Appendix B: Detailed summary and evaluation of the toxicological information for Lyc-O-Mato®

Contents

1. Introduction

Lyc-O-Mato®, a natural tomato oleoresin extracted from lycopene rich tomato hybrid cultivars, is submitted for a safety evaluation. In this appendix, a summary of toxicity studies with several batches of Lyc-O-Mato® (with varying percentage of lycopene and appearance) is presented. Furthermore, toxicological information on lycopene from public literature is presented. In Chapter 2 an overview of synonyms and batches used in the various toxicological studies is presented.

2. General substance information

2.1 Synonyms

Lyc-O-Mato® is also referred to as:

Lycopene oleoresin

Tomato extract containing lycopene

Lycopene is also referred to as ØØ-carotene.

2.2 Batches used in the studies

The batches used for analyses and stability in Chapter 3 of the safety evaluation report and the animal toxicity studies in Chapter 3 of this appendix have been summarised in Table 2.1.

Table 2.1 Batches of Lyc-O-Mato® used

Batch number	Lycopene content (%)
analyses	
630435	5
630436	5
630438	6.5
630441	5.5
630447	6.5
stability	
630442	5
510004	7
512003	7
405043	7
toxicity studies	
620106	5
620207	5
620209	5
511008	6

3. Toxicology and metabolism

3.1 Toxicokinetics

3.1.1 Absorption, distribution, excretion and metabolism

No OECD studies on the toxicokinetics of Lyc-O-Mato® or lycopene were available.

Additional studies with respect to toxicokinetics

Information on the toxicokinetics of lycopene was found in public literature and is summarised below. It should be noted that often no information is given in the literature concerning: cis/trans ratios, cis/trans positions, the presence of possible aldehydes (for example at position C25), and the presence of acids in the used test substances. Thus, it is unknown if the available toxicokinetic data as described below are represented for the toxicokinetic behaviour of lycopene in Lyc-O-Mato® 6%. It is not known whether the extent of isomerisation in rats during passage through the gastrointestinal tract is identical with that in humans.

3.1.1.1 Animal data

In an abstract (Wendt *et al*., 1996), the following animal data with regard to toxicokinetics of [^{14}C]-lycopene were reported:

Absorption

Lycopene, as other compounds of the carotenoid group, was only poorly absorbed on oral administration to rats. On the basis of expired air, urinary and biliary excretion and the percentage remained in the body, the absorption of lycopene after administration of a single dose of 2.0 mg/kg bw [^{14}C]-lycopene (in a simulated beadlet formulation) was at least 8.7% of the administered dose (Wendt *et al*., 1996).

Distribution

After administration of a single oral dose of 2.0 mg/kg bw [^{14}C]-lycopene, low absolute amounts of radioactivity (<1.0 mg eq/kg) were measured in organs/tissues, blood, and plasma of the rat at any time. The amounts of residual radioactivity were lower after prefeeding the animals for 14 days with unlabelled lycopene. Otherwise, no significant

differences in excretion pattern were observed either between males and females or between untreated and pretreated rats. Furthermore, no difference in excretion pattern were observed with a ten times lower dose of 0.2 mg/kg bw. Blood and plasma levels peaked at 2 hours following administration (Wendt *et al*., 1996).

Excretion

Within 96 hours, 91% of the administered single oral dose of 2.0 mg/kg bw [^{14}C]-lycopene was recovered in the faeces, 3.8% in the urine, 1.1% in the expired air, and 2.1% remained in the body of rats. Within 48 hours in two bile-duct cannulated male rats a mean of 1.7% was excreted into the bile indicating that biliary excretion was low and the radioactivity in the faeces represented mostly nonabsorbed material (Wendt *et al*., 1996).

From secondary literature (Clydesdale, 1999), the following animal data with regard to toxicokinetics of lycopene were derived.

Absorption

In monkeys, Mathews-Roth *et al*. (1990) showed that there was much individual variation in absorption of [^{14}C]-lycopene. Maximal absorption occurred between 8 to 48 hours after administration. The liver contained the largest amount of radioactivity 72 hours after oral intake of [^{14}C]-lycopene. Clearance of lycopene from monkey plasma appeared to be slow. Furthermore, Mathews-Roth *et al*. (1990) dosed a single amount of [^{14}C]-lycopene by gavage to rats. Maximal absorption was shown 4 to 8 hours after administration.

Distribution

In mammalian tissues, Bendich and Olson (1989) could not characterise specific binding proteins for carotenoids. In monkeys, Mathews-Roth *et al*. (1990) showed that there was wide variation among individuals concerning lycopene absorption into various organs. The liver contained the largest amount of radioactivity. Only one in five monkeys had detectable lycopene in the ovaries. Distribution was highest in liver, colon, intestine and spleen 48 hours after administration. Lycopene was distributed to various organs in rats fed with [^{14}C]-lycopene. Bladder and brain contained only trace amounts of radioactivity and the liver contained the highest amount of radiolabelled lycopene. In rat ovaries lycopene was measurable, and testes contained only trace amounts.

Metabolism

Mathews-Roth *et al*. (1990) could not detect any metabolic products of lycopene in rats or monkeys administered [^{14}C]-lycopene by gavage.

3.1.1.2 Human data

In secondary literature (Clydesdale, 1999), the following human data with regard to toxicokinetics of lycopene were presented.

Absorption

Johnson *et al*. (1997) reported that absorption of lycopene was improved (or clearance reduced) when ingested simultaneously with ß-carotene (n = 10 men; $p < 0.05$).

Absorption of lycopene was reduced with the ingestion of a "synthetic fat". Plasma concentrations of lycopene were reduced by 52% from baseline ($p = 0.0001$) when subjects were fed sucrose polyesters (SPE; 12.4 g/day) as a "synthetic fat" as part of their main vegetable-containing meal in a placebo controlled, 4-week crossover study. Plasma lycopene was reduced by 38% ($p = 0.0001$) when subjects were fed 3g SPE/day (Westrate and van het Hof, 1995).

Gartner e*t al*. (1997) found in a study with 5 subjects that the bioavailability of lycopene from tomato paste was higher than from fresh tomatoes. Ingestion of tomato paste increased concentrations of total and all-trans lycopene 2.5 times when compared with fresh tomatoes ($p < 0.05$) and the area under the curve (AUC) response was 3.8 times higher with tomato paste ($p < 0.001$). For cis-isomers, only the AUC response was significantly higher for tomato paste than for fresh tomatoes.

Stahl and Sies (1992) found that heating the tomato juice with a small amount of oil improves the absorption of lycopene from tomato juice. In contrast, intake of unheated juice did not increase serum lycopene concentrations. The increase of lycopene in the serum was maximal 24 to 48 hours after intake of heated tomato juice. Individual differences in increase (while consuming the same amount of juice) ranged from 80 - 350 nmol/l over basal concentration. The uptake of lycopene was dose dependent, but not strictly linear with dose. The absorption of cis-isomers was slightly better than alltrans isomers. The efficiency of lycopene absorption from the diet was higher at lower doses than at higher doses (Stahl and Sies, 1992).

Lycopene concentrations in plasma consistently declined from baseline in all groups except the tomato juice group in men on a controlled diet given defined daily doses of carotenoids from either broccoli, carrots or tomato juice, or ß-carotene from supplements (n = 30). The tomato juice group had a slightly positive average maximum change, which was significantly different from other groups (Micozzi *et al* (1992)).

Elinder *et al*. (1995) reported that the absorption of lycopene is affected by medication that lowers cholesterol. In hypercholesterolemic humans (n = 303) the use of cholestyramine reduced serum lycopene by 30% ($p < 0.001$). The addition of probucol to the treatment regime resulted in a combined reduction of 51%.

Distribution

In humans, lycopene is the predominant plasma carotenoid in most studies that distinguish among the carotenoids. In humans, carotenoids are transported from intestine into blood in association with lipoproteins, primarily chylomicrons (Gartner, 1997) and LDL (Forman, 1998). No specific binding proteins have been characterised in mammalian tissues (Bendich and Olson, 1989). Olmedilla *et al*. (1994) reported that there were no sex-related (n = 111 persons) or seasonal variations (n = 18 persons) in serum lycopene concentrations.

Micozzi *et al*. (1992) determined that in healthy men on self-selected diets, 43% of total plasma carotenoids comprised lycopene. Yong *et al.* (1994) found that in healthy women on self-selected diets, lycopene comprised 35% of total plasma carotenoids.

It was reviewed that there were negative correlations between serum lycopene and advancing age

(n = 29; $p < 0.05$) (Zarling, 1993) and plasma lycopene and age in non-smoking men (n = 110; $p < 0.05$) (Ascherio *et al*, 1992). Peng *et al*. (1995) reported that lycopene concentrations were inversely associated with age. In contrast, no correlations were found between serum lycopene and age by Aoki *et al*. (1987).

LeMarchand *et al*. (1994) showed that a large increase in lycopene intake (443%) resulted in only a moderate rise in plasma levels (25%). Furthermore, a positive correlation was found between serum lycopene and intake of green-yellow vegetables in men ($p < 0.05$) and women ($p < 0.001$) (n = 849, rural Japan, over 40 years of age) (Aoki *et al*., 1987).

Unlike other carotenoids, lycopene concentrations in plasma and buccal mucosal cells were stable and not affected by smoking or use of vitamin supplements in 96 subjects (Peng *et al*., 1995).

Lecomte *et al*. (1994) showed that plasma levels of lycopene were significantly lower in alcoholic men (n = 95) compared with controls (low alcohol intake; n = 118). However, after a 21-day withdrawal programme plasma lycopene increased again in the alcoholic men.

Schmitz *et al*. (1991) reported that lycopene concentrations were higher in human liver than in kidneys or lung (n = 20; range: 4 months to 86 years). Hepatic lycopene concentrations ranged from 0.2 to 20.7 nmol/g tissue. Lycopene concentrations ranged from 0.1 to 2.4 nmol/g tissue in the kidneys and < 0.1 to 4.2 nmol/g tissue in the lung.

Kaplan *et al*. (1990) reported that one analysis found that lycopene is highest in adrenals and testis, at ten times the liver concentrations. It is the predominant carotenoid in adrenals (63% of total carotenoids) and testis (80% of total carotenoids). Lycopene is distributed in minor amounts to subcutaneous fat, pancreas, kidneys, heart, thyroid, ovary and spleen (0.01 x adrenal concentration). Like Kaplan *et al*. (1990), Stahl *et al*. (1992) also found the highest levels of lycopene in adrenals and testis, but at 3.4 and 1.5 times liver concentrations, respectively. Lycopene was also present in ovary, fat and kidneys. Clinton *et al* (1996) showed that lycopene is the predominant carotenoid in prostate tissue. Lycopene concentrations in prostate tissue ranged from 0 to 2.58 nmol/g tissue (mean lycopene concentration: 0.80 ± 0.08 nmol/g tissue). Serum concentrations in men ranged from 0.60 to 1.9 nmol/ml.

In human plasma, Ashcerio *et al*. (1992) found mean concentrations of lycopene in men to be 0.82 µmol/l ±0.38 (n = 121) and in women 0.76 µmol/l ± 0.32 (n = 186).

Aoki *et al*. (1987) noted that the amount of smoking was weakly negatively associated with lycopene concentrations in men over 40 years of age from rural Japan ($p < 0.05$). Ascherio *et al*. (1992) presented that there was no association between current smoking status and plasma concentration of lycopene (n = 272).

Pamuk *et al*. (1994) showed that smoking was significantly associated with reduced circulating concentrations of lycopene after adjustment for consumption of supplements containing vitamin A. Vitamin A supplement use significantly increased serum lycopene.

Rock *et al*. (1992) observed a decrease in plasma lycopene between day 2 and 3 and day 14 and 15 in humans fed a low carotenoid diet ($n = 12$; $p < 0.0001$). Between day 63 and 64 of the study, lycopene had declined to 8% of initial values ($n = 8$). The plasma depletion half-life of lycopene was found to be between 12 and 33 days.

Stahl and Sies (1992) reported a half-life of lycopene in human serum was 2 to 3 days. In contrast, Brown *et al.*, (1989) estimated the half-life of lycopene to be approximately 14 days based on analysis of plasma lycopene concentrations in men with initial high lycopene levels after consuming a low carotenoid diet.

LeMarchand *et al*. (1994) found that plasma lycopene concentrations increased, but not statistically significantly ($p = 0.06$), in patients counselled to increase intake of fruits and vegetables to eight servings/day. Plasma lycopene correlated positively with intake of tomato products.

Leo *et al*. (1993) reported that patients with liver disease have lower total hepatic lycopene levels than controls without liver disease ($p < 0.001$). In serum, no significant differences could be detected between patients and controls. It is suggested that liver disease results in an interference with hepatic uptake, excretion and/or metabolism of carotenoids. A significant correlation (although weak) was observed between serum and liver values for lycopene.

Tanumihardio *et al*. (1990) reported that lycopene concentrations were not detectable in livers of subjects with biliary atresia or Byler's disease.

Metabolism

Stahl *et al*. (1992) reported that in human serum, cis-isomers account for more than 50% of total lycopene. Concerning human tissues, 9-cis-lycopene was present in similar amounts as all-trans-lycopene; 13-cis-lycopene contributed only 20% to the total; small amounts of 15-cis-lycopene were detected. In testes, all-trans-lycopene was present in 3 to 5-fold higher amounts than cis-isomers. Khachik *et al*. (1995/1997) characterised metabolites of lycopene (5,6-dihydroxy-5,6-dihydrolycopene and 2,6cyclolycopene-1,5-diol) in human serum/plasma. The authors concluded that these metabolites appear to result from oxidation of lycopene to an intermediate lycopene epoxide, which may undergo metabolic reduction to form the metabolite.

Cis isomers accounted for 79 to 88% of total lycopene in benign or malignant prostate tissues. All-trans isomers accounted for 12 to 21% of total lycopene. In serum, cis isomers accounted for 58 to 73% of total lycopene and all-trans isomers for 27 to 42% (Clinton *et al*., 1996).

Further studies (*in vitro* studies: more data are presented in Appendix A)

Levy *et al.* (1995) found that lycopene inhibited proliferation of human mammary and lung cancer cells at a half-maximal inhibitory concentration of 1 to 2 µM; lycopene was a more potent inhibitor of cell growth than á- or â-carotene.

Lycopene did not induce detoxifying phase II enzymes (NAD(P)H: quinone reductase) in human colon cancer cells (Wang and Higuchi, 1995).

He and Campbell (1990) observed that lycopene showed little or no inhibition (< 0.01% inhibition) of aflatoxin B1-induced mutagenesis of S. *typhimurlum* TA98 or TA100.

Bertram *et al*. (1991) observed that lycopene exhibited moderate activity to inhibit the production of transformed foci in a dose-dependent manner when continuously administered to methylcholanthrenetreated cultures 7 days after removal of the carcinogen. Lycopene was less potent than canthexanthin, â-carotene, and á-carotene. Although it demonstrated less activity in the transformation assay, lycopene caused equal or greater reduction in proliferation rate, as compared to the other carotenoids tested. The half-life of lycopene at 10^{-5} M was 354 hours.

Kim(Jun) (1990) found that lycopene inhibited lipid peroxidation ($p < 0.005$) and had more inhibitory effect than â-carotene or DL-á-tocopherol in rat liver microsomes; lycopene exhibited more efficient radical-quenching activity than lutein or á-carotene.

Serbinova *et al*. (1992) found that lycopene feeding conferred anti-oxidant protection to a liver homogenate (rat) continuously exposed to a source of peroxyl radicals. The correlation coefficient for lycopene and inhibition of lipid peroxidation was 0.497, which was intermediate between á-carotene and â-carotene.

Zhang *et al*. (1992) observed that lycopene at concentrations of 10^{-5} M resulted in large increases in *connexin43* levels in comparison with solvent-treated controls (mouse C3H/10T1/2 cells). The authors propose that carotenoids up-regulate gap junctional intercellular communication by increasing expression of *connexin43*, a gene encoding a gap junction protein.

3.1.2 Metabolic pathway

A metabolic pathway was not available. *In vivo* oxidation of lycopene to its metabolite (see suggestion under human metabolism) has not been found in animals yet (Khachik *et al*., 1995/1997).

3.1.3 Summary

A summary of the toxicokinetic data is given in the safety evaluation report, section 10.2.1.1.

3.2 Acute toxicity, including irritation and skin sensitisation

Several studies on acute toxicity, irritation, and skin sensitisation with Lyc-O-Mato® 6% are evaluated and are summarised below:

STUDY 1
Characteristics

Reference	: Dreher, 1994a	*Exposure*	: once by gavage
Type of study	: acute oral toxicity study (limit test)	**Doses**	: 5000 mg/kg bw[1]
Year of execution	: 1994	**Vehicle**	: none[2]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620207	**GLP statement**	: yes
Route	: Oral	**Guideline**	: in accordance with OECD guideline 401
Species	: rat, Sprague Dawley	**Acceptability**	: acceptable
Group size	: 5/sex	**LD_{50}**	: > 5000 mg/kg bw

[1] dose based on a range-finding study at 5000 mg/kg bw in which no adverse effects were observed.
[2] heated to 30-70 °C before use.

Study design

The study was performed in agreement with OECD guideline 401.

Results

Mortality: None.

Symptoms of toxicity: No signs of systemic toxicity were observed.

Body weight: Animals showed expected body weight gain during the study.

Pathology: No pathological changes were noted.

Acceptability

The study was considered acceptable.

Conclusions

The acute oral LD_{50} of the test substance for rats was found to be > 5000 mg/kg bw.

STUDY 2
Characteristics

Reference	: Dreher, 1994b	*Exposure*	: once by gavage
Type of study	: acute oral toxicity study (limit test)	**Doses**	: 5000 mg/kg bw[1]
Year of execution	: 1994	**Vehicle**	: none[2]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620106	**GLP statement**	: yes
Route	: Oral	**Guideline**	: in accordance with OECD guideline 401
Species	: rat, Sprague Dawley	**Acceptability**	: acceptable
Group size	: 5/sex	**LD_{50}**	: > 5000 mg/kg bw

[1] dose based on a range-finding study at 5000 mg/kg bw in which no adverse effects were observed.
[2] heated to 30-70 °C before use.

Study design

The study was performed in accordance with OECD guideline 401.

Results

Mortality: One female was found dead four hours after dosing. No other mortalities were found.

Symptoms of toxicity: In the female animal that died hunched posture, lethargy, decreased respiratory rate and laboured respiration were observed two hours after dosing. No signs of systemic toxicity were observed in the surviving animals. Incidents of brown-coloured staining of the fur were noted.

Body weight: Animals showed expected body weight gain during the study.

Pathology: Haemorrhagic lungs, dark liver and kidneys in the animal that died. Surviving animals did not reveal substance-related pathological changes.

Acceptability

This study was considered acceptable.

Conclusions

The acute oral LD_{50} of the test substance for rats was found to be > 5000 mg/kg bw.

STUDY 3

Characteristics

Reference/notifier	: Dreher, 1994c	*Exposure*	: 24 hour (semi-
Type of study	: acute dermal toxicity study (limit test)	**Doses**	: 2000 mg/kg bw
Year of execution	: 1994	**Vehicle**	: none[1]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620207	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 402
Species	: rat, Sprague Dawley	**Acceptability**	: acceptable
Group size	: 5/sex	**LD_{50}**	: > 2000 mg/kg bw

[2] heated to 30-70 °C before use.

Study design

The study was performed in accordance with OECD guideline 402.

Results

Mortality: None.

Symptoms of toxicity: No signs of systemic toxicity were noted during the study. 1 male and 1 female suffered from haemorrhage of the dermal capillaries 2 - 4 days after the treatment.

Body weight: Body weight gain was considered normal except for two females which lost weight during the first week.

Pathology: Examination did not reveal substance-related pathological changes.

Acceptability

The study was considered acceptable. It is noted, however, that staining of the fur (light brown) affected evaluation of skin responses 1 day after application.

Conclusions

The acute dermal LD_{50} of the test substance for rats was found to be > 2000 mg/kg bw.

STUDY 4
Characteristics

Reference	: Dreher, 1994d	*Exposure*	: 4 hours (semi-occlusive)
Type of study	: acute dermal irritation study	**Doses**	: 0.5 ml
Year of execution	: 1994	**Vehicle**	: none[1]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620207	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 404
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 2 females and 4 males	**Effect**	**: irritating to skin**

[2] heated to 30-70 °C before use.

Study design

The study was performed in accordance with OECD guideline 404. However, it should be noted that 6 rabbits were used instead of 3 rabbits.

Results

Table 3.1

Scores observed after	**1.0 hour**	**24 hours**	**48 hours**	**72 hours**	**7 days**
Erythema	1, 1, 2, 2, 2, 2	2, 2, 2, 2, 2.2	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	0, 0, 0, 0, 0, 0
Edema	2, 2, 2, 2, 2, 2	2, 2, 3, 2, 3, 3	2, 2, 3, 2, 3, 3	2, 2, 2, 2, 2, 2	0, 0, 0, 0, 0, 0

Loss of skin flexibility and/or elasticity was observed 72 hours after application in all test animals. Crust formation was noted 7 days after treatment in all test animals.

Acceptability

The study was considered acceptable.

Conclusions

The test substance is irritating to the skin of rabbits.

STUDY 5

Characteristics

Reference	: Dreher, 1994e	*Exposure*	: 4 hours (semi-[2]
Type of study	: acute dermal irritation study	**Doses**	: 0.5 ml
Year of execution	: 1994	**Vehicle**	: none[1]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620106	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 404
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 2 females and 4 males	**Effect**	**: irritating to skin**

[2] heated to 30-70 °C before use.

Study design

The study was performed in accordance with OECD guideline 404. However, it should be noted that 6 rabbits were used instead of 3 rabbits.

Results

Table 3.2

Scores observed after	**1.0 hour**	**24 hours**	**48 hours**	**72 hours**	**7 days**	**14 days**
Erythema	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2.2	2, 1, 2, 1, 3, 3	2, 1, 2, 1, 3, 3	0, 0, 0, 0, 0, 0	-, -, -, -, ?, -
Edema	2, 2, 2, 2, 2, 2	3, 2, 3, 2, 3, 3	2, 1, 2, 1, 3, 2	2, 1, 2, 1, 3, 2	0, 0, 0, 0, 0, 0	-, -, -, -, 0, -

Loss of skin flexibility, elasticity and thickening of the skin was observed 72 hours after application in 3 test animals. At this time point, fissuring was observed in 1 test animal and desquamation in 2 test animals. Crust formation and/or desquamation were noted 7 days after treatment in 5 test animals. Hardened light brown-coloured scrab prevented accurate evaluation of erythema and oedema in 1 test animal with crust formation 7 days after treatment, preventing accurate evaluation of erythema at the 14-day observation (see question mark in table 3.2.2).

Acceptability

This study was considered acceptable.

Conclusions

The test substance is severely irritating to the skin of rabbits.

STUDY 6

Characteristics

Reference	: Rees, 1996a	*Exposure*	: 4 hours (semi-
Type of study	: acute dermal irritation study	**Doses**	: 0.5 ml
Year of execution	: 1996	**Vehicle**	: none
Test substance	: tomato oleoresin containing 6% lycopene, lot no. 511008	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 404
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 6 females	**Effect**	: **not irritating to skin**

Study design

The study was performed in accordance with OECD guideline 404. However, it should be noted that 6 rabbits were used instead of 3 rabbits.

Results

No skin reactions were observed. Orange staining of the skin was observed in all test animals during the whole test period.

Acceptability

The study is considered acceptable.

Conclusions

The test substance is not irritating to the skin of rabbits.

STUDY 7
Characteristics

Reference	: Dreher, 1994f	*Exposure*	:single instillation in the lower conjunctival sac
Type of study	: acute eye irritation study	**Doses**	: 0.1 ml
Year of execution	: 1994	**Vehicle**	: none[2]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620207	**GLP statement**	: yes
Route	: ocular	**Guideline**	: in accordance with OECD guideline 405
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 6 females[1]	**Effect**	: **not irritating to eyes**

[1] test performed in 2 trenches (1 and thereafter 5 rabbits were committed to the study).
[2] heated to 35 °C before use.

Study design

The study was performed in accordance with OECD guideline 405.

Results

Table 3.3

Scores observed after	**1.0 hour**	**24 hours**	**48 hours**	**72 hours**	**7 days**
Cornea/opacity					
-degree	1, 1, -, -, -, -	0, 1, 1, 0, 1, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	-, 0, 0, -, 0, -
- area	2 ,4, 4, 4, 4 4	0, 2, 1, 0, 1, 1	0 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	-, 0, 0, -, 0, -
Iris	1, 1, 1, 1, 1, 1	1, 1, 1, 0, 1, 0	0, 1, 1, 0, 0, 0	0, 0, 0, 0, 0, 0	-, 0, 0, -, 0, -
Conjuctiva					
- redness	2, 2, 2, 2, 2, 2	2, 2, 2, 1, 2, 1	1, 2, 2, 0, 2, 1	0, 1, 1, 0, 1, 0	-, 0, 0, -, 0, -
- chemosis	2, 2, 2, 2, 2, 2	1, 2, 2, 1 2, 1	1, 1, 1, 0, 1, 0	0, 0, 1, 0, 1, 0	-, 0, 0, -, 0, -
-discharge	2, 3, 3, 2, 2, 2	0, 2, 2, 0, 3, 1	0, 0, 2, 0, 0, 0	0, 0, 0, 0, 0, 0	-, 0, 0, -, 0, -

Residual test material around the treated eye was observed in all test animals during the whole observation period. Staining in eyes (orange) noted in 5 test animals did not affect evaluation of ocular effects. One hour after treatment, dulling of the normal lustre of the corneal surface was observed in 4 test animals.

Acceptability

This study was considered acceptable.

Conclusions

The test substance is not irritating to the eyes of rabbits.

STUDY 8

Characteristics

Reference	: Dreher, 1994g	*Exposure*	:single instillation in the lower conjunctival sac
Type of study	: acute eye irritation study	**Doses**	: 0.1 ml
Year of execution	: 1994	**Vehicle**	: none[2]
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620106	**GLP statement**	: yes
Route	: ocular	**Guideline**	: in accordance with OECD guideline 405
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 5 females and 1 male[1]	**Effect**	: **not irritating to eyes**

[1] test performed in 2 trenches (1 and thereafter 5 rabbits were committed to the study).
[2] heated to 35 °C before use.

Study design

The study was performed in accordance with OECD guideline 405.

Results

Table 3.4

Scores observed after	1.0 hour	24 hours	48 hours	72 hours	7 days	14 days
Cornea						
-degree of opacity	1, -, 1, 1, -, 1	1, 1, 2, 2, 1, 1	0, 1, 2, 2, 1, 1	0, 1, 2, 2, 1, 1	0, 0, 1, 0, 0, 0	-, -, 0, 0, -, -
-area of opacity	3, 4, 4, 4, 4, 3	1, 3, 4, 4, 2, 3	0, 3, 4, 4, 2, 2	0, 1, 3, 3, 1, 1	0, 0, 1, 0, 0, 0	-, -, 0, 0, -, -
Iris	1, 1, 1, 1, 1, 1	1, 1, 1, 1, 1, 1	0, 1, 1, 1, 1, 1	0, 1, 1, 1, 1, 1	0, 0, 0, 0, 0, 0	-, -, 0, 0, -, -
Conjuctivae						
- redness	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	1, 2, 2, 2, 2, 2	0, 0, 0, 0, 0, 0	-, -, 0, 0, -, -
- chemosis	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	1, 2, 2, 2, 2, 2	0, 1, 1, 1, 2, 1	0, 0, 0, 0, 0, 0	-, -, 0, 0, -, -
-discharge	2, 3, 3, 3, 2, 3	2, 3, 3, 2, 3, 3	0, 2, 2, 0, 3, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	-, -, 0, 0, -, -

Residual test material around the treated eye was observed in all test animals during the whole observation period (except for 1 animal). Staining in eyes (orange) was noted in all test animals 1 hour after treatment. It did not affect evaluation of ocular effects. One hour after treatment dulling of the normal lustre of the corneal surface was observed in 2 test animals. Sloughing of the cornea was observed in 2 test animals during the 24-hour

observation. 7 days after treatment, slight vascularisation along the lower edge of the cornea was observed in 2 test animals.

Acceptability

This study was considered acceptable.

Conclusions

The test substance is not irritating to the eyes of rabbits.

STUDY 9

Characteristics

Reference	: Rees, 1996b	*Exposure*	:single instillation in the lower conjunctival sac
Type of study	: acute eye irritation study	**Doses**	: 0.1 ml
Year of execution	: 1996	**Vehicle**	: none
Test substance	: tomato oleoresin containing 6% lycopene, lot no. 511008	**GLP statement**	: yes
Route	: ocular	**Guideline**	: in accordance with OECD guideline 405
Species	: rabbit, New-Zealand White	**Acceptability**	: acceptable
Group size	: 6 females[1]	**Effect**	: **not irritating to eyes**

[1] test performed in 2 trenches (1 and thereafter 5 rabbits were committed to the study).

Study design

The study was performed in accordance with OECD guideline 405.

Results

Table 3.5

Scores observed after	**1.0 hour**	**24 hours**	**48 hours**	**72 hours**	**8 days**
Cornea					
-degree of opacity	1, 0, 0, 0, 0, 0	0, 1, 0, 0, 0, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
-area of opacity	4, 0, 0, 0, 0, 0	0, 1, 0, 0, 0, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Iris	1, 1, 1, 1, 1, 1	1, 0, 0, 0, 0, 1	1, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjuctivae					
-redness	2, 2, 1, 1, 1, 2	2, 1, 1, 1, 1, 2	2, 1, 1, 1, 1, 1	1, 1, 0, 0, 1, 1	0, 0, 0, 0, 1, 1
-chemosis	0, 1, 0, 0, 0, 1	1, 0, 0, 1, 0, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
-discharge	2, 1, 0, 0, 1, 1	2, 0, 0, 1, 0, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

[1] No ulceration or stippling observed
[2] No necrosis or ulceration observed
[3] In 2 test animals redness was still observed after 15 and/or 22 days post-treatment

Acceptability

This study was considered acceptable.

Conclusions

The test substance is not irritating to the eyes of rabbits.

STUDY 10
Characteristics

Reference	: Dreher, 1994h	*Exposure*	: intradermal and topical induction and topical challenge (occlusive 24h)
Type of study	: skin sensitisation study	**Doses**	: induction: 5% w/v intradermal, 25% w/w topical challenge: 2% and 5% w/w topical
Year of execution	: 1994	**Vehicle**	: arachis oil (intradermal induction) and petroleum jelly B.P. (topical induction and challenge)
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620207	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 406
Species	: guinea pig, Dunkin-Hartley	**Acceptability**	: acceptable
Group size	: 20 females in test group, 10 females in control group	**Effect**	: **sensitising to skin**

Study design

The study was performed in accordance with OECD guideline 406.

Results

24 hours after topical induction, erythema (score 1 (4/30), score 2 (2/30)) and oedema (score 1 (13/30), score 2 (2/30)) were observed. Due to staining of the induction site in almost all test animals, which prevented accurate evaluation, underestimation of the incidence of erythema is possible. Other skin reactions were bleeding and small superficial scabs. After the topical challenge period (24-hours observation) erythema of grade 1 (6/20), grade 2 (1/20) and oedema of grade 1 (1/20) were seen in response to the

test substance concentration of 5% w/w in petroleum jelly B.P. At the 48-hours observation, one test animal suffered erythema (grade 2). Upon challenge with 2% w/w of the test substance in petroleum jelly B.P, erythema of grade 1 (6/20) was observed in test animals (24-hours observation). No skin reactions or oedema were noted at the 48-hours observation. No skin reactions were observed in the control animals.

Acceptability

This study was considered acceptable. No data on individual skin reactions of the intradermal induction of the main study are present. Furthermore, the chosen topical induction concentration of 25% was not the highest to cause mild-to-moderate skin irritation in the sighting test.

Conclusions

The test substance is sensitising to the skin of guinea pigs.

STUDY 11
Characteristics

Reference	: Rees, 1996c	*Exposure*	: intradermal and topical induction and topical challenge (occlusive, 48h)
Type of study	: skin sensitisation study (maximization)	**Doses**	: induction: 30% v/v intradermal, 100% v/v topical. challenge: 5% and 30% v/v topical
Year of execution	: 1996	**Vehicle**	: paraffin oil and FCA (intradermal induction), none (topical induction), paraffin oil (topical challenge)
Test substance	: tomato oleoresin containing 6% lycopene, lot no. 511008	**GLP statement**	: yes
Route	: dermal	**Guideline**	: in accordance with OECD guideline 406
Species	: guinea pig, Dunkin-Hartley	**Acceptability**	: acceptable
Group size	: 10/sex in test group, 5/sex in control group	**Effect**	: **not sensitising to skin**

Study design

The study was performed in accordance with OECD guideline 406.

Results

After intradermal induction, erythema was observed in both control and test group (score 2 (10/10 and 20/20, respectively)) exposed to Freunds Complete Adjuvant (FCA). In the control and test group exposed to vehicle or test material in FCA, respectively, erythema was observed (score 1 (3/10, 19/20, respectively; score 2 (7/10, 1/20, respectively). Discolouration was observed in animals exposed to test material in FCA (9/10). Orange staining was observed in all test animals. After topical induction, exfoliation was observed in controls (3/10) and test animals receiving test material in vehicle (20/20). Furthermore, test animals exposed to test material in vehicle, showed eschar formation (8/20) and yellow/orange/red staining (19/20). 24 to 48 hours after challenge, (slight) yellow/orange/red staining was observed in all control animals and test animals exposed to 30% v/v test material in vehicle. Erythema was observed in controls (score 1(1/10)) and test animals (score 1 (4/20)). Exfoliation was observed in 1 test animal.

In control and test animals exposed to 5% v/v test material in vehicle, no erythema or oedema was observed. Slight yellow/orange/red staining was observed in both controls (4/10) and test animals (8/20).

Acceptability

This study was considered acceptable, although the undiluted test substance could have been examined in the sighting test. Furthermore, to the reviewer it is not clear why two different vehicles are used and a justification for the chosen vehicles is lacking.

Conclusions

The test substance is not sensitising to the skin of guinea pigs.

3.2.1 Summary

The results of the acute toxicity, irritancy and sensitisation studies are presented in table 3.6, 3.7, and 3.8, respectively.

Table 3.6 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

Table 3.7 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 5% lycopene, batch no. 620207.	Not irritating to skin	Rabbit, New Zealand White	Dermal	-	Rees, 1996a
Tomato oleoresin containing 5% lycopene, batch no. 620207	Not irritating to eyes	Rabbit, New Zealand White	Ocular	-	Dreher, 1994f
Tomato oleoresin containing 5% lycopene, batch no. 620106	Not irritating to eyes	Rabbit, New Zealand White	Ocular	-	Dreher, 1994g
Tomato oleoresin containing 6% lycopene, batch no. 511008	Not irritating to eyes	Rabbit, New Zealand White	Ocular	-	Rees, 1996b

Table 3.8 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 (see table 3.7) 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 postive 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, possible sensitising potential cannot be ruled out with a sufficient degree of certainty on the basis of the available information.

3.3 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. 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’.

3.4 Short-term toxicity

A 13-week oral toxicity study with a tomato oleoresin containing 5% lycopene was submitted by LycoRed and is summarised below:

STUDY 1

Characteristics

Reference/notifier	: East, 1995	*Exposure*	:13 weeks, by gavage
Type of study	: 13-week oral toxicity study	**Doses**	: 0, 45, 450 and 4500 mg/kg bw
Year of execution	: 1994	**Vehicle**	: corn oil (peroxide free)
Test substance	: tomato oleoresin containing 5% lycopene, batch no. 620209	**GLP statement**	: yes
Route	: oral	**Guideline**	: in accordance with OECD guideline 408
Species	: rat, CD-strain	**Acceptability**	: acceptable
Group size	: 20/sex/dose	**NOAEL**	: $\geq$ 4500 mg/kg bw/day

Study design

The study was performed in accordance with OECD guideline 408.

Results

Absorption analysis, after 13 weeks of treatment, showed that lycopene was absorbed and that plasma levels of lycopene were similar in males given 450 and 4500 mg/kg bw/d of tomato oleoresin (containing 5% lycopene). The remaining results are summarised in Table 3.9.

Table 3.9

Dose (mg/kg bw/day)	**0**		**45**		**450**		**4500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortalities								
Clinical signs - Orange/red stained faeces - Brown staining of tail - Red staining of tail - Salivation - Slow, irregular and gasping respiration			 +		 ++	 +	 ++ ++ ++ +[1] +[1]	 ++ ++	
Body weight	No treatment related findings								
Food consumption	No treatment related findings								
Ophthalmoscopy	No treatment related findings								
Haematology	No treatment related findings								
Clinical chemistry[1] - ALP - ALAT - Sodium - Urea			 dc[2]		 dc[2]		 dc[2] ic[2] dc[2]	 Ic[3]	
Urinalysis - Urine volume							 ic		
Organ wights - Lungs						 ic		 ic	
Pathology									
Macroscopy	No treatment related findings								
Microscopy	No treatment related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
m/f male/female
\+ present in one/a few animals
++ present in most/all animals present
[1] signs observed at day 1 only
[2] signs observed after six weeks of treatment
[3] signs observed at the end of the treatment period

Acceptability

This study was considered acceptable.

Conclusions

Staining of the faeces was considered treatment-related and was attributed to the presence of unabsorbed test material (opaque brown semi-solid) in the faeces. Furthermore, staining of the tail was associated with the accidental transfer of test material during dosing. Changes in organ weight (lungs) found in female rats receiving 450 and 4500 mg/kg bw/day of tomato oleoresin (containing 5% lycopene) were only absolute and not associated with histopathological changes. Besides, the effects (group mean values) found for clinical chemistry were not dose-related.

Taking the above into account, the No-Observed-Adverse-Effect Level (NOAEL) was considered to be the highest dose level tested, 24500 mg/kg bw/day (*i.e.* under the conditions of this study).

3.4.1 Summary

The results of the semichronic toxicity study is presented in table 3.10.

Table 3.10 Semichronic toxicity study

Duration	Species	NOAEL (mg/kg	LOAEL (mg/kg	Critical effects	Reference
13 weeks	rat, CD strain	$\geq$ 4500	-	-	East, 1995

3.5 Genotoxicity

3.5.1 *In vitro*

STUDY 1

Study design and results

Type of study: Reverse mutation assay in *Salmonella typhimurium* and *Escherichia coli,* with and without metabolic activation

Indicator cells	Endpoint	Res. - act.	Res. + act.	Activation		Dose range	Reference
				Tissue	inducer		
B*: S.typh.*				liver (rat)	Aroclor 1254	Plate incorporation. Tested concentrations exp. 1: 0, 8, 40, 200, 1000 and 5000 µg/plate in solvent distilled water. Tested concentrations exp. 2: 0, 312.5, 625, 1250, 2500, 5000 µg/plate solvent distilled water. No cytotoxicity occurred[1]	Thompsen, 1994
TA1535	Point mutations	-	-				
TA1537	Point mutations	-	-				
TA1538	Point mutations	-	-				
TA98	Point mutations	-	-				
TA100	Point mutations	-	-				
B*: E. coli*							
WP2uvrA-	Point mutations	-	-				
[1] No cytotoxicity was observed up to 5000 µg/plate. Precipitation was observed ≥ 5000µg/plate in both experiments Test substance: tomato oleoresin containing 5% lycopene, batch no. 620207 GLP statement yes According to OECD 471: yes							

Acceptability

This study was considered acceptable.

Conclusion

The test substance was found to be non-mutagenic *in vitro* both with and without metabolic activation under the conditions of the test.

Additional information on in vitro genotoxicity of lycopene:

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.

3.5.2 *In vivo*

No *in vivo* genotoxicity studies with Lyc-O-Mato® 6% are available.

Additional information on in vivo genotoxicity of lycopene:

Rauscher *et al*. (1998) stated that lycopene exerts antimutagenic properties in an *in vivo* mouse bone marrow micronucleus assay.

3.5.3 Summary

For a summary of the genotoxicity data, see the safety evaluation report, section 10.2.1.6.

3.6 Long-term toxicity and carcinogenicity

No chronic and carcinogenicity studies are available with Lyc-O-Mato® or lycopene.

3.7 Reproductive toxicity

3.7.1 Reproductive toxicity

No reproductive toxicity studies are available with Lyc-O-Mato®.

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.

3.7.2 Teratogenicity studies

No teratogenicity studies are available with Lyc-O-Mato® or lycopene.

3.7.3 Summary

For a summary of the reproductive toxicity, see the safety evaluation report, section 10.2.1.7.

3.8 Further toxicological studies

3.8.1 Toxicity studies on metabolites

No data of toxicity studies on metabolites are available.

3.8.2 Supplementary studies

No supplementary studies are available.

3.8.3 Medical data and information

No data concerning clinical cases and poisoning incidents are available.

4. Human Studies evaluating the Safety of Lycopene and Lycopene oleoresin

There have been a number of human clinical studies in which one of the reported parameters was the safety of either lycopene from tomato sources (*e.g*. tomato juice, pasta sauces *etc*.) or lycopene oleoresin from tomatoes.

4.1 Lycopene from Tomato Products

Dietary intervention studies with either tomato juice or tomato sauce have been carried out on human subjects of both sexes and covering a wide age range (21-86 years) These are summarised in Table 4.2. The highest lycopene intakes reported was by Watzl *et al.*, (2000), with a calculated intake of 47.1mg lycopene/day from tomato juice over a period of 8 weeks, with 53 elderly subjects in the age range 63-86 years. No adverse effects were reported and there were no changes in subject body weight during the study.

A study of 32 prostate cancer patients in the age range 60-74 years and receiving 30mg lycopene from tomato sauce based pasta products over a period of three weeks reported three reversible and minor gastrointestinal problems in three patients. However, these reported mild adverse effects are more probably related to the dietary change rather than the lycopene.

4.2 Lycopene from Lycopene Oleoresin

A number of human studies have been carried out using lycopene oleoresin supplementation either on its own or in combination with other carotenoids (â-carotene and lutein). The duration of these studies ranged from 26 days to 12 months. Levels of lycopene were from 15 to 75mg introduced via capsules.

The age range of subjects was from 18 to over 62 years, and whilst three studies were only on male subjects, Olmedilla *et al*., (2002) reported on a study of 175 male and 174

female subjects in the age range 25-45 years. This study, which included carotene-rich palm oil (15mg/day), lutein (15mgday) and lycopene oleoresin (15mg/day) reported that carotenodermia was observed in 25% of the subjects supplemented with lycopene compared with 95% of those supplemented with carotene and 40% supplemented with lutein.

A recent and as yet unpublished study (Hall *et al*., 2004), carried out at the Wake Forest School of Medicine, Winston-Salem, North Carolina, USA, treated 36 prostate cancer patients.

The study, which ran for twelve months, was a prospective dose-escalation trial which was conducted in 36 men with biochemical recurrence of prostate cancer after prostatectomy (n=15) or radiation therapy (n=21). Patients were treated with lycopene (Lyc-O-Mato® tomato extract oleoresin) at one of six dose levels between 15 and 120 mg/day for a planned 12 months. Primary study endpoints were PSA response, changes in serum lycopene level, and impact on intermediate biomarkers of DNA damage, antioxidant status, and immune responsiveness before and after treatment. Tolerability and potential toxicity were also monitored.

The results showed that lycopene was extremely well-tolerated; one patient was taken off the study due to diarrhoea. Serum lycopene levels did not differ significantly between patients on different doses (p=0.82). Lycopene serum levels reached a plateau between 6-9 months at levels approximately 60% above baseline.

The conclusions of the study were that lycopene administered over a wide-dose range for one year was well-tolerated but did not result in significant PSA response in men with PSA-only failure following definitive local treatment for prostate cancer. Attainable serum lycopene levels were not significantly different among patients over the dose range of 15 to 120mg/day. This is consistent with other studies which show that lycopene uptake does not increase significantly at doses above 15mg/day

Table 4.1 Lycopene Supplementation via Capsules (15-75mg Lycopene/day)

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Fuhrman *et al*., 1997		3 months	6 healthy males	30 to 35 years	60mg lycopene/day as lycopene oleoresin from tomatoes		14% reduction in plasma LDL cholesterol concentrations. No adverse effects were reported.
Agarwal and Rao, 1998	Randomised, cross-over dietary intervention study 1	8 weeks (1 week/ treatment separated by 1 week wash-out periods)	19 healthy subjects (10 male and 9 female)	25 to 40 years	39.2mg lycopene/day from spaghetti sauce	Plasma lipids (total, HDL- and LDL-cholesterol levels, triglyceride levels). Anthropometric measurements and verbal communication regarding tolerance of supplemented doses assumed.	No effects on plasma lipid levels. All subjects maintained their body weights and no adverse symptoms were reported throughout the duration of the study.
					50.4mg lycopene/day from tomato juice		
					75.0mg lycopene/day from tomato oleoresin capsules		
					0mg lycopene/day (placebo)		
Wright *et al*., 1999	Double-blind, placebo-controlled supplementation study.	26 days	23 healthy male volunteers	18 to 60 years	15mg lycopene/day (lycopene-rich tomato extract in capsule form) *versus* placebo (corn oil)	Plasma fatty acid profile.	Compared with placebo, lycopene treatment significantly decreased plasma linoleic acid and significantly decreased the polyunsaturated:saturated fatty acid ratio.
Hininger *et al*., 2001	Placebo-controlled single blind study	3 months	175 healthy male volunteers	25 to 45 years	β-carotene (15mg/day via capsules)	Verbal communication regarding tolerance of supplemented doses assumed.	No adverse biological effects were reported.
					Lutein (15mg/day via capsules)		
					Lycopene (15mg/day via capsules)		
					Placebo (15mg/day via capsules)		

Table 4.1 Lycopene Supplementation via Capsules (15-75mg Lycopene/day) continued

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Mohanty *et al*., 2001		1 year	50 infertile male patients		8mg lycopene/day as lycopene oleoresin from tomatoes		Improvement of sperm count and functional sperm concentration followed by improvement in sperm motility percentage and sperm motility index. No adverse effects were observed.
Corridan *et al*., 2001		12 weeks	17 healthy volunteers	65 to 83 years	13.3mg lycopene/day from tomato oleoresin capsules		Supplementation with these relatively low levels of β-carotene or lycopene was not associated with either beneficial or detrimental effects on several aspects of cell-mediated immunity.
Kucuk *et al*., 2001	Randomised clinical trial	3 weeks	26 patients with newly diagnosed prostate cancer	62 years (mean)	15mg lycopene twice daily (15 patients) or no supplementation (11 patients)	Adverse event report, complete physical examination, complete blood count and chemistry profile	No adverse effects were reported, no abnormalities were observed in blood counts or chemistries.

Table 4.1 Lycopene Supplementation via Capsules (15-75mg Lycopene/day) continued

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Olmedilla *et al*., 2002	Placebo-controlled supplementation study	16 weeks	175 male and 174 female volunteers (French, Irish, Danish and Spanish cohorts)	25 to 45 years	Carotene-rich palm oil (15mg/day via capsules) with and without vitamin E	Plasma lipids (total, HDL- and LDL-cholesterol levels), biochemical and haematological profiles, adverse event report (Spanish cohort only; 32 male and 32 female).	No significant changes in plasma lipid levels, general biochemical or haematological profiles. Carotenodermia was reported in 25% of the subjects supplemented with lycopene (compared with 95% of those supplemented with carotene and 40% of those supplemented with lutein).
					Lutein (15mg/day via capsules) with and without vitamin E		
					Lycopene (15mg/day via capsules) with and without vitamin E		
					Placebo (15mg/day via capsules) with and without vitamin E		

Table 4.1 Lycopene Supplementation via Capsules (15-75mg Lycopene/day) continued

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Ansari and Gupta, 2003	Randomised clinical trial	24-28 months	54 male patients with histologically confirmed metastatic prostatic cancer		Orchidectomy plus 4mg lycopene/day (27 patients – OL group) or orchidectomy alone (27 patients - control)		All the patients in the OL group tolerated the drug well and there were no adverse reactions. During the study period 44% of the control group and 26% of the OL group died.
Heinrich *et al*., 2003		12 weeks	12 healthy adults	22 to 55 years	24mg mixed carotenoids/day, comprised of a combination of 8mg β-carotene, 8mg lycopene (4% tomato oleoresin) and 16mg lutein esters, stoichiometrically equivalent to 8mg lutein.		Reduction in skin erythema after exposure to UV light. No adverse effects were reported.
Aust *et al*., 2004		12 weeks	12 healthy adult volunteers		10mg lycopene/day as lycopene oleoresin from tomatoes (enhanced with phytoene and phytofluene)		38% and 48%, respectively, reduction in skin erythema after exposure to UV light. No adverse effects were reported.
Hall *et al*., 2004	Prospective dose-escalation	1 year	36 patients		Lycopene oleoresin (Lyc-O-Mato®) 6 dose levels between 15 and 120mg/day	Serum lycopene levels impact on intermediate biomarkers of DNA. Adverse event reporting.	Lycopene was extremely well tolerated. One patient taken off study due to diarrhoea. No toxicity deduced from findings.

Table 4.2 Lycopene Supplementation via Food Sources (5-47mg Lycopene/day)

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Micozzi *et al*., 1992	Placebo-controlled supplementation study	6 weeks	30 healthy males (6 groups of 5)	20 to 45 years	30mg carotenoids/day from â-carotene capsules or 272g carrots 12mg â-carotene/day from â-carotene capsules or 12mg lycopene/day from 180g tomato juice 6mg carotenoids/day from 300g broccoli Placebo (capsules)	Verbal communication regarding tolerance of supplemented doses assumed.	No gastric discomfort was reported.
Bohm and Bitsch, 1998	Randomised supplementation study	6 weeks	22 female volunteers (3 groups of 6 to 8)	Mean 21 years	5mg lycopene/day from tomato oleoresin soft gel capsules 5mg lycopene/day from tomato juice 5mg lycopene/day from raw tomatoes	Plasma lipids (total and HDL-cholesterol levels, triglyceride levels).	No effects on plasma lipid levels.
Watzl *et al*., 2000	Placebo-controlled dietary intervention study	8 weeks	Healthy elderly subjects (33 female and 20 male)	63 to 86 years	47.1mg lycopene/day from tomato juice Mineral water	Anthropometric measurements. Immunomodulatory activity (concanavalin-stimulated lymphocyte proliferation, cytokine secretion, number and lytic activity of NK cells, assessment of delayed-type hypersensitivity)	No changes in body weights throughout the study. No differences in immune function between treatment groups.

Table 4.2 Lycopene Supplementation via Food Sources (5-47mg Lycopene/day) continued

Authors	Study Type	Duration	Subjects	Age Range of Subjects	Treatment	Safety Related End Points	Findings
Chen *et al*., 2001	Non-randomised, whole food intervention arm of a clinical trial	3 weeks	32 prostate cancer patients	60 to 74 years	Approximately 30mg lycopene/day from tomato sauce-based pasta dishes	Record of gastrointestinal adverse effects (checklist including constipation, burping, gas and/or flatulence, nausea, bloating, diarrhoea, cramping and heartburn).	Intervention was well accepted by patients. 3 patients reported minor gastrointestinal problems (not specified), which resolved within a few days.
Stahl *et al*., 2001		10 weeks	9 healthy adults	26 to 67 years	16mg lycopene/day from 40mg tomato paste		A 40% reduction in skin erythema after exposure to UV light was found. No adverse effects were reported.

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