lycopene content), but also some Z (cis-)isomers, mainly as 5Z, 9Z, 13Z & 15Z in percentages varying between 1-22% (Schierle *et al.*, 1997). Therefore, Lyc-O-Mato® mainly contains the all-trans-isomer of Lycopene that is naturally present in the tomato.

Purity and composition

See above.

4.2 Identity of the formulations

Not applicable.

4.3 Physical and chemical properties of the active substance

Description for lycopene

Colour:	red
Physical state:	solid
Appearance:	crystalline
Odour:	neutral

Solubility in water

Solubility in water:	practically insoluble

Other relevant physical and chemical properties of lycopene
Melting point: 172-173 °C

4.4 Stability

Stability of lycopene in tomato oleoresin

Study design

The stability of lycopene in Lyc-O-Mato® was evaluated. Nine manufactured batches of Lyc-O-Mato® were examined under different storage conditions (room temperature and at 4°C) over a period ranging from 18 to 37 months. Analyses were done by spectrophotometry and HPLC.

Results

Under the different storage conditions, the lycopene content was as follows:

Batch no.	Storage conditions	Lycopene concentration (%)[1]			Lycopene Concentration (%)[2]		
		T=0	T=months	Change (%)	T=0	T=months	Change (%)
630442	Room temp.	4.95	5.18 [37]	4.4			
630442	4ºC	4.95	4.95 [37]	0.0	4.20	4.24 [37]	0.9
510004	Room temp.	6.82	6.98 [25]	2.3			
510004	4ºC	6.82	6.88 [25]	0.9	6.20	6.32 [25]	1.9
512003	Room temp.	6.73	6.60 [24]	-2.0			
512003	4ºC	6.73	6.67 [24]	-0.9	6.03	6.12 [24]	2.0
405043	Room temp.	6.56	6.64 [18]	-1.2			
405043	4ºC	6.56	6.61 [18]	0.8	6.05	6.05 [18]	0.0
#003120	Room temp.	10.81%	10.67{24)	-1.3	10.21	10.08{24)	-1.3
#003120	4ºC	10.81%	10.73 {24}	-0.7		10.13 {24}	-0.7
902172	Room temp.	10.65	10.45{24}	-1.9	10.1	10.02{24}	-0.8
902172	4ºC	10.65	10.53 {24}	-1.1		10.06 {24}	-0.4
710023	Room temp.	11.23	11.18 {18}	-0.4	10.64	10.62 {18}	-0.2
710023	4ºC	11.23	11.27 {18}	0.3		10.67{18}	0.3
705151	Room temp.	16.05	16.01 {18}	-0.3	15.6	15.5 {18}	-0.64
705151	4ºC	16.05	15.95 {18}	-0.6		15.56 {18}	-0.25
703127	Room temp.	15.3	15.38 {18}	0.5	14.91	15.01 {18}	0.67
703127	4ºC	15.3	15.27 {18}	-0.2		14.86 {18}	0.33

1. analyzed by spectrophotometry

2. analyzed by HPLC

(LycoRed Natural Products Industries Ltd.)

Conclusions

The lycopene concentration in Lyc-O-Mato® did not show relevant changes upon storage at 4ºC and at room temperature up to 37 months.

Former uses

For the production of lycopene as a colour additive, the preparation Lyc-O-Mato® 6%-15% is further processed by removal part of the tomato lipids by physical means to form the concentrated product Lyc-O-Mato® 60-70%, of which the final colour additive formulations are prepared. Hence, the colour formulations as such cannot be seen as former use of Lyc-O-Mato® 6%-15% oleoresin. In addition to red colour formulations, Lyc-O-Mato can be used in a similar manner to β-carotene, as a food colour in the yellow to orange range.

Stability of the formulations

Not applicable.

4.5 Analysis

HPLC method (Tordjman, 1996)

The method is used to quantify lycopene in tomato extracts on a weight (% w/w) basis using a lycopene standard stock solution. The sample was first dissolved in dichloromethane, stabilised with BHT.

The HPLC separation was performed on an RP-C8 column using VIS-detection (472 nm), the mobile phase being acetnitrile:methanol:dichloromethane:hexane (85:10:2.5:2.5 v/v) with a typical retention time of lycopene of 6-8 minutes.

The HPLC method has been validated with the following characterisation:

linearity - mean correlation coefficient of 0.9996 over a range of 20-100 ppm lycopene;

precision - system precision results gave a relative standard deviation (RSD) of 0.48% and the method precision gave an RSD of 2.6%. The within day and day

by day precision of the linearity results showed an RSD of <2% and <5%, respectively;

accuracy - a mean recovery of 101% was found for the oleoresin recovery test and 98.2% for the crystalline lycopene addition test;

specificity - absence of chromatographic interference.

5. (II) Effects of the production process applied to Lyc-O-Mato® 6%

- ***Does Lyc-O-Mato® undergo a production process?***

Yes, Lyc-O-Mato® is produced by crushing lycopene-rich tomatoes to pulp. The tomato pulp is then separated from the serum and extracted with ethyl acetate. Following the extraction, the extract is separated from the tomato pulp. Lyc-O-Mato® oleoresin is obtained after the solvent is removed by evaporation under vacuum.

- ***Is there a history of use of the production process for the food?***

The Lyc-O-Mato® production process is identical with the production of tomato lycopene colour formulations (E160d). Yet, the colour formulations are processed further than Lyc-O-Mato® oleoresin via the concentrated product Lyc-O-Mato® 60-70%. Hence, the colour formulations cannot be referred to as former use of Lyc-O-Mato® oleoresin.

- ***Does the process result in a significant change in the composition or structure of the Novel Food Ingredient compared to its traditional counterpart?***

Not applicable, since there is no traditional counterpart. For completeness, the scheme is completed as if the answer were 'yes'. Otherwise the information from scheme III would have been sufficient.

- ***Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?***

Yes, there is sufficient information available to assess the possible nutritional, microbiological and toxicological hazards of the production process (see schemes XI, XII, and XIII).

- ***Are the means identified for controlling the process to ensure that the Novel Food Ingredient complies with its specification?***

Yes. Analytical methods are available to quantify the components of Lyc-O-Mato® and possible impurities (organic volatile impurities, microbial impurities, acid-soluble metals). Certificates of analysis for Lyc-O-Mato® are present for a range of five batches. The statistical confidence of the proposed specifications cannot yet be confirmed on the basis of the available batch certificates.

The production process takes place under conditions that are in accordance with the principles of Food Good Manufacturing Practices (21 CFR Part 110 in the USA and the Institute of Food Science and Technology Guidelines in Europe).

- ***Has the process the potential to alter the levels in the Novel Food Ingredient of substances with an adverse effect on public health?***

No. No potentially toxic contaminants or micro-biological hazards were detected or are to be expected in Lyc-O-Mato®

- ***After processing is the Novel Food Ingredient likely to contain microorganisms of adverse public health significance?***

No. No micro-organisms or remains of micro-organisms that may jeopardise general health were detected or are to be expected.

Further detailed information is given in the following sections.

5.1 Method of production and/or preparation of Lyc-O-Mato®

5.1.1 Technological process

The manufacturing of the product is partly identical to the production of the food additive *i.e.*:

1. Tomato cultivation.
2. The LycoRed process uses conventional physical unit operations to separate the ripe tomatoes into serum and pulp. The pulp is then extracted in a similar manner as various oils are being produced from their corresponding plant sources.
3. Since the tomato is a seasonal (summer) crop, the process is conducted in two stages. The pulp is prepared in the season, kept frozen and under vacuum, and used throughout the year to extract the tomato oleoresin.
4. In the processing plant the tomatoes are thoroughly washed and processed into tomato pulp. The tomato pulp separated from the serum is packed under vacuum and kept frozen (-18ºC) until it is extracted in a specially designed modern facility. The proprietary process uses conventional unit operations approved for the food industry. There is no chemical intervention in the process.

5.1.2 Manufacturing Process and Specifications

Lyc-O-Mato® Oleoresin is manufactured by a process involving solvent extraction of naturally-selected, non-genetically modified, hybrid, high lycopene content (LRT) (150-250 ppm) variety of tomato (*Lycopersicon lycopersicum* (L.) Karst. Ex. Farwell; trade names Lyc-O-Mato® LRT). The extract (oleoresin) contains 6% - 15% lycopene on average, with the balance made up of other tomato lipids and carotenoids (Table 1).

It is important to note that the Lyc-O-Mato® process is based on extraction from tomato pulp. Tomato pulp, as a starting point, has significant advantages over processes based on extraction from other tomato sources such as tomato paste and tomato waste.

There are distinct advantages in using tomato pulp for lycopene extraction when compared to other starting points. The pulp is free from partially broken down and oxidized lycopene degradation products which are formed in tomato paste and are present in tomato waste. Literature shows that in the production of tomato paste, about 30% of lycopene is lost due to oxidation. Tomato pulp on the other hand is produced in a very gentle conditions (This is assured by minimal agitation, low temperatures and very short exposure to air) Thus the use of tomato pulp as the material for producing the oleoresin allows for a better control over the quality of the raw material whilst providing a high lycopene content. In addition, lycopene can be extracted from the pulp using single solvent extraction whilst the other sources require a multi-solvent extraction process. Studies have shown that there is less deterioration of the lycopene during production and storage using the pulp as a starting point when compared to the other major sources.

Table 4a. Difference between alternatives of lycopene production

Parameter	Extracted Raw Material (average)			
	Tomato paste	Tomato waste (peels & seeds)	Tomato pulp	LRT pulp
Lycopene content in tomatoes used to produce the raw material	120 ppm	120 ppm	120 ppm	170 ppm
Lycopene content in the raw material to be extracted	450-550 ppm	150 ppm	1400 ppm	2300 ppm
Lycopene recovery	65-75%	80-85%	>95%	>95%
Concentration ratio to obtain oleoresin (10% lycopene)	180-220	600-700	70	43
Total lycopene recovery (based on original tomatoes used)	40-50%	60-70%	85%	85%

Table 4b. Advantages of tomato pulp

Advantages	Disadvantages
Good control over the quality of the raw material	Frozen storage required for pulp preservation
High lycopene content (1400-2300ppm)	
Low soluble solids (sugar) content	
Very low deterioration of lycopene during production and storage	
Single stage extraction	
Lycopene in the extract is stable and does not oxidize readily	
The extracted oleoresin is clear and has relatively low viscosity	

Production of the tomato lycopene oleoresin is carried out in two steps. In the first step, high lycopene content tomatoes are used to prepare tomato pulp (Figure 1). After sorting and washing, the tomatoes are crushed and screened. The resulting tomato juice is passed through a heat exchanger and centrifuged to produce the tomato pulp. Pulp with a satisfactory lycopene content is cooled, packed into laminate bags under vacuum, then into drums, and stored at -18°C until extracted. The production process introduces no exogenous substances to the tomato pulp, protects the tomato phytonutrients from oxidation and assures that the extraction is conducted on unchanged and undeteriorated raw material

In the second step, lycopene is extracted from the tomato pulp (Figure 2). The tomato pulp is crushed and extracted. Throughout the production special care is taken to protect the

lycopene from high temperatures and from prolonged contact with the air. The treatment is milder than that used in conventional tomato processing so that the lycopene is protected from isomerisation and degradation. Strict quality control accompanies the various stages of production, from cultivating the tomatoes in the field to the standardising of the oleoresin for production of various formulations. The processing is ISO-9002 certified and GMP approved. Each production batch is continuously tracked so that it can be traced back all the way to a particular lot of tomatoes in the field.

The tomato extract, or oleoresin, consists of tomato lipids. It contains high concentration of lycopene, partially dissolved and mostly dispersed in tomato oil, as well as several other important phytonutrients.

The ratio between the lipid phytonutrients that is found in the ripe tomato is preserved in the oleoresin.

The resulting oleoresin is then analysed for lycopene content as well as for solvent residues, pesticides, water content, microbiological contamination and metals (Table 2). Only those lots that meet the specifications are packaged. Lots not meeting specifications are reprocessed. Lots may be mixed to achieve the desired lycopene concentration.

Lycopene levels can be increased by partial removal of the tomato oil by physical separation. No carrier oil or additives are added.

The production process is fully controlled and allows traceability from the seeds through cultivation in the field to the finished products.

The final product, Lyc-O-Mato® Oleoresin, is available in 1, 10 and 25 kg aliquots packed under nitrogen in aluminium, HDPE, or plastic coated metal containers and stored at 4°C.

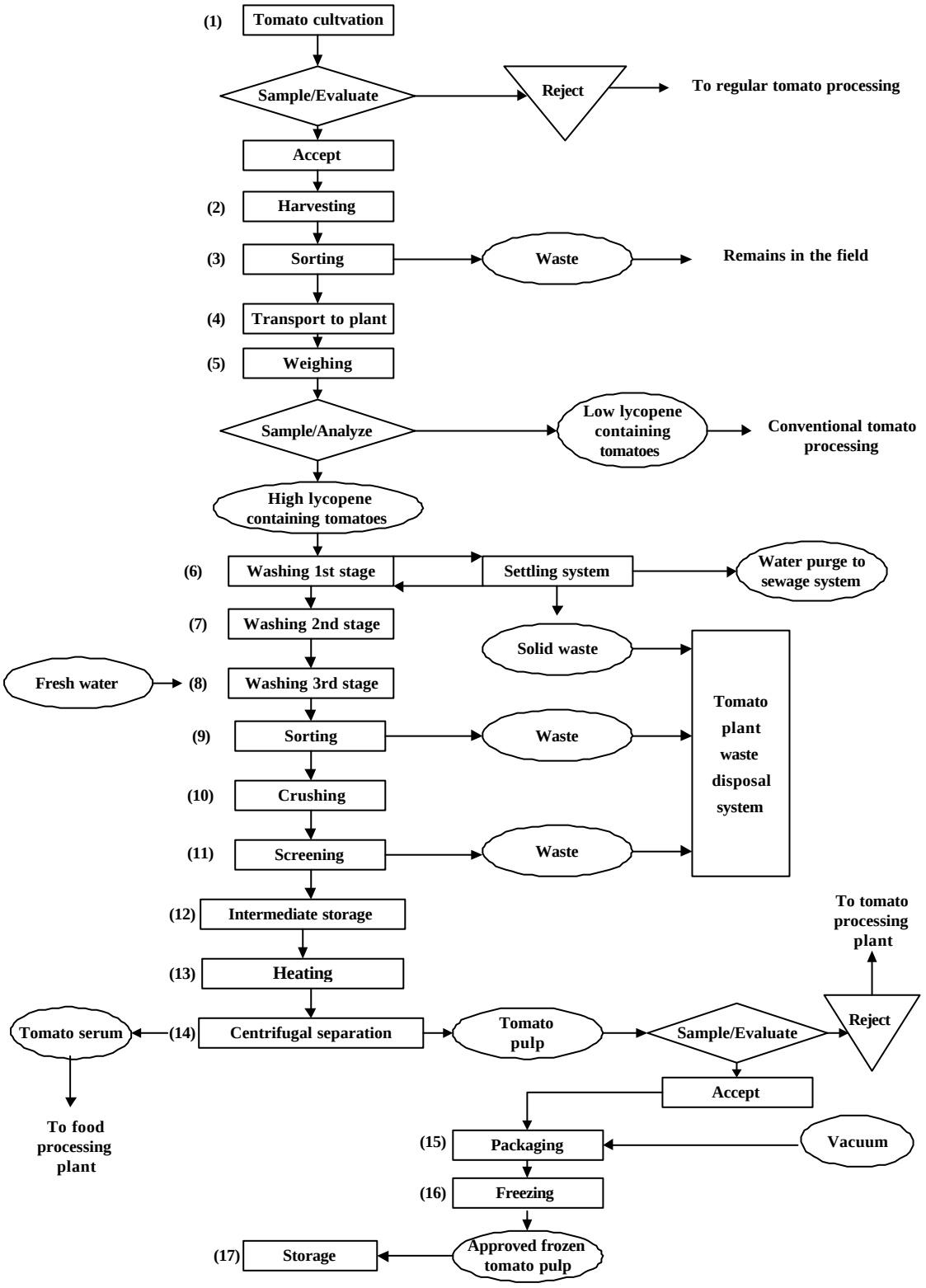


Figure 1. Pulp production scheme

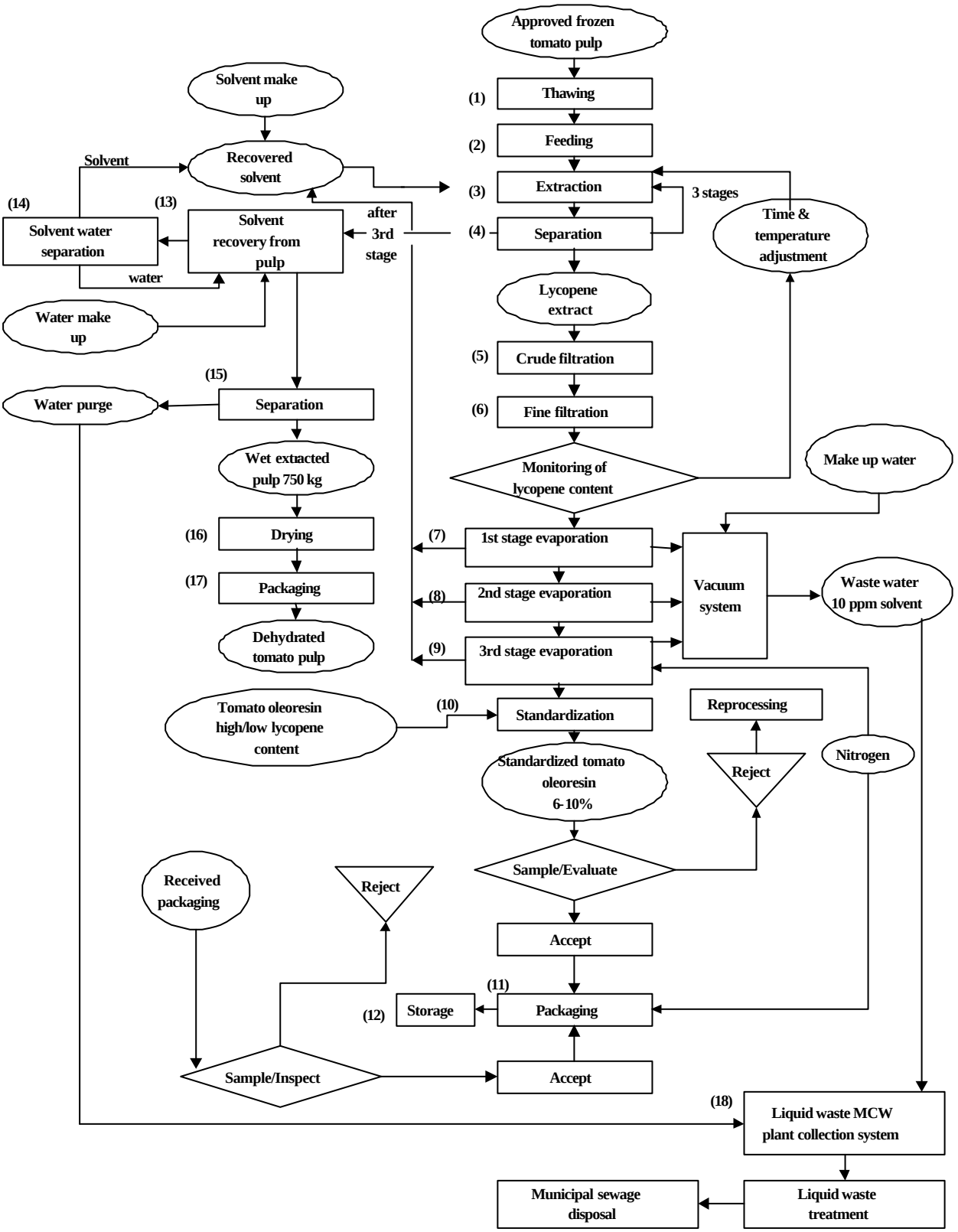


Figure 2. Lyc-O-Mato® Oleoresin 6% production scheme

5.1.3 Influence of the process on the composition of Lyc-O-Mato® (major nutrients, toxicants and pathogens)

The production process which is the extraction and standardisation of Lyc-O-Mato® oleoresin results in material which meets the chemical composition given in Table 1 and the specification in Table 2.

The controls and limits given in Table 2 for lycopene content heavy metals (Pb, As, Mo, Cd, Ni and Hg), solvent residue and microbial content are applied to every production batch.

From an evaluation of the process and controls it is concluded that the production process is not expected to result in relevant levels of toxicants or pathogens.

5.1.4 Introduction of contaminants and by-products

There are no data that indicate that the production process may lead to the introduction of relevant levels of contaminants and by-products. See also section 5.1.3.

6. (III) History of the organism used as the source of Lyc-O-Mato®

- ***Is Lyc-O-Mato® obtained from a biological source, i.e. plant, animal or micro-organism?***

Yes, lycopene is extracted from tomato hybrid cultivars. Traditional and conventional breeding methods without using transgenic modification by genetic engineering, utilising the natural gene pool of the genus *Lycopersicon* have been applied in order to create a tomato plant with a high content of lycopene.

- ***Has the organism used as the source of Lyc-O-Mato® been derived using GM?***

No, the organism used as the source of Lyc-O-Mato® has not been derived using GM.

- ***Is the source organism characterised?***

Yes, lycopene is derived from tomato hybrid cultivars. These hybrids originate from the natural gene pool of the genus *Lycopersicon*.

- ***Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?***

Yes, toxicity reports are available. Furthermore, it is generally accepted that the tomato does not present a hazard for human health through its long history of safe use.

7. (IX) Anticipated intake/extent of use of Lyc-O-Mato®

- ***Is there information on the anticipated uses of Lyc-O-Mato® based on its properties?***

Yes, LycoRed is interested in using Lyc-O-Mato® both as a food supplement and 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% and smaller amount when oleoresin with higher lycopene content is used. In case of food fortification, products will be formulated in such a way that they will provide ca. 5 mg lycopene (83 mg Lyc-O-Mato® 6% and less when lycopene content is higher) per recommended daily portion.

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. As indicated in Chapter 1 of Appendix A, the estimated daily (background) intake from natural food sources in the Netherlands is on average 1.05 mg in men, and 1.33 mg in women (Goldbohm *et al*., 1998). An exact estimate of 'natural' dietary intake is difficult as part of the lycopene comes from tomato sauce/ketchup added to, or present in many constituent food products such as pizzas. One tomato (average weight is 70 gram) may contain 3-10 mg lycopene. The average tomato consumption by users of the product in the Netherlands is 40 gram raw tomato per day (ca. 2-6 mg lycopene), 27 gram cooked tomato per day (ca. 1-4 mg lycopene), 22 gram canned tomato (concentrated) (ca. 1-3 mg lycopene) and 39 gram stewed tomato per day (ca. 2-6 mg lycopene). Total tomato intake will probably be higher as there are more dietary products that contain tomato/lycopene (soups, sauces, fruit, beverages *etc.*). Therefore, depending on the actual product use daily lycopene intake can be as high as 6-19 mg from normal daily servings. Lycopene is also used as a food colouring agent (E160d) in the EU. Taking a level of 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. Total daily lycopene intake among product users might therefore vary between ca. 1 and maximum 30 mg. In case of supplement use and/or lycopene fortified products, total intake might be as high as ca. 45 mg/day (range 6-45 mg/day). This latter estimated higher daily intake level, due to combined intake of supplements and for instance 2 fortified products, on top of a high background intake from natural sources, appears however very unlikely and an exceptional case.

- ***Is there information to show anticipated intakes for groups predicted to be at risk?***

Not applicable. There are no indications of groups at risk, however, for completeness the following questions will be answered.

- ***Will introduction of Lyc-O-Mato® be restricted geographically?***

No, geographical restriction is not an issue.

- ***Will Lyc-O-Mato® replace other foods in the diet?***

Yes, in case of fortification Lyc-O-Mato®- enriched products may replace other (non-enriched) products.

- ***Are any of the replaced foods significant nutritional sources?***

Yes. The replaced foods may be significant nutritional sources, but the Lyc-O-Mato® - enriched foods are expected to be about equivalent in the amount/sort of nutrients.

- ***Does the probable level of substitution have a nutritional significance for any population groups?***

Yes. For population groups at large, use of the fortified products will result in a higher lycopene blood level. The potential benefits of increasing lycopene intake (blood levels) is discussed in Appendix A.

8. (X) Information from previous human exposure to Lyc-O-Mato® or its source

- ***Is there information from previous direct, indirect, intended or unintended human exposure to Lyc-O-Mato® or its source which is relevant to the Community situation with respect to production, preparation, population, lifestyles and intakes?***

No, there is no information available on previous human exposure to Lyc-O-Mato®. However, tomatoes and tomato products (*i.e*. as tomato paste, sauces, *etc.*) are natural sources of lycopene. Watermelon, red palm oil, guave and red grapefruits also contain lycopene, but their contribution to the total lycopene intake is likely to be relatively small in the usual (European) diet. The estimated intakes from food sources in the Netherlands is on average 1.05 to 1.56 mg in men, and 1.33 to 1.88 mg in women (Goldbohm *et al*., 1998). Vegetables were the main source of lycopene (66%), followed by nonalcoholic beverages (25%) and soups (5%). Maximum intakes for men and women were respectively 26.1 and 18.6 mg/day. Tomato juice was the major contributor to the maximum lycopene intake in both men and women (25 and 17 mg/day).

Data from the US show higher mean intakes (Forman *et al*., 1993), a daily intake of lycopene of about 3.7 mg/day was found. Depending on the actual product use, daily intake can be as high as 15-30 mg from normal daily servings (or even higher in case of red palm oil use which may contain up to 20g lycopene per 100g).

In a British study (Scott *et al*., 1996) the daily consumption of lycopene-rich food was equivalent to a lycopene intake of about 1.1 mg/day. A lower intake was determined in the Finnish Mobile Clinic Health Examination Survey with a mean intake of 0.7 mg/day for men and 0.9 mg/day for women (Jarvinen, 1995). The Nordic Council of Ministers (Strube and Dragsted,1999) report an estimated lycopene intake for Sweden and Finland of 0.34 and 0.26 mg/day.

Besides, lycopene is allowed as a food colouring agent (E160d) in the EU. It is only allowed as an extract of red tomatoes (*Lycopersicon esculentum* L.). This product also contains minor amounts of other carotenoid pigments, as well as oils, waxes and flavour components naturally occurring in tomatoes. The contribution from this source is difficult to estimate as no information is available as to which product this food colour is added, and/or whether this added lycopene is (partly) included in the data from food tables and

intake estimates. Taking a level of 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.

The dietary lycopene intake and sources are also discussed in Appendix A.

9. (XI) Nutritional information on Lyc-O-Mato®

- ***Is there information to show that Lyc-O-Mato® is nutritionally equivalent to existing foods that it might replace in the diet?***

Yes. Lyc-O-Mato® is a tomato oleoresin (from *Lycopersicon lycopersicum* L. Karst. *Ex*. Farw) which is the lycopene enriched lipid fraction from tomato. In relation to the levels and ratios of carotenoids this lycopene containing lipid extract may be considered nutritionally equivalent to natural tomatoes and tomato products, as well as to tomato extracts, used as a food colourant. However, small differences may occur in the amount of other carotenoids and other natural components present, because of different varieties, and/or effects of processing.

Yet, it is to be noted that higher intakes are anticipated with the currently intended use in comparison with the traditional (existing) exposure.

The variety used for preparation of the Lyc-O-Mato® product (*Lycopersicon lycopersicum* L. Karst. *Ex*. Farw) has been especially selected because of its high lycopene yield. Other differences with existing (natural) products may relate to the physical appearance, *i.e*. crystal size (in Lyc-O-Mato® for 90% < 5 ìm), and to isomer composition. Further information on isomerism is described in Appendix A. In the event that Lyc-O-Mato®-enriched products will replace the equivalent (non-enriched) products, the effect on the intake of other nutrients is considered to be insignificant as the Lyc-O-Mato®- enriched foods are expected to be about equivalent in the amount and type of nutrients.

Because of the potential health benefits of lycopene, LycoRed is interested in using Lyc-O-Mato® both as a food supplement and as a food ingredient, in order to raise the lycopene intake. Although further information from scheme XI is not requested in the case of nutritional equivalency this information is still provided because the total lycopene intake will be increased which might have a nutritional impact.

- ***Is there information to show that Lyc-O-Mato® does not affect the bioavailability of nutrients from the diet or have any adverse physiological effects?***

Carotenoid interactions have been reported (van den Berg, 1999). In case of lycopene no interactions have been reported with respect to other carotenoids, or other components. There is one report of an *in vitro* study showing competitive inhibition of the â-carotene-15,15'-dioxygenase activity, the enzyme responsible for cleavage of provitamin A carotenoids into retinal, in rabbit intestinal cells (Ershov et al, 1993). However, this was not confirmed in another study with the rat intestinal enzyme (Wendt et al, 1996).

- ***Is there information to allow an assessment to be made of the nutritional impact of the introduction of Lyc-O-Mato®?***

Yes. There is information from which the effects of the introduction of Lyc-O-Mato® in the diet can be evaluated. The potential health beneficial effects are discussed in detail in Appendix A and summarised below. It should be noted that the findings from observational and experimental studies, as summarised below, are supportive, but need further extension and confirmation in larger scale, controlled intervention studies.

- *Lycopene and risk of cancer*

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. If the protective effect of tomatoes on prostate cancer risk is indeed due to lycopene and confirmed in other (intervention) trials, then the protective lycopene intake level would be at least 6.5 mg/day.

In addition to free radical scavenging, other mechanisms have been hypothesised, such as antiproliferative effects (through inhibition of insulin-like growth factor [IGF-1] or up-regulation of gap-junction communication by increasing expression of *connexin43*). Recently, a study was reported showing a marked decrease in endogeneous levels of DNA strand breaks (using the Comet assay) in lymphocytes after supplementation with tomato products (60 mg/day lycopene).

- *Lycopene and cardiovascular disease (CVD)*

Lycopene, as tomato oleoresin, has been demonstrated, both *in vitro* as *in vivo*, to inhibit LDL oxidation and have an inhibitory effect on cholesterol synthesis. These findings support the hypothesis that lycopene may reduce the risk factors for cardiovascular disease.

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.

Low levels of serum lycopene are associated with higher incidence of cardiovascular diseases.

- *Antioxidant action*

Experimental studies show that lycopene is an excellent singlet oxygen quencher and has antioxidant capacity. Lycopene has been shown to be effective in scavenging of NO radicals in lymphocytes, and increased consumption of tomatoes, a rich source of lycopene, had a positive effect on biomarkers of oxidative stress, *i.e*. DNA damage and lipid oxidation.

An increased lycopene intake might positively affect the antioxidant capacity of the organism.

- *Lycopene and UV protection of skin*

Animal studies showed that lycopene ameliorated toxicity from total body irradiation. UV exposure of the skin is associated with photosensitivity and increased free radical and singlet oxygen generation. In one study, UV exposure to the skin resulted in a reduction in skin lycopene concentration.

- *Supportive evidence from studies with tomato oleoresin*

1) Reduced susceptibility of LDL oxidation demonstrated in *in vitro* studies with mice and in (human) *in- vivo* studies (dose level: 30 mg).
2) Synergistic effect of lycopene with tocotrienol, vitamin E and camosic acid in inhibition of *in vitro* lipid peroxidation measured as TBARS formation.
3) Inhibition of cholesterol biosynthesis and augmentation of LDL receptor activity after incubation of human macrophage cell lines with lycopene (10 µM).
4) Reduction in plasma LDL content after (non-controlled) supplementation with 60 mg lycopene/day for 3 months.

10. (XII) Microbiological information on Lyc-O-Mato®

- ***Is the presence of any micro-organisms or their metabolites due to the novelty of the product/process?***

No, Lyc-O-Mato® is produced without the aid of microbiological processes. Great care is taken throughout the production process to prevent microbial contamination Therefore the production process is not expected to result in the presence of micro-organisms or their metabolites other than would be present with current uses or mentioned in the specifications.

11. (XIII) Toxicological information on Lyc-O-Mato®

- ***Is there a traditional counterpart to Lyc-O-Mato® that can be used as a baseline to facilitate the toxicological assessment?***

Yes. Lyc-O-Mato® is extracted and purified from tomato hybrid cultivars with a high lycopene content. As such, the tomato and tomato products may be considered a traditional counterpart. Besides, lycopene is abundant in nature and naturally present in many varieties of fruits and vegetables ingested by humans. Lyc-O-Mato® is also used as a starting material for the production of lycopene as a permitted food colourant (E160d).

- ***Compared to the traditional counterpart does Lyc-O-Mato® contain new toxicants or changed levels of existing toxicants?***

No. Lyc-O-Mato is a pure tomato product without any additives. There are no chemical reactions involved in its production. The specifications of Lyc-O-Mato® do not suggest the presence of new toxicants or changed levels of existing toxicants compared to the traditional counterpart as such. No information is available on the tomatin levels in Lyc-O-Mato®. On the other hand, LycoRed use only ripe tomatoes for the production of Lyc-O-Mato®. In literature, it has been found that concentration of tomatin diminishes with increasing maturity. Practically ripe tomatoes do not contain tomatin.

- ***Is there information which suggests that Lyc-O-Mato® might pose an allergenic risk to humans?***

According to LycoRed, there are no indications for an allergenic risk to humans. However, one batch of tomato oleoresin containing 5% lycopene was found to be sensitising in a sensitisation study (Maximisation test). An explanation was provided by LycoRed which indicates that the problem was due to lactic fermentation of the pulp from which the particular lot of oleoresin was extracted .The lactic acid formed was also extracted by the ethyl acetate and this have caused the sensitising potential in the sensitisation study. However, it is noted that there are no distinct data available to substantiate this explanation. Therefore, the possibility of an allergenic potential cannot be excluded on the basis of the available information. See page 39 for a more detailed discussion on this issue.

Further detailed information is given in the following sections.

11.1 Components relevant for assessment

Tomato oleoresin containing 5 or 6% lycopene (Lyc-O-Mato® 6%) has been tested for its toxicological properties. It consists of various components which are specified in Chapter 3.

11.2 Complete toxicological profile of Lyc-O-Mato® 6%

A toxicological dossier was presented. Acute toxicity, irritation, sensitisation and semichronic toxicity studies were performed, as well as a mutagenicity study (*in vitro*). Furthermore, toxicokinetic data on lycopene could be derived from open literature. The toxicity studies were performed with several batches of Lyc-O-Mato® 6% (see Appendix B, paragraph 2.2).

The individual toxicological studies have been summarised in Appendix B of this dossier. The following main conclusions were derived.

11.2.1 Animal data

11.2.1.1 Toxicokinetics

Absorption of [^{14}C]-lycopene was at least 8.7% after oral administration of 2.0 mg/kg bw to rats, on the basis of expired air, urinary and biliary excretion and body residues. Within 96 hours, 91% of the radiolabeled lycopene was recovered in faeces. After 48 hours, in two bile-duct cannulated rats only 1.7% was excreted in bile, indicating a low biliary excretion and the radioactivity in the faeces represented mostly unabsorbed material. It should be noted that this information was obtained from secondary literature and is not based on a thorough evaluation of original papers or study reports.

No indications for differences in toxicokinetics of lycopene between humans and rats were found. No information on the toxicokinetics of Lyc-O-Mato® 6% was available. For detailed information on the toxicokinetics of lycopene, see Appendix B (section 3.1).

11.2.1.2 Acute toxicity, irritation, and skin sensitisation

The results of the acute toxicity studies, irritancy and skin sensitisation studies, which are suitable for the toxicological evaluation, are presented in tables 11.1, 11.2 and 11.3.

Table 11.1 Acute toxicity studies

Test substance	LD_{50} (mg/kg bw)	Species	Route	Reference
Tomato oleoresin containing 5% lycopene, batch no. 620207	> 5000	Rat (Sprague Dawley)	Oral (gavage)	Dreher, 1994a
Tomato oleoresin containing 5% lycopene, batch no. 620106	> 5000	Rat (Sprague Dawley)	Oral (gavage)	Dreher, 1994b
Tomato oleoresin containing 5% lycopene, batch no. 620207	> 2000	Rat (Sprague Dawley)	Dermal (semi-occlusive)	Dreher, 1994c

The tomato oleoresin containing 5% lycopene exhibits low acute oral (LD50 levels > 5000 mg/kg bw) and dermal (LD50 levels > 2000 mg/kg bw) toxicity.

Table 11.2 Eye-and skin-irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
Tomato oleoresin containing 5% lycopene, batch no. 620207	Irritating to skin	Rabbit, New Zealand White	Dermal	-	Dreher 1994d
Tomato oleoresin containing 5% lycopene, batch no. 620106	Irritating to skin	Rabbit, New Zealand White	Dermal	-	Dreher 1994e
Tomato oleoresin containing 6% lycopene, lot no. 511008	Not irritating to skin	Rabbit, New Zealand White	Dermal	-	Rees, 1996a
Tomato oleoresin containing 5% lycopene, batch no. 620207	Not irritating to skin	Rabbit, New Zealand White	Dermal	-	Dreher 1994f
Tomato oleoresin containing 5% lycopene, batch no. 620106	Not irritating to skin	Rabbit, New Zealand White	Dermal	-	Dreher 1994g
Tomato oleoresin containing 6% lycopene, lot no. 511008	Not irritating to skin	Rabbit, New Zealand White	Dermal	-	Rees, 1996b

Lyc-O-Mato® 5% was found to be irritating to the skin. However, the skin irritation studies with the positive results were performed in 1994 when the fermentation problem existed. For an explanation of the fermentation problem see below at the sensitisation studies. No eye irritating properties were observed of both tomato oleoresin containing 5 and 6% lycopene.

No analytical data of batch numbers 620207 and 511008 were submitted. Therefore, the equivocal results of the skin irritation studies cannot be explained on the basis of the available documentation.

Table 11.3 Sensitisation studies

Test substance	Effect/ Classification	Species	Route	Vehicle	Reference
Tomato oleoresin containing 5% lycopene, batch no. 620207	Sensitising to skin (Maximisation)	Guinea pig, Dunkin-Hartley	Dermal	Arachis oil, petroleum jelly B.P.	Dreher, 1994h
Tomato oleoresin containing 6% lycopene, batch no. 511008	Not sensitising to skin (Maximisation)	Guinea pig, Dunkin-Hartley	Dermal	Paraffin oil, FCA	Rees, 1996c

The sensitisation studies were performed with two different types of tomato oleoresin (batch numbers 620207 and 511008). It is conceivable that dermal sensitisation in guinea pigs occurred after exposure to a product derived from a partly fermented material (batch number 620207).

LycoRed has the following clarification on the positive result of the sensitisation study with batch number 620207:

In 1994, when LycoRed started to produce tomato oleoresin, there was a problem of fermentation of the pulp prior to extraction. At that time the acidity of the tomato oleoresin was very high (above 3%) and pH values were low (below 3.5). The Institute for Food Microbiology found that the fermentation was caused by the development of lactic acid bacteria. Since then LycoRed has changed the production procedure, improved the sanitation and cleaning schedule and frequency, increased heat treatment and avoided holdups in the processing. These measures prevented the lactic fermentation. In order to follow up on this problem, two analytical parameters were introduced in the quality control schedule. Each batch of tomato oleoresin was checked for acidity (titration and expressing the results in citric acid equivalent) and pH (after diluting and stirring with water).

Following the above described changes, the acidity dropped to below 0.5% and pH increased to above 4.5. This level of acidity is due to the natural content of citric and other organic acids that are present naturally in the tomato and are extracted by ethyl acetate.

Lactic fermentation in addition to elevated acidity can cause formation of substances that have sensitising potential. This could explain the positive result of the first sensitisation study (batch number 620207, Dreher 1994) and negative results in the second sensitisation study (batch number 511008, Rees 1996) after the above described changes were introduced.

However, it is noted that there are no distinct data available to substantiate this explanation. Therefore, the possibility of a sensitising potential of Lyc-O-Mato® 5% cannot be fully excluded on the basis of the available information.

11.2.1.3 Allergenicity

To date, there is very little in the published literature about the nature of tomato allergens. Whilst by no means considered a major allergenic source, tomatoes are known to produce allergic reactions in some individuals.

Whilst IgE cross-reactive profilins have been suggested to account for the symptoms in patients suffering from tomato allergy, a recent study, (Westphal et al 2004) concludes that tomato profilin is a minor allergen in tomato fruit with biological activity as confirmed by in vitro histamine release assays with human basophils. This has the potential to account for clinical symptoms in tomato-allergic patients.

Attempts to carry out the Bradford assay for proteins on the Lyc-O-Mato® oleoresin have been unsuccessful as have attempts with other similar methods. The Bradford test is a dye-binding assay which gives a blue colour reaction with proteins that can be quantitatively determined with a spectrophotometer. The method works well with protein dissolved in a water phase but does not react when the protein is dispersed in a very dark coloured oleoresin or in an organic solvent.

The solvent used for the extraction of Lyc-O-Mato® is ethyl acetate and expert opinions are that as most natural proteins are known to undergo denaturation when in contact with organic solvents it is unlikely that structural proteins will be extracted by the solvent and present in the lypophylic ethyl acetate phase.

Although nitrogen can be determined at low levels in the oleoresin (Table 1), this cannot be taken to represent a protein component and has been shown to be contributed by other nitrogen containing substances such as phosphatidyl ethanol amine or free amino acids extracted by the ethyl acetate.

Since the oleoresin is practically free of proteins, it is doubtful whether it contains any tomato allergens. To date there have been no complaints of an allergic reaction caused by tomato oleoresin

However, in view of the fact that the oleoresin originates from tomatoes which can induce allergenic responses in some individuals, it is suggested that Lyc-O-Mato® is described in ingredients for foods and supplements as:

'tomato extract containing lycopene'.

11.2.1.4 Short term toxicity

A 13-week oral toxicity study with Lyc-O-Mato® 6% (batch number 620209) was performed in the rat. The results of the semichronic study are summarised in Table 11.4. No analytical data of batch number 620209 were submitted.

Table 11.4 Semichronic study

Duration	Species	Route	NOAEL	LOAEL	Critical effects	Reference
13 weeks	Rat, CD-strain	Oral gavage	≥ 4500	-	-	East, 1995

11.2.1.5 Chronic toxicity/carcinogenicity

No data are available.

11.2.1.6 Genotoxicity

The results of the genotoxicity study with the tomato oleoresin containing 5% lycopene is summarised in Table 11.5.

Table 11.5 Genotoxicity study

Type of study		**Result**		**Reference**
Indicator colts	Endpoint	Without	With activation	
Salmonella				
TA1535	point mutation	Negative	Negative	Thompsen, 1994
TA1537	point mutation	Negative	Negative	
TA1538	point mutation	Negative	Negative	
TA98	point mutation	Negative	Negative	
TA100	point mutation	Negative	Negative	
Eschericia coli				
WP2uvrA	point mutation	Negative	Negative	

Collins *et al*. (1998) did not find an increase in DNA damage in human lymphocytes after ingestion of 15 mg lycopene per day by 8 volunteers for 12 weeks. Riso *et al*. (1999) assumed that the consumption of tomato products may reduce the susceptibility of human lymphocyte DNA to oxidative damage. Rauscher *et al*. (1998) stated that lycopene exerts antimutagenic properties in an *in vivo* mouse bone marrow micronucleus assay.

It is concluded that there is not a full package of information concerning genotoxicity of Lyc-O-Mato® 6% to allow a full evaluation. However, there are no indications for genotoxicity of Lyc-O-Mato®.

11.2.1.7 Reproductive / developmental toxicity, and teratogenicity

No reproductive / developmental toxicity and teratogenicity studies with Lyc-O-Mato® were submitted.

Additional information on reproductive toxicity of lycopene:

One reproduction toxicity study in rats was reviewed by Strube and Dragsted (1999). It was reported that lycopene did not exhibit significant effects on fertility, pregnancy, the number of litters produced, pup growth or the incidence of overt malfunctions when male and female rats were fed with 10-20 mg lycopene/kg bw/day for a prolonged period prior to mating and throughout pregnancy.

It is concluded that there is not a full package of information concerning reproductive/developmental toxicity and teratogenicity of Lyc-O-Mato® to allow a full evaluation. However, there are no indications for reproductive/developmental toxicity and teratogenicity of Lyc-O-Mato®.

11.2.2 Human data

No adverse effects of lycopene in humans have been reported. An overview of studies conducted to investigate the possibilities of clinical and pharmacological application of lycopene can be found in Appendix A.

12. Evaluation and conclusion

Introduction

LycoRed is interested in extending the use of Lyc-O-Mato® from its current use as a food supplement to include its use as a food ingredient. 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.

This dossier contains the information needed to obtain approval for the use of Lyc-O-Mato® as a food ingredient in addition to use in food supplements in accordance with the requirements of the Novel Food Regulation (EC) no. 258/97 and Commission Recommendation 97/618/EC.

Intended intake levels

The dose range of lycopene used in dietary supplements is 5 to 15 mg. This amount is equivalent to 83-250 mg Lyc-O-Mato® 6% and proportionally less when higher concentration is used. In the 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% and proportionally less when higher concentration is used) per daily portion per product.

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 in the Netherlands is 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 a level of 5-10 ppm as a typical level used in beverages, 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 per day. This latter estimated higher daily intake level appears however very unlikely and an exceptional case.

Nutritional assessment

Experimental (*in vitro*) studies show that lycopene is an excellent singlet oxygen quencher and has antioxidant capacity. Lycopene has been shown to be effective in scavenging of NO radicals in lymphocytes, and increased consumption of tomatoes, a rich source of lycopene, had a positive effect on biomarkers of oxidative stress, *i.e.* DNA damage and lipid oxidation.

The protective, health beneficial effects of lycopene might be related to its antioxidant potential. 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.

Besides its antioxidant properties, lycopene was demonstrated (*in vitro*) to have an effect on cell-cell communication, and cell growth/differentiation.

Experimental and observational (epidemiological) studies indicate that consumption of tomato products, containing lycopene, are associated with lower cancer risk, especially in the 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.

The health beneficial effects of fruits and vegetables, especially from tomato products, might be attributed to lycopene, but 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.

Studies performed with the intended product Lyc-O-Mato® show that the lycopene contained in the tomato oleoresin is absorbed and results in a significant increase in serum lycopene level, at least comparable with that obtained with equivalent amounts of processed tomatoes (tomato puree), using intake levels of at least 5 mg. This preparation was also found to be effective in inhibition of *in vitro* LDL oxidation and *in vivo* LDL cholesterol lowering.

Nutritional risk assessment

Lycopene has a history of 'safe use', *i.e.* no adverse effects of lycopene in humans have been reported to our knowledge, nor is there any indication for groups at risk. However, with the exceptions of a 12 month study (as yet unpublished) using high levels of lycopene on prostate cancer patients, no other data are as yet available on long term exposure, *i.e.* controlled studies with higher dosages, nor from studies performed in children and pregnant/lactating women.

There is no evidence for interaction at the level of absorption or postprandial metabolism of high dose lycopene intake with other carotenoids or other fat soluble compounds. Although pro-oxidant effects cannot be excluded, these are not anticipated to occur at intake levels in the nutritional/dietary range (ca. 6 up to a maximum 45 mg/day).

No safe upper limit of intake for humans has been established by nutritional authorities.

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® and 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. Lyc-O-Mato® was found to have skin irritating properties. A skin sensitising potential was established for a batch of tomato oleoresin containing 5% lycopene. An explanation was provided by LycoRed which indicates that the problem was due to fermentation, which might have caused the irritating properties and/or sensitising potential in two irritation and one sensitisation study. However, it is noted that there are no distinct data available to substantiate this explanation. Therefore, the possibility of a skin sensitising potential cannot be excluded on the basis of the available information. Although tomatoes are known to be allergenic for a small number of individuals, a review of the process method indicates that it is doubtful whether the oleoresin contains any tomato allergens.

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® 6%. Lyc-O-Mato® 6% 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® 6% 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 4500 mg Lyc-O-Mato® 6%/kg bw (highest dose tested) is considered relevant to the intended use of Lyc-O-Mato® 6% and is proposed as the overall NOAEL. Comparison of this overall NOAEL (4500 mg Lyc-O-Mato® 6%/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 has been performed and indicates that the evidence provided for a beneficial health effect of Lyc-O-Mato® 6% at the intended dose level is supportive and 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, the possibility of a sensitising/allergenic potential cannot be fully excluded.

13. Annex I

A Novel food category and related essential information

A.1 Categories of Novel Food covered by the EU Novel Foods Regulation

Categories A-F are categories based on the EU-guideline

Classes 1-6 are based on the recommendations of the Scientific Committee for Human Nutrition

		1	**2**	**3**	**4**	**5**	**6**
A	Foods and food ingredients containing or consisting of genetically modified organisms within the meaning of Directive 90/220/EEC			x	x	x	
B	Foods and food ingredients produced from, but not containing genetically modified organisms			x	x	x	
C	Foods and food ingredients with a new or intentionally modified primary molecular structure	x					
D	Foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae	x	x				
E	Foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and which have a history of safe use	x	x				
F	Foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients, which affect their nutritional value, metabolism or level of undesirable substances						x

Class 1 Pure chemicals or simple mixtures derived from not genetically modified sources

Class 2 Complex Novel Foods derived from not genetically modified sources

Class 3 Genetically modified plants and their products

Class 4 Genetically modified animals and their products

Class 5 Genetically modified micro-organisms and their products

Class 6 Foods produced using a novel process

Class 1 through 5 have two additional subclasses on the basis of previous use:

The source has been used previously for food consumption within the EU

The source has not been used previously for food consumption within the EU

A.2 Requirements related to Novel Foods according to class/category

	Flowcharts	Novel food class									
		1.1	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**	**5.1**	**5.2**	**6**
I	Specification of the Novel Food.	x	x	x	x	x	x	x	x	x	x	x
II	Effects of the production process applied to the Novel Food.	x	x	x	x	x	x	x	x	x	x	x
III	History of the organism used as the sources of the Novel Food.	x	x	x	x	x	x	x	x	x	x	x
IV	Effect of the genetic modification on the properties of the host organism.					x	x	x	x	x	x	
V	Genetic stability of the GMO.					x	x	x	x	x	x	
VI	Specificity of expression of novel genetic material.					x	x	x	x	x	x	
VII	Transfer of genetic material from the GMO.					x	x	x	x	x	x	
VIII	Ability to survive in and colonise the human gut.									x	x	
IX	Anticipated intake/extent of use of the novel Food.	x	x	x	x	x	x	x	x	x	x	x
X	Information from previous human exposure to the Novel Food or its source.	x		x		x		x		x		x
XI	Nutritional information on the Novel Food.	x		x		x		x		x		x
XII	Microbiological information on the Novel Food.	x		x		x		x		x		x
XIII	Toxicological information on the Novel Food.	x		x		x		x		x		x

GMO Genetically modified organism

GMM Genetically modified micro-organism