

**ADDITIONAL LYC-O-MATO[®]
INFORMATION**

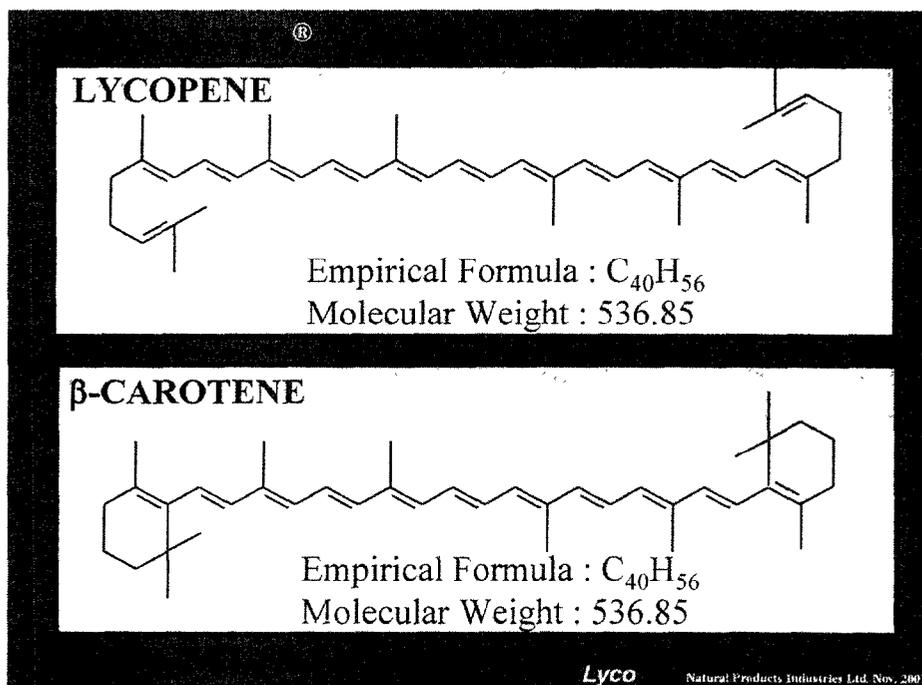
Industrial Production, Properties and Uses of Tomato Lycopene

By Dov Hartal Ph.D. and Lorri Danzig

Introduction

A large body of scientific evidence associates diets rich in fruit and vegetables with good health and long life. Scientists believe that this association is due to the beneficial effect of certain phytochemicals and are conducting extensive research in order to trace and evaluate these health-preserving substances.

The carotenoids are a family of compounds that received special attention in the framework of these investigations. More than 600 carotenoids were found in fruits, vegetables and in green plants and therefore they are part of the human diet. About 20 carotenoids are found in significant amounts in human plasma and tissues. Lycopene, the red pigment that gives the ripe tomato its bright red color, is one of them. Recently, lycopene received growing attention in the scientific community because of its unique chemical structure that is responsible for its being described as the "most efficient biological carotenoid singlet oxygen quencher"¹. All carotenoids possess a certain common chemical structure that consists of a long chain of conjugated double bonds. In the case of lycopene, this chain is longer and consists of more double bonds than in any other carotenoid.



This configuration is responsible for the potent free radical scavenging ability of lycopene and for the important part it plays in the human organism defense system that protects us from degenerative diseases.

Although lycopene was first isolated by Hartsen in 1873², only recent studies have shown its importance to human health and well being.

Lycopene and prevention of disease

Epidemiological studies combined with recent clinical trials have pushed lycopene-rich tomatoes and tomato products from the culinary to the healthcare arena. Physicians are increasingly exploring the benefits of tomato phytonutrients as both a preventative and adjunctive therapeutic agent, complementing established treatment protocol. Dr. Edward Giovannucci of the Department of Medicine of the Brigham and Women's Hospital and Harvard Medical School reviewed 72 epidemiological studies looking at the role of tomatoes,

Dr. Giovannucci's review found that 57 of the 72 studies showed an inverse relationship between blood lycopene level (or tomato consumption) and cancer risk at a defined anatomical site. The evidence of the benefit of tomatoes was strongest for cancers of the prostate, lung and stomach. The epidemiological work does not support any definitive conclusions regarding any one particular tomato phytonutrient acting in isolation, rather, the studies, evaluating the role of tomatoes as a component of the diet, pointed to the complex of tomato phytonutrients acting together as the key player in reducing the risk of cancer.³

Though much of the epidemiological work on lycopene has looked at its effect on cancer, there is growing evidence that lycopene is protective in several chronic diseases including cardiovascular disease⁴ and age related macular degeneration⁵, as well as in the protection of the skin from erythema due to UV radiation.⁶

Scientists researching the effects of tomato lycopene are increasingly convinced that it is the synergism between lycopene and other tomato phytonutrients that enhances lycopene's ability to curb degenerative diseases. This synergistic effect of the natural composition of tomato phytonutrients was demonstrated in several studies including clinical research by Dr. Omer Kucuk with prostate cancer patients⁷; a blind, placebo-controlled study by Esther Paran, MD, evaluating the effect of Lyc-O-Mato[®], a standardized natural lycopene complex on 35 mildly hypertensive patients⁸, Dr. Aviram's research on the effects of tomato extract on LDL cholesterol^{9,10}; and in vitro work by Drs. Sharoni and Levi, demonstrating the effective synergy of tomato phytonutrients in reducing proliferation of prostate and breast cancer cells¹¹. This last work, quite effectively demonstrates that lycopene is not therapeutically effective when present at the relatively low levels normally found in the blood even after supplementation. Effectiveness at the low lycopene concentrations that are possible to attain, occurs only when the lycopene is presented along with other phytonutrients naturally present in tomato and tomato extract. Also important from a practical health perspective are recent scientific findings that indicate that lycopene bioavailability is enhanced by the presence of the other natural phytonutrients that are present in the tomato extract.

Industrial production of tomato lycopene

Although lycopene is found in watermelon, in red citrus fruit, as well as in smaller quantities in other fruits and vegetables, it is the tomato which is the major source of this carotenoid in nature. Therefore, the tomato is the obvious raw material to be chosen for lycopene production. The fact that tomato is a major industrial crop, mechanically harvested and used extensively by the food industry, further enhance its position as the best candidate. Nonetheless, although the ripe tomato is the best natural source of lycopene, still it contains only 80-120 ppm of this carotenoid. It is not economical to extract such minute quantities of Lycopene directly from the tomato, and a step of concentration is required prior to the extraction.

There are three possible sources of concentrated tomato based material as a raw material for lycopene production:

- I. Tomato industry waste
- II. Tomato paste
- III. Tomato pulp

The three alternative raw materials have higher lycopene content than the tomato and each has certain advantages and disadvantages as a source for industrial lycopene production.

Alternative I - Tomato industry waste

The only obvious advantage of this alternative is the very low cost of tomato waste. This raw material, however, has numerous disadvantages when used for lycopene production. Tomato industry waste consists mainly of tomato peel and seeds and therefore is highly contaminated with agrochemicals. In the extraction process these contaminants are extracted with the tomato lipids and are very difficult to remove from the oleoresin. As a raw material, tomato waste is very variable, and often starts to ferment before it is collected. Practically no control is possible over its quality and composition. Industrial tomato waste has relatively low lycopene content (about 150 ppm), and it is difficult to collect and preserve it from microbial spoilage and from oxidation. In addition, the extraction requires a complicated multi-solvent process and the lycopene in the extract is not very stable.

Table I – Tomato industry waste

<i>Advantages</i>	<i>Disadvantages</i>
Very low cost raw material	High contamination with agro-chemicals
	Difficult to collect and to preserve
	Low stability
	Multi-solvent extraction required
	Low lycopene content
	No control over the quality of raw material
	Frozen storage required for waste preservation
	The extracted oleoresin is turbid and very viscous

Alternative II - Tomato paste The tomato paste is a much better choice than tomato industry waste as a source for lycopene production.. It is not contaminated with agrochemicals, has a relatively high lycopene concentration, and is usually preserved by heat and therefore is both shelf stable and a readily available product. However, tomato paste is relatively expensive and has several disadvantages as a raw material for lycopene extraction. Conventional tomato paste production involves prolonged heating and agitation in the presence of air which results in very high losses of lycopene (more than 30%) which is oxidized and broken down into smaller molecules. These products of degraded Lycopene, are extracted from the tomato paste along with other broken down phytochemicals and lipids. The toxicity of these unknown chemicals has not been investigated. Their presence in the tomato paste is very small, but when concentrated by extraction a few hundred times, a toxicological evaluation should be conducted to prove that their presence in the oleoresin does not, cause a health hazard. The high concentration of sugars in the tomato paste poses a technical difficulty and requires the use of a complicated multi-solvent extraction process.

Table II- Tomato paste alternative

<i>Advantages</i>	<i>Disadvantages</i>
Readily available	Possible presence of lycopene degradation products
High concentration of lycopene (350-550ppm)	Low stability of extracted lycopene
Shelf stable	High lycopene losses in production (>30%)
	Multi-solvent extraction required
	The extracted oleoresin is turbid and very viscous

Alternative III - Tomato pulp

The third, and the best alternative for lycopene production, is based on tomato pulp that is specially prepared for this purpose. By separating the tomato pulp from the tomato serum, we increase the concentration of lycopene 10 fold, and decrease accordingly the amount of sugars in the pulp to be extracted. In comparison to the tomato paste, the lycopene content is increased by removal of water by vacuum concentration. This process also increases the concentration of soluble solids, mainly sugars, in the same ratio. Thus tomato paste has 30 deg. Brix, while tomato pulp less than 5 deg. Brix. The presence of sugars complicates the extraction process and requires the use of a multiple solvent system. In comparison simple, one solvent extraction can be used in the case of tomato pulp. The lycopene in the frozen tomato pulp does not deteriorate and the oleoresin extracted from the pulp is remarkably stable to oxidation. As a raw material, tomato pulp is tailor made for lycopene production. It can be prepared, as will be discussed later, from tomatoes rich in lycopene specially bred and cultivated for this purpose. The gentle handling of the pulp assures minimal losses of lycopene and is instrumental in production of a very high quality product.

The pulp is separated from the crushed tomatoes in a very gentle and rapid process. Minimal heating and oxidation are required and therefore there are practically no changes in lycopene due to oxidation and degradation. The pulp contains high levels of lycopene and very little sugars. Therefore, an effective single stage extraction process is possible. In order to increase even more the efficiency of the pulp alternative, new tomato cultivars, with more than double the lycopene content have been developed. These lycopene Rich Tomatoes (LRT) were developed employing only conventional breeding methods, without the use of genetic

engineering. Physical comparison of oleoresins (extracts) prepared from tomato paste and from tomato pulp shows that the first one is turbid and viscous, while the one extracted from tomato pulp is clear, has much lower viscosity and has different composition of fatty acids.

Table III - Tomato pulp

<i>Advantages</i>	<i>Disadvantages</i>
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	

Table IV - Comparison between alternatives for production of tomato lycopene

Parameter	Raw material used		
	Tomato paste	Tomato waste	Tomato pulp
Lycopene recovery	Low	Low	High
Lycopene deterioration	High	High	Low
Contamination with agrochemicals	Low	High	Low
Extraction method	Multi solvent extraction	Multi solvent extraction	Single solvent extraction
Viscosity of extract	High	High	Medium-low

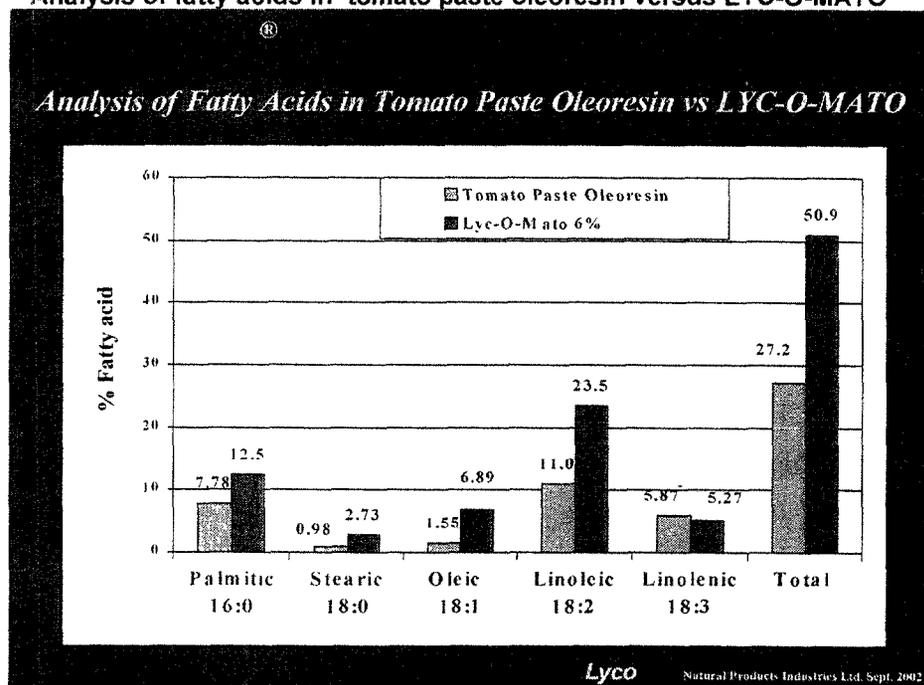
Table V - Lycopene levels in extracted raw material

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%	75-80%	> 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 VI- Viscosity and clarity of oleoresin produced from tomato paste versus Lyc-O-Mato® 6% extracted from tomato pulp

Tomato Paste Oleoresin				LYC-O-MATO® 6%			
Sample no.	Lot no	Viscosity in cp 50°C	Clarity of 1% solution in chloroform	Sample no.	Lot no.	Viscosity in cp 50°C	Clarity of 1% solution in chloroform
1	20011020	11700	Turbid	1	901140	1400	Clear
2	20011021	9600	Turbid	2	907003	810	Clear
3	20020527	7900	Turbid	3	907009	1060	Clear
4	20020528	12500	Turbid	4	908030	1420	Clear
5	02037801	11200	Turbid	5	910048	1650	Clear
				6	911065	820	Clear
				7	802116	1030	Clear
				8	803127	1320	Clear
				9	804138	1290	Clear
				10	805151	1380	Clear
Average		10580.0				1218.0	
n		5				10	
ST.DEV.		1834.94				276.40	
RSD		17.34				22.69	

Analysis of fatty acids in tomato paste oleoresin versus LYC-O-MATO®



The LycoRed process

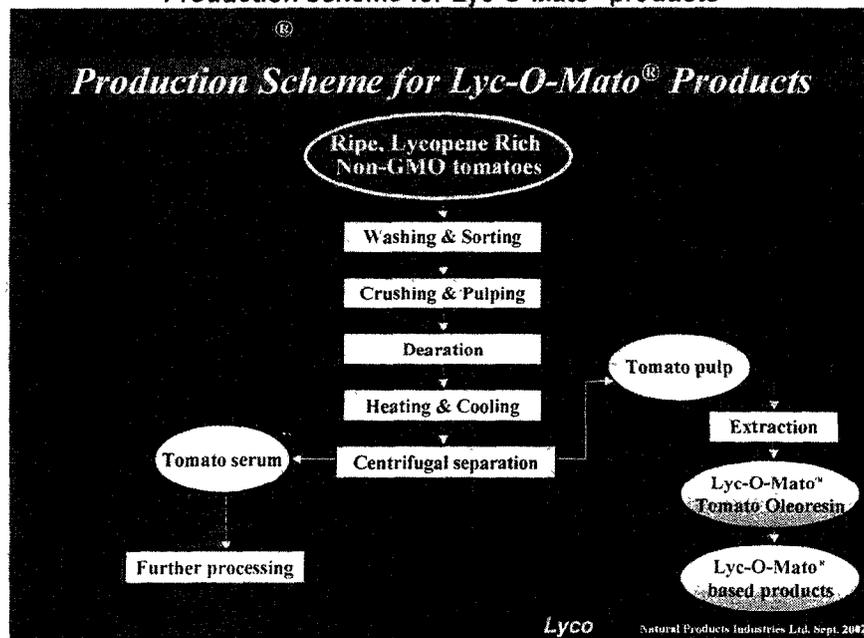
Twelve years ago, LycoRed Natural Products Industries, Ltd. launched an ambitious program directed at producing a high quality tomato lycopene for the food industry to be used both as a natural color and as an ingredient for food fortification. The first stage of the project was to develop hybrid tomato cultivars yielding fruit especially high in lycopene content. Only conventional breeding methods were used without applying genetic engineering techniques. The tomato varieties that were developed yield two-to-three-fold higher Lycopene content than conventional tomatoes. These tomatoes are cultivated under strict supervision. Only approved agrochemicals are used and their application schedule irrigation etc. is carefully designed and observed. The ripe tomatoes are mechanically harvested and transported to the

processing plant. Only red, ripe tomatoes are selected; the green are rejected with the help of an electric eye control. The ripe tomatoes are then transported to the processing plant. The harvested tomatoes are sampled and analyzed for the presence of agrochemicals. Only those with a low level of agrochemicals are used for lycopene production.

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 (-18°C) until it is extracted in a specially designed modern facility. The proprietary (US Patent No. 5,837,311) process uses conventional unit operations approved for the food industry. There is no chemical intervention in the process.

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 isomerization and degradation. Strict quality control accompanies the various stages of production, from cultivating the tomatoes in the field to the standardizing 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.

Production scheme for Lyc-O-Mato® products



Lyc-O-Mato® range of products

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 oleoresin is standardized to the desired lycopene content and is used in various formulations under the brand name of Lyc-O-Mato®. These formulations are used as a source of lycopene in functional foods and in dietary supplements. In most Lyc-O-Mato® products, lycopene is delivered in natural tomato oil which has good bioavailability. Lyc-O-Mato® contains other important phytonutrients as well, such as phytoene, phytofluene, β-carotene, tocopherols and phytosterols. The synergy of these phytonutrients enhance Lyc-O-Mato®'s biological activity.

Lyc-O-Mato®, lycopene color formulations

Coloring materials have long been used in the food, pharmaceutical and cosmetic industries. Most colors used are synthetic materials produced by the chemical industry. In recent years, however, a growing public awareness and concern for health has resulted in a controversy surrounding the use of synthetic food additives. There has been an increasing consumer demand for a return to natural food ingredients. Legislation has not been far behind, and several artificial colors have been banned for use in foods. The legal limitations, and the

public's growing resentment toward the use of artificial food coloring, has caused an inevitable demand for natural pigments. Unfortunately, most of the available natural colors are plagued by serious drawbacks. They are usually unstable to heat and to acidic or alkaline pH values. Some have offensive off-flavors and poor coloring ability. Also natural pigments are available in rather limited color range and are usually effective only in high concentrations. B-carotene is one of the few good natural colors available. Being effective in the yellow to orange color range, and not suffering from most of the disadvantages of other natural pigments, it is widely used as a food-color. However, only a small percentage of the β -carotene used in the various industries is, in fact, truly natural. Most of it is a "nature identical", chemically synthesized all trans isomer of the pigment.

Tomato lycopene, being very similar in chemical composition to β -carotene, has all the natural advantages necessary to make it an excellent food color. It is also stable to heat and to the extreme pH values encountered in food processing. In addition, lycopene has a much wider color range than β -carotene from yellow through orange to red and is effective at very low concentrations. Because lycopene is derived from tomatoes by conventional means, it is a truly natural color, its coloring ability depends on its concentration, the method of dispersion and formulation used. Lyc-O-Mato[®] formulations can be used both as a source of lycopene and in food fortification to color food products. They are specially designed for the various requirements of food industry and are tailor made for specific applications.

Safety of Lyc-O-Mato[®] products

The Lyc-O-Mato[®] oleoresin is extracted from the ripe tomato in a gentle physical process without chemical intervention. The lycopene and other tomato phytonutrients in the oleoresin are not affected by the process. Lycopene extracted from certain molds as well as synthetic lycopene are both available in the market. Both products have different isomer composition from that of tomato lycopene, contain undefined chemicals, and their use in foods is not allowed. The Lyc-O-Mato[®] line of products contains only natural tomato lycopene – the same carotenoid that has been consumed safely in tomatoes and in tomato products by generations of humans. Only tomato lycopene has been investigated in numerous epidemiological and clinical studies and shown to have beneficial effect on prevention of degenerative diseases. In spite of the fact that Lyc-O-Mato[®] 6% is a pure tomato product, it was subjected to a 13-week oral toxicity study which showed no adverse effects in the animals. The No-Observed-Adverse-Effect-Level (NOAEL) for rats was found to be ≥ 4500 mg/kg body weight. Lyc-O-Mato[®] 6% was also found to be negative in the Ames study and successfully passed various toxicological assessments.

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Safety Evaluation of Lyc-o-mato[®] 6% Derived From Lycopene Rich Tomatoes

R.A. Matulka^a, A.M. Hood^a, J.C. Griffiths^a

^aThe Burdock Group, 780 U.S. Highway 1, Vero Beach, FL 32962, USA

Abstract:

Experimental and epidemiological studies indicate that consumption of tomato products containing high amounts of lycopene is associated with lower cancer risk. Consumption analysis of lycopene-containing foods determined the mean daily intake of lycopene to be estimated at 8.2 mg/day. The protective effects of lycopene are postulated to be related to its antioxidant potential. Lycopene, as tomato oleoresin, has been demonstrated to inhibit oxidation. Lyc-o-mato 6[®]% is a purified tomato oleoresin containing 6% lycopene as produced naturally from lycopene-rich tomatoes. Lyc-o-mato 6[®]% was evaluated for toxicological effects. The 50% lethal dose (LD₅₀) derived from the acute oral toxicity study was found to be greater than 5000 mg/kg bodyweight. The no- observed-adverse-effect level (NOAEL) derived from the 13-week study was 4500 mg/kg/day. Acute dermal toxicity study of Lyc-o-mato 6[®]% found no toxicity at the level of 2000 mg/kg bodyweight. Lyc-o-mato 6[®]% lacked dermal irritation in the rabbit model, but was found to have moderate eye irritant capabilities. Lyc-o-mato 6[®]% tested at 5% (w/w) in petroleum jelly was found to be a moderate sensitizer in the guinea pig model. There was no evidence of mutagenic potential at doses up to 5000 µg/plate, as determined by the Ames assay. The results of these studies demonstrate the inability of Lyc-o-mato 6[®]% to produce oral, dermal or mutagenic toxicity in animal models at doses greater than 1500-fold over normal human lycopene consumption.

1. Introduction:

Evidence of the importance of diet and the maintenance of human health is well documented; and anti-oxidant compounds are strongly associated with increasing the bodily defenses against disease (Ziegler, 1991, van Poppel and Goldbohm, 1995, Gann *et al.*, 1999). Consumption of carotenoid-rich vegetables has also been shown to reduce genetic damage (Pool-Zobel *et al.*, 1997, Porrini *et al.*, 2002). Specifically, tomatoes and tomato-based products containing high levels of carotene and lycopene have been associated with increased cardiovascular health, a lower risk of cancers and decreased oxidative damage to DNA in humans (Bowen *et al.*, 2002, Porrini and Riso, 2000, Willcox *et al.*, 2003). Lycopene from processed tomatoes has been shown to be more bioavailable than from fresh tomatoes (Bohm and Bitsch, 1999, Gartner *et al.*, 1997, Porrini *et al.*, 1998). In addition, lipid concentrations, as well as the type of lipid involved, may regulate the amount of lycopene absorbed into plasma (Bohm, 2002, Lee *et al.*, 2000). The estimated daily (background) consumption of lycopene from natural food sources is approximately 5 mg/day for the United States (Schweitzer *et al.*, 1999), which is calculated to be 0.071 mg/kg/day for a 70 kg (154 lb) man.

Unfortunately, Schweitzer *et al.* (1999) reported their results in abstract form, thus detailed information regarding the analysis is not available. Therefore, a consumption analysis was performed to estimate the daily intake of lycopene from foods in which it is a known constituent. Food consumption data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996 database was used. Foods in which lycopene is a known constituent were identified using the USDA-NCC¹ Carotenoid database as reported by Holden *et al.* (1999). The USDA-NCC Carotenoid database also provided the concentration of lycopene in these foods. A total of 26 foods and food products were selected that included fruits, vegetable products, meat products, as well as tomato and tomato products (see Table 1). Food and lycopene consumption was estimated by (1) weighting of the data for estimation of consumption by individuals in the entire United States population, and (2) multiplying the estimated food consumption (g food/day) with the lycopene concentration (mg lycopene/g food).

One study indicates the benefits of lycopene consumption by a decrease in lymphocyte DNA damage at a dose of approximately 7 mg lycopene (in the form of a tomato puree) for 14 days (Porrini and Riso, 2000). Other studies indicate benefits of lycopene at a consumption level of approximately 30-75 mg lycopene *per day* (Chen *et al.*, 2001, Lee *et al.*, 2000, Paetau *et al.*, 1998). These studies indicate that doses of lycopene between 30-75 mg/day were well-tolerated,

¹ United States Department of Agriculture-Nutrition Coordinating Center

with only minor gastrointestinal problems reported which were resolved within a few days. The only reports of adverse reactions of the consumption of large amounts (up to two liters per day) of tomato products results in lycopenemia, a carotenoid-induced skin color alteration (Reigh, *et al.*, 1960; La Placa *et al.*, 2000). The objective of the present study was to determine the oral, dermal and genetic effects of lycopene as a 6% solution in tomato oleoresins, derived from lycopene-rich tomatoes.

2. Materials and Methods:

Production of Lyc-o-mato[®]6% oleoresin (also referred to as “Lyc-o-mato[®]6%”) is intended for use as a food ingredient or dietary supplement and, is initiated by crushing of lycopene-rich tomatoes (*Lycopersicon lycopersicum* L. Karst. Ex. Farw) to form a pulp. Following extraction with ethyl acetate, the extract is separated from the tomato pulp. Lyc-o-mato[®]6% is obtained after the solvent is removed by evaporation under vacuum, and standardized with oleoresins to provide a consistent concentration (6%) of lycopene. The standardized Lyc-o-mato[®]6% is sampled and analyzed for lycopene content, solvent residue, agrochemicals, water and pH. Independent batch formulations were tested for lycopene concentration of in Lyc-o-mato[®]6%, with the average of 5.8% (range of 5 –7%) lycopene for 13 samples processed (data not shown).

2.1. Toxicity Studies

The safety evaluation of Lyc-o-mato[®]6% was initiated by Lyco-Red, but the individual safety studies were carried out by independent and experienced contractor(s). All study protocols were followed in compliance with Good Laboratory Practice (GLP) standards.

2.1.1. Acute oral toxicity in rats (Dreher, 1994a,b)

Sprague-Dawley rats (Harlan, U.K.) at five to eight weeks of age were used for this study, being acclimatized for at least five days and, housed in groups of up to five by sex in solid-floor polypropylene cages furnished with wood flakes. Rats received food *ad libitum*, except for an overnight fast immediately before dosing and for approximately two hours after dosing. The animal rooms were maintained at 19 – 22° C and relative humidity of 36 – 62% under a twelve-hour light/dark cycle. The study was initiated with a range-finding study, wherein one male and one female rat received by gavage a variable volume of compound in order to receive a dose of 5000 mg Lyc-o-mato[®]6% preparation/kg bodyweight (5.24 ml/kg, based on density studies (data not shown)). The rats were observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and, once daily for five days. Based on the results of the range-finding study, the

main study was initiated with one group of five male and five female rats given a dose of 5 g/kg body weight.

During the main study, each rat was observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. Individual bodyweights were recorded prior to dosing on Day 0 and on Days 7 and 14. At the end of the study, the animals were killed by cervical dislocation and subjected to gross pathological examination.

2.1.2. Subchronic oral toxicity study in rats (East, 1995)

A repeated-dose, 13-week oral toxicity study was conducted in 80 male and 80 female CD rats obtained from Charles River (UK). The males weighed 99 – 132 g and the females weighed 96 – 117g four days prior to the start of the study. The rats were 33 to 40 days of age when treatment started.

Lyc-o-mato[®]6% was diluted in peroxide-free corn oil (control) and administered daily by gavage at dose levels of 0 (control), 45, 450 and 4500 mg/kg body weight/day. The dose was given by gavage based on the weight measured immediately prior to each administration. The dose volume used was 10 ml/kg body weight, except on Day 1 of treatment, when 5 ml/kg bodyweight was used for Groups 1, 2 and 3. Due to the high viscosity of the highest concentration formulation (Group 4), a volume-dosage of 5 ml/kg body weight was not possible and, consequently, a volume-dosage of 10 ml/kg bodyweight/day was used in all Groups from Day 2 of treatment. Treatment was performed daily for at least 13 weeks, depending on the day of necropsy.

All of the animals were inspected at least twice daily for evidence of reaction to treatment or ill health. Any deviations from normal were recorded at the time of onset, duration and progress of the observed condition, as appropriate. Individual daily observations of all animals were made before and shortly after each dose; at the end of dosing each Group; 1 to 2 hours after completion of dosing and again as late as possible in the working day, daily during the first week of treatment, twice weekly during Weeks 2 to 4 and then once weekly during Weeks 5 to 13. In addition, a more detailed weekly examination was conducted that included palpation and an assessment of feces color (subjective observation) for each animal. Each animal was weighed during the acclimation period, on the day that treatment started, at weekly intervals thereafter and before necropsy. Weekly food consumption *per rat* (mean) was obtained by determination of the weight of the food supplied to each cage, minus the food remaining, with an estimate of the amount spilled, for each week. Hematology, clinical chemistry, ophthalmoscopy, urinalysis and, proof of absorption investigations were performed at various time points during the study, and on

completion of the treatment period all animals were subjected to macroscopic examination and organ weight analysis. Microscopic examination of a comprehensive list of tissues was also undertaken.

Proof of absorption was examined during week 13 of treatment, when blood samples were drawn from the retro-orbital sinus of ten male rats and ten female rats. The rats were held under halothane/nitrous oxide anesthesia, using lithium heparinate anticoagulant in the tubes. The animals were not fasted prior to sampling. The samples were centrifuged and kept at -80°C . Samples from the male rats were then analyzed for concentrations of lycopene, retinol (vitamin A) and α -tocopherol (vitamin E), according to Khachick *et al.*, (1992a, b).

2.1.3. Acute dermal toxicity study in rats (Dreher, 1994c)

Five male and five female Sprague-Dawley rats (Harlan, U.K.) at ten to fourteen weeks of age, were used for this study (males weighing 251 – 271 g and females weighing 227 – 233 g). The rats were acclimatized for at least five days prior to the start of the study and, housed in solid-floor polypropylene cages furnished with wood flakes. The animals were housed individually during the 24-hour exposure period and in groups of five, by sex, for the remainder of the study. Rats received food *ad libitum* throughout the study. The animal rooms were maintained at $19 - 23^{\circ}\text{C}$ and relative humidity of 39 – 54% under a twelve-hour light/dark cycle.

On the day before treatment, the back and flanks of each animal were clipped free of hair to expose a skin area of approximately 5 x 4 cm. The calculated volume of Lyc-o-mato[®] 6%, to obtain a dose level of 2 g/kg body weight, was applied uniformly to an area of shorn skin approximating 10% of the total body surface area using a graduated syringe. Surgical gauze measuring 7 x 4 cm was placed over the treatment area and semi-occluded with a piece of self-adhesive bandage, which was further secured with a piece of Blenderm[™] (3M, UK) wrapped around each end.

The animals were observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. After the 24-hour contact period, the bandage was removed and the treated skin and surrounding hair wiped with cotton wool moistened with arachis oil (a hypoallergenic plant oil derived from *Oleum arachis*) to remove any residual test material. The test sites were examined for evidence of primary irritation and scored according to the scale from Draize (1977). At the end of the study the animals were killed by cervical dislocation and subjected to gross pathological examination, consisting of external examination and opening of the abdominal and thoracic cavities. Any abnormalities were recorded.

2.1.4. Acute dermal irritation study in rabbits (Huntingdon, 1996)

The acute dermal irritation study was performed to assess the irritancy potential of Lyc-o-mato®6% following a single, 4-hour, semi-occluded application to intact rabbit skin, according to OECD guidelines (OECD, 1992). Twelve New Zealand White rabbits (David Percival Ltd, UK), weighing 2.31 – 2.68 kg at the start of the study and approximately 12 – 20 weeks of age, were acclimatized for at least five days prior to the study. The animals were individually housed in suspended metal cages and had free access to food and water throughout the study. The animal rooms were maintained at 17 – 22°C and relative humidity of 51 – 59% under a twelve-hour light/dark cycle.

Approximately 24 hours prior to the start of testing, each of a group of six rabbits (two groups) was clipped free of fur from the dorsal/flank area using veterinary clippers. Only animals with a healthy intact epidermis were utilized for the study. At the start of the study, 0.5 ml of Lyc-o-mato®6% was placed onto the shorn skin and a 2.5 x 2.5 cm gauze patch was placed over the solution. The patch was secured in position with a strip of surgical adhesive tape (Blenderm™) and, the trunk of each rabbit was wrapped in an elasticized corset (Tubigrip®, SSL International, UK) and the animals returned to the cages for the duration of the exposure period. Four hours after application, the corset and patches were removed from each animal and residual test material removed with cotton wool soaked in diethyl ether.

One hour following the removal of the patches and approximately 24, 48 and 72 hours after patch removal, the test sites were examined for evidence of primary irritation and scored according to Draize (1977). The scores for erythema and edema at the 24 and 72-hour readings were totaled for the six test rabbits (24 values) and were then divided by 12 to give the primary irritation index of the test material.

2.1.5. Acute ocular irritation study in rabbits (Dreher, 1994d,e)

The acute ocular irritation study was performed to assess the irritancy potential of Lyc-o-mato®6% following a single, 4-hour, semi-occluded application to the rabbit eye. Twelve New Zealand White rabbits (David Percival Ltd, UK), weighing 2.13 – 2.58 kg at the start of the study and approximately 12 – 20 weeks of age, were acclimatized for at least five days prior to the study. The animals were individually housed in suspended metal cages and had free access to food and water throughout the study. The animal rooms were maintained at 18 – 21°C and relative humidity of 44 – 56% under a twelve-hour light/dark cycle.

On the day prior to the study, both eyes of six provisionally selected test rabbits (six *per* group, two groups *per* experiment) were examined under ultra-violet light, after treatment with

sodium fluorescein B.P. (Fluorets: Smith & Nephew Pharmaceuticals Limited, UK). The cornea, conjunctive and iris were examined for lesions. Immediately before treatment, the rabbit eyes were again examined with the aid of a light source and any animals showing evidence of ocular lesions were rejected and replaced. In order to minimize pain on instillation of the test material, one drop of local anaesthetic ("Ophthaine®", 0.5% proxymetacaine hydrochloride, E.R. Squibb & Sons, Ltd., UK) was added into both eyes of all animals one – two minutes before test material treatment.

One rabbit was initially treated. A volume of 0.1 ml of the test material was instilled into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second immediately after instillation, to prevent loss of the test material and, then released. The left eye remained untreated and was used for control purposes. An initial pain reaction assessment was made immediately after administration of the test material. After consideration of the ocular responses produced in the first treated animal, five additional animals were treated.

Assessment of ocular damage/irritation was made at approximately 1, 24, 48 and 72 hours following treatment, according to the numerical evaluation given in Appendix I of Draize (1977). Any other ocular effects were also noted. Examination of the eye was facilitated by use of a standard ophthalmoscope. An additional observation was made on day seven to assess the reversibility of the ocular effects.

2.1.6. Magnusson and Kligman maximization study in guinea pigs (Dreher, 1994f)

The Magnusson and Kligman maximization study was performed to assess the skin contact sensitization potential of Lyc-o-mato® 6% exposure in guinea pigs. Thirty-six female, albino Dunkin-Hartley guinea pigs (David Hall Ltd, UK), weighing between 332 – 407 g at the start of the main study and approximately 8 – 12 weeks of age, were acclimatized for at least five days prior to the study. The animals were housed individually or in pairs in solid-floor polypropylene cages with wood flakes and, had free access to food and water throughout the study. The animal rooms were maintained at 20 – 23°C and relative humidity of 32 – 58% under a twelve-hour light/dark cycle.

The concentrations of Lyc-o-mato® 6% to be used at each stage of the study were determined by range-finding tests in which several guinea pigs were treated with various concentrations of Lyc-o-mato® 6%. For selection of the concentration for intradermal induction, two animals were intradermally injected with preparations of test material (1 or 5% w/v in arachis oil British Petroleum (B.P.)). The highest concentration that did not cause local necrosis,

ulceration or systemic toxicity was selected for the intradermal induction stage of the main study. To select the concentration for topical induction, two guinea pigs (intradermally injected with Freund's Complete Adjuvant fifteen days earlier) were treated with undiluted Lyc-o-mato[®]6% and three dilutions (75, 50 and 25% in petroleum jelly B.P.). The highest concentration producing only mild to moderate dermal irritation after a 48-hour occlusive exposure was selected for the topical induction stage of the main study. The concentration for topical challenge was selected by applying four preparations of Lyc-o-mato[®]6% (5, 10, 25 and 50% w/w in petroleum jelly B.P.) occlusively to the flanks of two guinea pigs for a period of 24 hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The highest concentration considered least likely to produce irritant responses at challenge and one lower concentration were selected for the topical challenge stage of the main study.

The maximization test involves two main procedures: a) an induction of the response and b) a challenge of that response. Induction of the Lyc-o-mato[®]6% test guinea pigs was as follows: Prior to treatment on Day 0, the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal. A row of three injections (0.1 ml each) was made on each side of the mid-line. The first injection was Freund's Complete Adjuvant plus distilled water (1:1 ratio). Second injection was a 5% w/v dilution of test material in arachis oil B.P. The third injection was a 5% w/v dilution of test material in a 1:1 preparation of Freund's Complete Adjuvant plus arachis oil B.P. The control animals were treated identically, except they received the control of arachis oil B.P. in place of Lyc-o-mato[®]6% in the injections.

One week later (Day 7), the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of 25% w/w Lyc-o-mato[®]6% in petroleum jelly B.P. (test animals) or vehicle (control animals). The Lyc-o-mato[®]6% was applied on Whatman No. 4 filter paper and held in place by surgical adhesive tape and covered with an overlapping length of aluminum foil. The patch and foil were further secured by a strip of elastic adhesive bandage (Elastoplast[®], Beiersdorf, Germany) wound in a double layer around the torso of each animal and kept in place for 48 hours. The degree of erythema and edema was quantified one and 24 hours following removal of the patches according to Draize (1977).

Prior to treatment on Day 21, the challenge phase of this test was initiated by clipping free of hair, an area approximately 50 mm x 70 mm on both flanks of each animal. A solution containing Lyc-o-mato[®]6% (5% w/w in petroleum jelly B.P.) was applied to the shorn right flank of each animal (0.1 – 0.2 ml) on a square of filter paper (Whatman No. 4) held in place by a strip

of surgical adhesive tape. To ensure that the maximum non-irritant concentration was used at challenge, a 2% Lyc-o-mato[®]6% solution (w/w in petroleum jelly B.P.) was also similarly applied to a separate skin site on the right shorn flank. The vehicle alone was applied to the left shorn flank and covered. The patches were occluded with an overlapping length of aluminum foil and secured by a strip of elastic adhesive bandage wound in a double layer around the torso of each animal.

After 24 hours, the dressing was carefully cut, removed and discarded. The challenge and vehicle sites were swabbed with cotton wool soaked in diethyl ether. The position of the treatment sites was identified by using a black indelible marker. Any regrown hair on the flanks was clipped before evaluation of the skin reactions. Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and edema was quantified using a scale according to Draize (1977).

2.2. Bacterial gene mutation (Thompson, 1994)

The mutagenic effect of Lyc-o-mato[®]6% was assessed by exposing five strains of *Salmonella typhinurium* (TA98, TA100, TA1535, TA1537, and TA1538) and one strain of *Escherichia coli* (WP2uvrA) to five different concentrations of the test material, based on the *in vitro* technique described by Ames *et al* (Ames *et al.*, 1970, Ames *et al.*, 1975, McCann *et al.*, 1975). It is known that some chemicals do not exert a mutagenic effect in this system unless they are activated by mammalian enzymes. The metabolic activation is accomplished by incubating the bacteria together with the test compound and S-9 mix, consisting of rat liver enzymes supplemented with salts and co-factors (Sprague-Dawley rats livers prepared by the British Industrial Biological Research Association). Two independent tests were performed, utilizing all five bacterial strains with and without S-9. A solvent treatment group was used as the negative control and the positive control materials were as follows: 4-Nitroquinoline-1-oxide 0.2 µg/plate for TA98; 3 µg/plate for TA100 and 5 µg/plate for TA1535; 9-Aminoacridine (9AA) 80 µg/plate for TA1537; 4-Nitro-*o*-phenylenediamine (4-NOPD) 5 µg/plate for TA1538 and N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) 2 µg/plate for WP2uvrA. In addition, for the S9 series of plates, the materials, benzo(*a*)pyrene (BP) 5 µg/plate for TA98, TA100, TA1537 and TA1538; and 2-Aminoanthracene (2AA) 2 µg/plate for TA1535 and 10 µg/plate for WP2uvrA were used as positive controls.

In order to decrease any unwarranted bacterial growth, Lyc-o-mato[®]6% was accurately weighed (after autoclaving for 10 min at 121°C) and suspended in sterile distilled water by warming in a 37°C incubator and mixing on an autovortex mixer and ultrasonic bath, with 1%

Tween80 (a surfactant) added to aid dispersal. Appropriate dilutions were then prepared on the day of each experiment to obtain five concentrations of the test material to be assayed in triplicate. Lyc-o-mato[®]6% was assayed against each tester strain using the direct plate incorporation method. Aliquots (0.1 ml) of each bacterial suspension were dispensed into sets of sterile test tubes containing 2.0 ml of molten trace histidine (or tryptophan in the case of WP2uvrA⁻) supplemented top agar at 45°C. These sets were comprised of two test tubes for each bacterial test strain. Appropriately diluted test material or solvent control solution (0.1 ml) was also added to each of the two tubes followed by either 0.5 ml of the S9 liver microsome mix or 0.5 ml of pH 7.4 buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner agar plates (one tube/plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material. The plates were incubated at 37°C for 48 hours and the number of revertant colonies counted. For a chemical to be considered positive in this test system, it should have induced a dose-related significant increase in mutation rate (of at least twice the spontaneous reversion rate) in one or more strains of bacteria in the presence and/or absence of the S9 microsomal enzymes in both experiments at sub-toxic dose levels.

3. Results

3.1. Toxicity studies

3.1.1. Acute oral toxicity in rats (Dreher, 1994a,b)

Two separate studies were performed to determine the acute toxicity of Lyc-o-mato[®]6%. In both range-finding studies there were no deaths or clinical signs of toxicity to Lyc-o-mato[®]6% given at 5 g/kg bodyweight.

In the main studies, one female was found dead after four hours of dosing. This female rat had clinical signs of hunched posture, lethargy, decreased respiratory rate and labored respiration two hours after dosing. This death was consistent with a non-treatment-related adverse effect. Abnormalities noted at necropsy of this animal were hemorrhagic lungs, dark liver and dark kidneys. The surviving animals appeared normal throughout the study, with the only noted effects of dosing were incidents of brown-colored staining of the fur. No abnormalities were noted during necropsy at the end of the studies.

3.1.2. Subchronic oral toxicity study in rats (East, 1995)

The bodyweight gain, food intake and food conversion efficiency of treated animals were similar to the controls. An increased incidence of orange-stained feces in the males receiving 45 mg/kg/day and animals receiving 450 mg/kg/day while red-stained feces were noted in animals receiving 4500 mg/kg/day. Throughout the treatment period, the bodyweight gains of treated animals were similar or marginally superior to the controls, as was the case of the females treated with Lyc-o-mato[®]6% at all doses (Figure 1). There were no ocular lesions, nor any unusual hematology findings related to treatment with Lyc-o-mato[®]6% at any doses. Urinalysis investigations after 11 weeks of treatment revealed no treatment-related abnormalities. When compared with the controls, the males given 4500 mg/kg/day Lyc-o-mato[®]6% revealed a marginally higher urinary output; however, this was not seen to be dose-related and similar findings were not observed in the females. Non-significant reductions in sodium concentrations were noted among the treated males, though this was not dose-related. Low plasma alkaline phosphatase activities were apparent after six weeks in males given 450 or 4500 mg/kg/day, while alanine amino-transferase activities were slightly higher in males given 4500 mg/kg/day Lyc-o-mato[®]6% (data not shown). Alkaline phosphatase activity at the end of the study (13 weeks) was slightly lower in treated males and females receiving 450 or 4500 mg/kg/day Lyc-o-mato[®]6% (Table 1). This reduced activity was dose-related in the males only, but not statistically significant in either males or females. Platelet values in the females at the end of the thirteen week period were slightly higher than controls at all Lyc-o-mato doses, but was not a dose-related increase (Table 2).

Analysis of blood for lycopene concentrations after 13 weeks of treatment found that plasma levels of lycopene were similar in males given 450 or 4500 mg/kg/day (57.33 µg/l and 57.43 µg/l, respectively), although a lower level was found in males given 45 mg/kg/day (a range from 0-29.37 µg/l).

One male rat dosed with 450 mg/kg/day Lyc-o-mato[®]6% had papillary hyperplasia of the urinary bladder, which is an unusual finding in rats of this age and strain. This lesion lacked invasive growth and showed evidence of an associated severe inflammatory cell infiltrate in the genito-urinary tract and, further investigation revealed a chronic infection of the genito-urinary tract and that the papillary hyperplasia is considered to be reversible and regenerative in nature, rather than neoplastic and is not considered related to the treatment with Lyc-o-mato[®]6%.

3.1.3. Acute dermal toxicity study in rats (Dreher, 1994c)

All animals survived this study, with no signs of systemic toxicity at the dose of 2 g/kg bodyweight. Light brown-colored staining was commonly noted at the treatment sites of all

animals during the study, which prevented an accurate evaluation of erythema at the treatment sites of all animals one day after dosing, but did not hinder the rest of the study. Hemorrhaging of the dermal capillaries was noted at the treatment site of one male and one female two to four days after dosing. No other signs of skin irritation were noted. All animals experienced weight gain during the study, except two females that showed nonsignificant weight loss during the first week of treatment. At the end of the study, necropsy was performed and no abnormalities were noted (data not shown).

3.1.4. Acute dermal irritation study in rabbits (Huntingdon, 1996)

One study was undertaken to determine the irritant capability of Lyc-o-mato[®]6% on rabbit dermis. Application of Lyc-o-mato[®]6% (0.5 ml) on the shaved skin of rabbits caused a yellowing to a light brown staining at the treated skin sites, which did not affect evaluation of the skin responses. The control sites did not show any response to the control procedure. The mean values for erythema and edema recorded 24, 48 and 72 hours after treatment did not equal or exceed the limit values considered to indicate a significant inflammatory response to treatment and, therefore Lyc-o-mato[®]6% was considered to be a non-irritant to the skin.

3.1.5. Acute ocular irritation study in rabbits (Dreher, 1994d,e)

Two separate acute eye irritation studies with Lyc-o-mato[®]6% were performed. In both studies, residual test material was noted around the treated eye of all animals throughout the study. Orange-colored staining was noted in five treated eyes one hour after treatment in the first study, while the same type of staining was noted in all the treated eyes in the second study.

In the first study, a dulling of the normal luster of the corneal surface was noted in four treated eyes one hour after treatment. Diffuse corneal opacity was noted in two treated eyes one hour after treatment and in four treated eyes at the 24-hour observation. No other corneal effects were noted. Iridial inflammation was noted in all treated eyes one hour after treatment, in four treated eyes at the 24-hour observation and in two treated eyes at the 48-hour observation. No other iridial effects were noted. Moderate conjunctival irritation was noted in all treated eyes one hour after treatment, with minimal to moderate conjunctival irritation at the 24-hour observation. Minimal to moderate conjunctival irritation was noted in five treated eyes at the 48-hour observation. Minimal conjunctival irritation was noted in three treated eyes at the 72-hour observation (Table 3). No ocular effects were noted 72 hours or seven days after treatment.

The second study noted a dulling of the normal luster of the corneal surface in two treated eyes one hour after treatment. Areas of diffuse corneal opacity were noted in four treated eyes

one hour after treatment. Areas of diffuse to translucent corneal opacity were noted in all treated eyes at the 24-hour period and in five treated eyes at the 48- and 72-hour observations. Diffuse corneal opacity was noted in one treated eye at the 7-day observation. Sloughing of the cornea was noted in two treated eyes at the 24-hour observation. Slight vascularization along the lower edge of the cornea was noted in two treated eyes at the 7-day observation. Iridial inflammation was noted in all treated eyes one and 24 hours after treatment and in five treated eyes at the 48- and 72-hour observations. No other iridial effects were noted. Moderate conjunctival irritation was noted in all treated eyes one and 24 hours after treatment with minimal to moderate conjunctival irritation at the 48- and 72-hour observations (Table 3). No ocular effects were noted seven or fourteen days after treatment.

3.1.6. Magnusson and Kligman maximization study in guinea pigs (Dreher, 1994f)

Based on a range-finding study (data not shown), the following concentrations of Lyc-o-mato[®]6% were used for the different parts of the study: Intradermal induction: 5% test article w/v in arachis oil B.P.; Topical induction: 25% w/w in petroleum jelly B.P.; topical challenge: 5% and 2% w/w in petroleum jelly B.P.

During the main study, brown/orange or yellow/orange-colored staining was noted at the induction sites of all test group animals at the one-hour observation. This staining prevented accurate evaluation of erythema at the induction sites of sixteen test group animals at the 1-hour observation and, prevented accurate evaluation of erythema at the induction sites of seventeen test group animals at the 24-hour observation. Very slight to well-defined erythema was noted at the induction sites of four test group animals at the 1-hour observation and in three test group animals at the 24-hour observation. Very slight to moderate edema was noted at the induction sites of nineteen test group animals at the 1-hour observation with very slight to slight edema in fifteen test group animals at the 24-hour observation. Other skin reactions noted were bleeding and small superficial scabs. In comparison, very slight to well-defined erythema with or without very slight edema was noted at the treatment sites of all control group animals at the 1-hour observation. Very slight erythema was noted at the treatment sites of three control group animals at the 24-hour observation.

Topical challenge resulted in incidents of yellow/orange-colored staining at the challenge sites of test and control group animals at the 24 and 48-hour observations, which did not affect evaluation of the skin responses. The topical challenge with 5% w/w Lyc-o-mato[®]6% in petroleum jelly B.P. resulted in positive skin responses, very slight to well-defined erythema (grades 1 or 2) with or without very slight edema in seven test group animals at the 24-hour

observation time point. Well-defined erythema persisted at the challenge site of one test group animal at the 48-hour observation. Six guinea pigs topically challenged with 2% Lyc-o-mato[®]6% in petroleum jelly B.P. showed positive skin responses, with very slight erythema (grade 1) at the challenge sites. No skin reactions were noted at the challenge sites of test group animals at the 48-hour observation.

3.2. Bacterial gene mutation (Thompson, 1994)

Two separate experiments were conducted to determine the mutagenicity of Lyc-o-mato[®]6%. The results of the assessments for characteristics, viability and spontaneous reversion rate for each tester strain were all found to be satisfactory. No toxicity was exhibited by any of the strains of bacteria used. A precipitate was observed at 5000 µg/plate in both experiments, however this did not interfere with the scoring of revertant colonies. No significant increases in the numbers of revertant colonies of bacteria were recorded for any of the strains of bacteria used, at any dose level, either with or without metabolic activation. For test confirmation, the positive control substances all produced marked increases in the number of revertant colonies and the activity of the S9 fraction was found to be satisfactory (Table 4).

4. Discussion

A recent review of the literature failed to reveal acute oral toxicity in humans or animals resulting from lycopene consumption. In the present acute toxicological study of lycopene (in an oleoresin mixture) administration *via* gavage to rats, no signs of systemic toxicity at any of the doses given were noted, with the animals having expected body weight gain (Figure 1) and no abnormalities noted at the time of necropsy; therefore, it can be concluded that the acute oral median lethal dose (LD₅₀) of Lyc-o-mato[®]6% in the Sprague-Dawley rat was found to be greater than 5000 mg/kg bodyweight. Long-term exposure to higher doses of lycopene-rich tomato products has been found to produce a reversible cutaneous change called lycopenemia. Two case studies have documented the symptoms and probable causes of this change (Reigh, *et al.*, 1960; La Placa *et al.*, 2000). In both cases studies, each of the women consumed large amounts of tomato products (up to 2 liters of tomato juice) daily for several years. The symptoms of lycopenemia include a "yellow-orange" discoloration of the skin and abdominal pain due to deposits of lycopene in focal areas of the liver, which may form fatty cysts and provoke microscopic and macroscopic alterations of the parenchyma, but no critically toxic outcomes have been cited. In the same manner, oral administration of Lyc-o-mato[®]6% to CD rats for 13 weeks at doses including 4500 mg/kg/bodyweight/day was generally well tolerated, with no

deaths or evidence of toxicity. Treatment-related signs were restricted to staining of the feces. The plasma alkaline phosphatase activities of the treated animals tended to be lower (nonsignificant) than those of the controls. This effect may be associated to the volumes of corn oil given to the controls versus the treatment groups (Young *et al.*, 1982). Since the test material supplied was a semi-solid and the doses were high, the volume of corn oil used for dilution for the treated animals was considerably less than that given to the controls. The low plasma alkaline phosphatase activities found in the treated groups may have been a consequence of the smaller volume of corn oil used in the treatment groups. The significance of the slightly lower sodium concentrations in the treated males, the nonsignificant elevation of alanine amino-transferase activities after six weeks of treatment in males given 4500 mg/kg/day and the high urea concentrations after 12 weeks of treatment in females at the highest dose is unclear, but is not an adverse effect.

Initial determination of the concentration of lycopene found in the bloodstream after treatment with Lyc-o-mato[®]6% for 13 weeks suggests that the absorption of Lyc-o-mato[®]6% was similar at both 450 and 4500 mg/kg/day. Therefore, 450 mg/kg/day of Lyc-o-mato[®]6%, although not a toxic level, may represent a level at which plasma concentrations of lycopene in the circulation are maximal. Based on the findings of this subchronic study, no definitive evidence of toxicity has been revealed and, the no-observed-adverse-effect level (NOAEL) of Lyc-o-mato[®]6% is 4500 mg/kg/day (270 mg lycopene/kg body weight/day). This amount is in agreement with a previous study utilizing synthetic lycopene, which determined a NOAEL level of approximately 300 mg lycopene/kg body weight/day (Mellert *et al.*, 2002). A recent study with synthetic lycopene tested oral dosing of lycopene up to 600 mg/kg/day for 14 weeks in the rat, with no overt toxicity findings displayed (Jonker *et al.*, 2003).

No deaths occurred, nor any signs of systemic toxicity or abnormalities were noted at necropsy, at the 2000 mg/kg dose evaluated in the current acute dermal toxicity study in the rat. Hemorrhaging of the dermal capillaries was noted at the treatment sites of two animals 2-4 days after dosing, but no other signs of skin irritation were noted. Therefore, the acute dermal median lethal dose (LD₅₀) of Lyc-o-mato[®]6% in the Sprague-Dawley rat strain was found to be greater than 2000 mg/kg bodyweight.

Lyc-o-mato[®]6% tested on the rabbit was found to lack dermal irritation, while having moderate eye irritant capabilities (Table 3). Evaluation of the sensitizing ability of Lyc-o-mato[®]6% on guinea pig skin found slight to well-defined erythema and very slight to slight edema formation, classifying Lyc-o-mato[®]6% as a moderate sensitizer.

Lyc-o-mato[®]6% contains 6% lycopene from tomatoes, which is known to be a potent antioxidant (Di Mascio *et al.*, 1989, Miller *et al.*, 1996). Stahl *et al.* (2001) has shown that dietary addition of tomato paste inhibits photooxidative stress formation (as measured by erythema) induced by UV-irradiation. A previous paper reported lycopene preventing oxidative DNA damage in green monkey kidney fibroblasts by ferric nitrilotriacetate *in vitro* at extracellular concentration of $2.6 \pm 0.6 \mu\text{M}$ (Matos *et al.*, 2000). In the current studies, Lyc-o-mato[®]6% was tested in six different strains of bacteria for mutagenic capability, both with and without metabolic activation of the compound and, was found to be non-mutagenic under the conditions initially set forth by the Ames test (Table 4). In fact, the higher doses of Lyc-o-mato[®]6% (2500 and 5000 $\mu\text{g}/\text{plate}$) decreased the number of revertant mutations to below control levels. Lycopene (*via* a tomato puree) ingestion in humans has been shown to effect cellular antioxidant capacity by decreasing lymphocyte DNA damage by 33-42% after *ex vivo* treatment with hydrogen peroxide (Riso *et al.*, 1999).

The current consumption analysis done presently of all individuals that consume one or more of the lycopene-containing foods listed in Table 1 indicates that the mean daily intake (90th percentile) for lycopene is estimated to be 8.2 (15.7) mg/day. Foods that contributed significantly to this level of lycopene consumption were (1) spaghetti with tomato sauce meatless, (2) tomato catsup, (3) watermelon raw and (4) tomatoes raw. These foods contain relatively high amounts of lycopene (30-170 $\mu\text{g}/\text{g}$ food), consumption is at a relatively high level (8.3-36.6 g/day) and are consumed by a significant proportion of the total individuals (3.4-53.8%). The estimated daily intake for lycopene in the current analysis is comparable to the lycopene daily intake reported by Schweitzer *et al.* (2002), which is 5.08 mg/day and based specifically on "tomatoes and tomato catsup." One possible reason for the slightly higher daily intake in the current analysis is the inclusion of many additional foods known to contain lycopene, which may be more accurate in evaluating daily consumption of lycopene.

5. Conclusion

Lyc-o-mato[®]6% showed no significant acute toxic effects, with an LD₅₀ in excess of 5000 mg/kg body weight. Acute dermal toxicity (LD₅₀) in the rat was found to be greater than 2000 mg/kg bodyweight. Subchronic toxicity testing in the rat concluded the NOAEL to be greater than 4500 mg/kg bodyweight. Based on this NOAEL from a subchronic study in rats, a tolerable intake of Lyc-o-mato[®]6% in humans can be calculated. Utilizing a safety factor of 10 for intraspecies differences and a factor of 10 for interspecies differences, a tolerable intake for

ingestion of Lyc-o-mato®6% by humans of 45 mg/kg *per day* is posited. Lyc-o-mato®6% lacks dermal irritation in the rabbit, while eliciting moderate eye irritation in the rabbit and moderate sensitizing capability in the guinea pig. Mutagenic toxicity as tested in the Ames assay determined that Lyc-o-mato®6% was non-mutagenic at the highest dose tested (5000 µg/plate).

Given the centuries of consumption of tomatoes and tomato products and, that no significant oral toxicity findings were observed in these studies, it can be concluded that Lyc-o-mato®6%, a tomato-based oleoresin containing 6% lycopene, can be considered safe for human oral consumption at a level of 45 mg/kg/day.

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Table 1. Consumption analysis of daily lycopene intake

Food Description	Lycopene (µg/g)	Consumption of Food (g/day)	% Individuals Eating The Food
Beef Stew w/potatoes & vegetables (carrots and dark green vegetables), in gravy	3.02	7.17	1.44
Beef sliced with vegetables & potatoes in sauce (Frozen meal)	2.85	0.02	0.01
Meatloaf in tomato sauce with potatoes & vegetables (Frozen meal)	9.30	0.18	0.06
Pizza with meat, thin crust	44.49	18.06	10.83
Pizza with meat & vegetables thin crust	20.71	8.45	4.10
Spaghetti with tomato sauce, meatless	159.90	15.62	3.38
Past with tomato sauce & cheese, canned	31.62	4.89	1.58
Lasagna with cheese & meat sauce (diet frozen meal)	77.5	0.19	0.07
Grapefruit, raw (include grapefruit, nonfrozen)	14.62	8.34	4.33
Apricots, raw	0.05	0.12	0.22
Apricots, cooked or canned, drained solids	0.65	0.08	0.12
Persimmons, raw	1.58	0.37	0.09
Watermelon, raw	48.68	24.28	6.75
Tomatoes, raw	30.25	36.55	53.84
Tomatoes cooked from fresh, no specifics as to method	44	0.53	0.63
Tomatoes, canned, low sodium	97.08	0.15	0.03
Tomato & vegetable juice, mostly tomato (including V-8 [®] juice)	96.6	3.49	1.28
Tomato catsup	170.08	8.28	38.42
Tomato sauce	159.16	1.62	2.35
Tomato paste	293.3	0.05	0.05
Tomato puree'	166.7	0.09	0.12
Tomato soup, canned, undiluted	109.2	0.13	0.07
Green peppers & onions, cooked (fat added in cooking)	30.92	0.37	0.61
Vegetable soup, canned, undiluted	19.3	0.03	0.01
Minestrone soup, home recipe	14.8	1.14	0.32
Vegetable beef soup prepared with water	3.64	2.46	0.66

Figure 1. Group mean bodyweight vs. period of treatment with Lyc-o-mato®6%

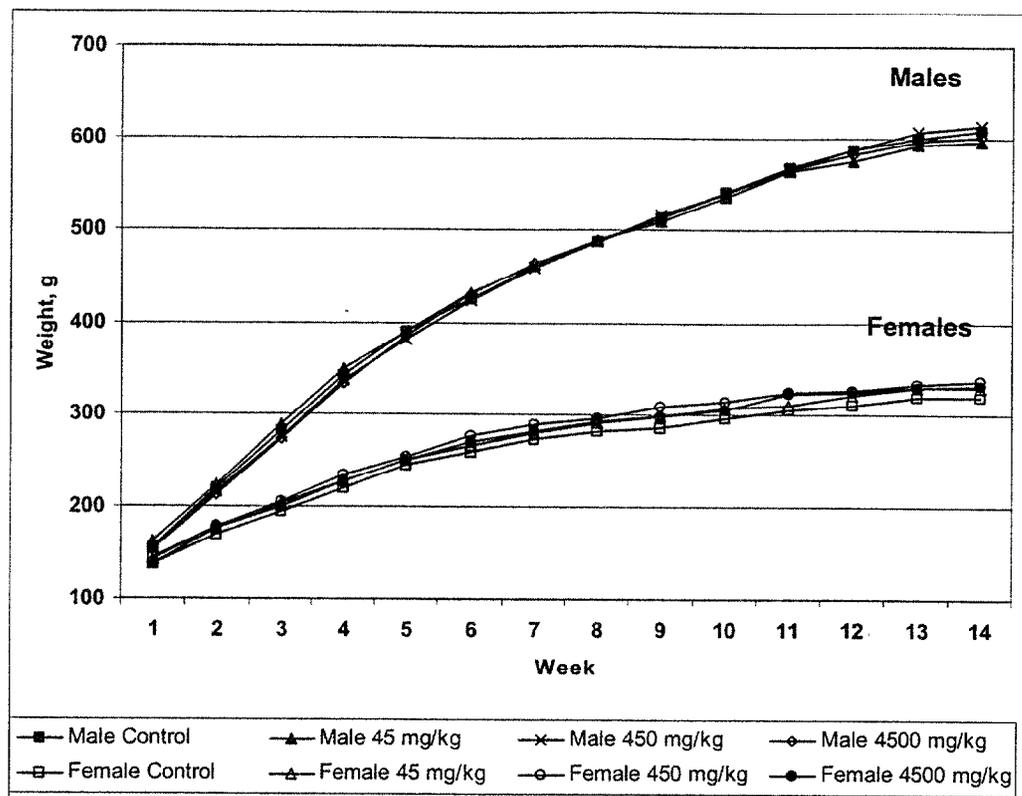


Table 2. Blood Chemistry Values of Rats Treated 13 Weeks with Lyc-o-mato@6%

Blood Chemistry	Males (mg/kg/day)				Females (mg/kg/day)			
	Control	45	450	4500	Control	45	450	4500
AP (iu/l)	292±161	258±150	231±44	208±50	134±83	129±30	104±27	111±64
ALT (iu/l)	36±7	38±7	37±3	41±6	35±7	32±4	29 ^b ±2	37±6
AST (iu/l)	71±12	77±9	69±8	68±15	67±12	65±12	58±9	68±10
GGT (iu/l)	1±1	1±1	1±1	1	1±1	1±1	1	1±1
Urea (mg%)	17±2	15±2	16±3	18±4	23±3	24±3	24±4	27 ^a ±2
Glucose (mg%)	113±12	115±12	114±8	117±9	131±17	132±6	147 ^a ±17	142±13
Total Bilirubin (mg%)	0.11±0.03	0.11±0.03	0.10	0.11±0.03	0.17±0.05	0.18±0.04	0.18±0.04	0.13±0.05
Cholesterol (mg%)	66±13	70±12	65±9	62±8	70±7	67±8	66±11	76±15
Total Plasma Proteins (g%)	6.1±0.3	6.1±0.2	6.1±0.2	6.0±0.4	6.5±0.4	6.4±0.4	6.3±0.2	6.5±0.2
Albumin (g%)	3.8±0.2	3.9±0.1	3.8±0.1	3.8±0.1	4.5±0.4	4.3±0.3	4.2 ^a ±0.2	4.4±0.2
A/G ratio ("":1)	1.7	1.7	1.7	1.7	2.2	2.1	2.0	2.1
Sodium (mmol/l)	138±1	138±1	138±1	137±1	139±1	140±2	138 ^a ±1	139±1
Potassium (mmol/l)	3.9±0.2	4.1±0.2	4.1±0.2	4.1±0.2	3.7±0.2	3.7±0.2	3.5±0.2	3.6±0.2
Chlorine (mmol/l)	104±1	103±1	105±1	103±1	104±1	105±2	105±1	104±2
Calcium (mmol/l)	2.8±0.1	2.7	2.7	2.7 ^c	2.9±0.1	2.9±0.1	2.8±0.1	2.9±0.1
Potassium (mmol/l)	2.2±0.1	2.1±0.1	2.2±0.1	2.2±0.1	1.9±0.2	1.8±0.2	1.8±0.2	1.8±0.2

*Values ± Standard Deviation. Significant when compared with respective control group a-p<0.05; b-p<0.01; c-p<0.001. A/G=Albumin/Globulin ratio; ALT=alanine aminotransferase; AP=alkaline phosphatase activity; AST=aspartate aminotransferase; GGT=gamma-glutamyl transferase;

Table 3. Hematological Values of Rats Treated 13 Weeks with Lyc-o-mato®6%

Hematology	Males (mg/kg/day)				Females (mg/kg/day)			
	Control	45	450	4500	Control	45	450	4500
PCV (%)	45±1	45±1	45±2	46±2	43±1	43±2	42±2	44±1
HB (g%)	15.6±0.5	15.5±0.4	15.5±0.6	15.8±0.5	15.5±0.4	15.3±0.8	15.2±0.6	15.9±0.4
RBC (mil/cmm)	8.41±0.39	8.61±0.41	8.48±0.37	8.51±0.45	8.04±0.24	8.08±0.46	8.01±0.24	8.20±0.23
MCHC (%)	35±1	35±1	35	35±1	36±1	35±1	36±1	36±1
MCV (Cμ)	54±3	52±2	53±2	54±2	54±2	53±1	53±1	54±2
MCH (pg)	19±1	18±1	18±1	19±1	19±1	19±1	19	19±1
Total WBC (1000/cmm)	14.9±3.8	14.0±2.1	13.8±2.6	15.2±2.9	9.9±2.4	10.1±3.7	10.1±2.4	12.1±3.0
Platelets (1000/cmm)	930±101	972±98	936±113	1014±84	873±135	1017 ^b ±120	983 ^a ±80	964±96
PT (secs)	12.4±0.8	13.1±0.6	12.6±0.8	12.7±0.6	13.1±0.9	12.9±0.7	13.1±1.4	13.4±1.1

*Values ± Standard Deviation. Significant when compared with respective control group a-p<0.05; b-p<0.01; c-p<0.001.

HB=hemoglobin; MCH=mean cell hemoglobin; MCHC=mean cell hemoglobin concentration; MCV=mean cell volume; PCV=packed cell volume; PT=prothrombin time; RBC=erythrocyte count; WBC=white blood cells

Table 4. Individual Total Scores and Group Mean Scores for Ocular Irritation*

Rabbit number and sex**	Individual Total Scores At:				
	1 Hour	24 Hours	48 Hours	72 Hours	7 Days
28 Female	27	11	4	0	-
90 Female	39	27	11	2	0
104 Female	19	22	15	4	0
116 Female	17	4	0	0	-
118 Female	17	24	6	4	0
138 Female	17	11	2	0	-
Group Total	136	99	38	10	0
Group Mean Score	22.7	16.5	6.3	1.7	0.0
Rabbit number and sex	1 Hour	24 Hours	48 Hours	72 Hours	7 Days
26 Female	32	22	6	2	0
53 Female	19	34	32	16	0
56 Female	39	59	57	41	5
78 Male	39	57	53	41	0
80 Female	17	29	29	18	0
82 Female	34	34	23	16	0
Group Total	180	235	200	134	5
Group Mean Score	30.0	39.2	33.3	22.3	0.8
	1 Hour	24 Hours	48 Hours	72 Hours	7 Days

*= Based on the numerical evaluation given in Appendix I of Draize (1977). **= Each rabbit treated with 0.1 ml of test material.

Table 5. Number of bacterial revertants (number of colonies/plate) after incubation with

Strain	Dose ($\mu\text{g}/\text{plate}$)						Positive Control
	0	312.5	625	1250	2500	5000	
(-) TA100*	163.3	158.7	161.3	154.7	143.3	137.7	491.7
(-) TA1535*	35.3	13.0	22.7	21.7	14.7	14.0	166.3
(-) WP2uvrA-*	24.7	26.7	21.7	30.0	25.0	21.3	112.7
(-) TA98#	17.7	21.7	19.0	18.3	22.3	20.3	135.7
(-) TA1537#	12.3	14.0	13.0	14.0	14.0	13.7	339.3
(-) TA1538#	27.7	28.0	29.7	26.7	26.0	30.0	416.3
(+) TA100*	131.3	137.3	147.0	138.7	132.7	123.0	513.3
(+) TA1535*	27.0	12.0	10.0	10.3	12.7	7.7	195.7
(+) WP2uvrA-*	31.7	28.3	33.0	25.7	30.7	21.7	144.7
(+) TA98#	37.3	38.3	31.0	35.7	30.0	38.3	133.7
(+) TA1537#	15.0	16.3	17.0	15.3	13.7	12.7	104.7
(+) TA1538#	39.3	31.3	33.3	29.0	28.7	23.3	206.3

(-) = bacteria and compound incubated in absence of S9 mix; (+) = bacteria and compound incubated in presence of S9 mix; * = Base-pair substitution type mutation; # = Frameshift type mutation

U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
May 22, 2003

Agency Response Letter GRAS Notice No. GRN 000119

Herbert D. Woolf, Ph.D
Technical Manager
BASF Corporation
3000 Continental Drive North
Mount Olive, NJ 07828-1234

Re: GRAS Notice No. GRN 000119

Dear Dr. Woolf:

The Food and Drug Administration (FDA) is responding to the notice, dated November 13, 2002, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938, April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on November 20, 2003, filed it on November 27, 2003, and designated it as GRAS Notice No. GRN 000119.

The subject of the notice is synthetic lycopene. The notice informs FDA of the view of BASF Corporation (BASF) that synthetic lycopene is GRAS, through scientific procedures, for use as a direct food ingredient in breakfast cereals (ready-to-eat and cooked), drinks (juice drinks, energy drinks, and dairy fruit drinks), instant soup, low fat dressings, meal replacements, meatless meat products, nutrient bars, salty snacks, crackers and yogurt at levels ranging from 0.5 percent to 7.0 percent.

For clarity, in this letter FDA uses the terms "lycopene," "synthetic lycopene," and "natural lycopene" as follows:

- We use the term "lycopene" to denote the chemical entity identified as Chemical Abstracts Service Registry Number 502-65-8. We use this term when describing the inherent properties of lycopene, regardless of the source of the lycopene.
- We use the term "synthetic lycopene" to denote the crystalline lycopene that is the subject of the notice and is produced by chemical synthesis.
- We use the term "natural lycopene" to denote the pigment that is produced during biosynthetic processes in developing plant tissue, such as that of tomato.

As part of its notice, BASF includes the report of a panel of individuals (BASF's GRAS panel) who evaluated the data and information that are the basis for BASF's GRAS determination. BASF considers the members of its GRAS panel to be qualified by scientific training and experience to evaluate the safety of substances added to food. BASF's GRAS panel evaluated estimates of dietary exposure, method of production, and product specifications as well as published and unpublished studies. Based on this review, BASF's GRAS panel concluded that synthetic lycopene that meets its established food grade specifications is GRAS under the conditions of its intended use.

BASF describes generally available information about lycopene. Lycopene is an aliphatic hydrocarbon containing thirteen double bonds. Its molecular formula is $C_{40}H_{56}$. It is a member of the carotenoid class of compounds and is the most abundant carotenoid in ripe tomatoes. In tomatoes, as well as other fruits and vegetables, the all-*trans* isomer of lycopene predominates. However, storage, cooking, processing and exposure to light results in isomerization of the all-*trans* form to various *cis* forms.

BASF describes synthetic lycopene as a crystalline material derived from chemical synthesis and describes a three-stage process for this chemical synthesis. (1) Stage one produces an organic solution of C_{15} phosphonium methanesulfonate in dichloromethane (DCM), and stage two produces an organic solution of C_{10} dialdehyde in toluene. In stage three, the intermediates produced in stages one and two are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. Glacial acetic acid and deionized water are added, the mixture is stirred vigorously, the aqueous and organic phases are allowed to separate, and the organic phase containing DCM and crude lycopene is extracted with water. Methanol is added to the organic phase. DCM is removed via distillation under reduced pressure, the crude methanolic lycopene solution is heated and then cooled to a crystalline slurry that is filtered and washed with methanol, and the lycopene crystals are then recrystallized and dried under heated nitrogen. BASF notes that synthetic lycopene is stored under nitrogen or suspended in an aqueous solution containing antioxidants to prevent oxidation and isomerization of lycopene.

BASF prepares three commercial products from synthetic lycopene: Lycopene 10 Percent (tablet grade), Lycopene 10 Cold Water Dispersion (CWD), and Lycopene Dispersion 20 Percent (lycopene in vegetable oil). Lycopene 10 Percent (tablet grade) is a powder consisting of spherical particles of synthetic lycopene in a food starch coated matrix of gelatin and sucrose. The powder is stabilized with sodium ascorbate and ascorbyl palmitate and contains tricalcium phosphate as a flow-aid. Lycopene 10 CWD is a powder consisting of pulverized synthetic lycopene imbedded in a matrix of gelatin and glucose and stabilized with di-alpha-tocopherol, ascorbyl palmitate and ascorbic acid. Lycopene Dispersion 20 Percent is a liquid that contains pulverized synthetic lycopene dispersed in oil. BASF notes that the three commercial lycopene products may be used in any of the food products described in its notice, although each commercial lycopene product has a distinctive formulation characteristic that would be more suitable for certain food products. For example, BASF states that Lycopene Dispersion 20 Percent is more suitable for food products in which there is an oil/fat phase.

BASF provides product specifications for synthetic lycopene. These specifications include limits on total carotenoids, arsenic, lead, copper, zinc, and heavy metals, loss on drying and residue on ignition. BASF also provides specifications for the concentration of total carotenoids in the three commercial products. BASF provides typical values for percentages of *cis* and *trans* lycopene, but does not set specifications for these isomers. BASF reports that synthetic lycopene contains a minimum of 96 percent lycopene, although typical batches contain approximately 98 percent lycopene. BASF reports that the majority of lycopene in synthetic lycopene is in the form of *trans* isomers (70 to 84 percent) and notes that the *trans* isomer content reported for natural extracts of lycopene ranges from 67 to 98 percent. BASF also reports that some *cis* isomers of lycopene are also present in synthetic lycopene. BASF discusses lycopene-related substances and process residuals present in synthetic lycopene and identifies which substances are also present in extracts of tomatoes.

Using its proposed use levels and data from the United States Department of Agriculture 1994-1996 Continuing Surveys of Food Intakes by Individuals and 1998 Supplemental Children's Survey, BASF estimates that the intake of synthetic lycopene would be approximately 5 milligrams per person per day (mg/person/day) at the mean and approximately 11 mg/person/day at the 90th percentile. BASF notes that this estimate is comparable to the intake of lycopene from plant sources reported in the Third National Health and Nutrition Examination Survey and by the United States Department of Health and Human Services, National Center for Health Statistics, and Nutrition Coordinating Center.

BASF discusses published and unpublished studies regarding the potential toxicity of synthetic and natural lycopene. BASF describes results from a published 13-week oral toxicity study conducted in rats fed BASF's commercial synthetic lycopene products. BASF concludes that results of the study support a no-observed-adverse-effect-level (NOAEL) for synthetic lycopene of 324 milligrams per kilogram body weight per day (mg/kg bw/day). BASF notes that this amount is approximately 4000-fold higher on a body weight basis than the mean estimated dietary intake (EDI) of synthetic lycopene. BASF also states that no adverse effects were reported in an unpublished⁽²⁾ developmental toxicity study conducted in rats and rabbits fed BASF's commercial synthetic lycopene products, and that no mutagenic effects were observed in unpublished genotoxicity studies conducted with BASF's commercial synthetic lycopene products. In addition, BASF notes that no adverse effects were observed in several published clinical studies conducted with either BASF's commercial synthetic lycopene products or natural lycopene.

BASF discusses published human studies related to the bioavailability of natural lycopene and BASF's commercial synthetic lycopene products. BASF reports that results of these studies showed no adverse effects on the absorption of other carotenoids. BASF notes, however, that excessive intake of food or dietary supplements high in carotenoids or lycopene has been associated with carotenodermia⁽³⁾ and lycopodermia⁽⁴⁾, respectively.

Potential requirement for a color additive petition

In its notice, BASF notes that synthetic lycopene imparts color to food. As such, the use of synthetic lycopene in food products may constitute the use of a color additive under section 201(t)(1) of the Federal Food, Drug and Cosmetic Act (FFDCA) and FDA's implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), the term color additive means a material that is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source and that is capable (alone or through reaction with another substance) of imparting color when added or applied to a food; except that such term does not include any material which the Secretary,⁽⁵⁾ by regulation, determines is used (or intended to be used) solely for a purpose or purposes other than coloring. Under 21 CFR 70.3(g), a material that otherwise meets the definition of color additive can be exempt from that definition on the basis that it is used or intended to be used solely for a purpose or purposes other than coloring, as long as the material is used in a way that any color imparted is clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned. Given the construct of section 201(t)(1) of the FFDCA and 21 CFR 70.3(f) and (g), the use of a substance that is capable of imparting color may constitute use as a color additive in addition to use as a food additive or GRAS substance. For example, beta-carotene is both approved for use as a color additive (21 CFR 73.95) and affirmed as GRAS for use as a nutrient supplement (21 CFR 184.1245); in some food products, beta-carotene is used for both purposes. Importantly, if the use of synthetic lycopene constitutes use as a color additive within the meaning of section 201(t)(1) of the FFDCA and FDA's implementing regulations in 21 CFR 70.3(f) and (g), section 721(a) of the FFDCA requires premarket review and approval of that use by FDA. Under section 402(c) of the FFDCA, a food product that contains an unapproved color additive would be deemed adulterated.⁽⁶⁾

In its notice, BASF acknowledges that it intends to submit a color additive petition for uses of synthetic lycopene that would constitute use as a color additive, but is not explicit about its view on whether any of the uses already described in its GRAS notice would constitute use as a color additive. In a telephone conversation on February 25, 2003, between FDA and BASF, FDA requested that BASF present its view on this issue. In an amendment received by FDA on March 10, 2003, BASF presents its reasons for concluding that all of the intended uses

of synthetic lycopene would be exempt from the definition of color additive under section 201(t) of the FFDCFA and FDA's implementing regulations in 21 CFR 70.3(f) and (g). Importantly, FDA's response to GRN 000119 does not include any comment by FDA about BASF's view on this issue. If, after receipt of this letter, BASF has any specific questions about this issue, we recommend that you contact the Office of Food Additive Safety (OFAS), Division of Petition Review (HFS-265), 5100 Paint Branch Parkway, College Park, MD 20740. You can also reach this division by telephone at (202)418-3035

Potential labeling issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FFDCA), a food is misbranded if its labeling is false or misleading in any particular. Section 403(r) of the FFDCA lays out the statutory framework for a health claim. In describing the intended use of synthetic lycopene and in describing the information that BASF relies on to conclude that synthetic lycopene is GRAS under the conditions of its intended use, BASF raises potential labeling issues under these provisions of the FFDCA. These labeling issues consist of BASF's assertion that synthetic lycopene has physiological effects that BASF views as beneficial. If products that contain synthetic lycopene bear any claims about such benefits on the label or in labeling, such claims are the purview of the Office of Nutritional Products, Labeling, and Dietary Supplements (ONPLDS) in the Center for Food Safety and Applied Nutrition (CFSAN). OFAS neither consulted with ONPLDS on these labeling issues nor evaluated the information in BASF's notice to determine whether it would support any claims made about synthetic lycopene on the label or in labeling.

Standards of Identity

In its notice, BASF states its intention to use synthetic lycopene in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity. If you have any questions about the use of synthetic lycopene in standardized foods that would be marketed in the United States, you should contact the staff in ONPLDS, Division of Food Labeling and Standards, 5100 Paint Branch Parkway, College Park, MD 20740. You can also reach this division by telephone at (301)436-2375.

Conclusions

Based on the information provided by BASF, as well as other information available to FDA, the agency has no questions at this time regarding BASF's conclusion that synthetic lycopene is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of synthetic lycopene. As always, it is the continuing responsibility of BASF to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements. In particular, we note that any use of synthetic lycopene that constitutes use as a color additive requires premarket review and approval by FDA.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter, as well as a copy of the information in your notice that conforms to the information in proposed 21 CFR 170.36(c)(1), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at <http://www.cfsan.fda.gov/~lrd/foodadd.html>).

Sincerely,

/s/
Alan M. Rulis, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

(1) FDA notes that steps in the three stage process for the synthesis of synthetic lycopene have been described in U.S. patents (issued to BASF and other companies) and journal articles. FDA notes that the Wittig reaction scheme for carotenoid synthesis is generally known and has been described in many books.

(2) Although BASF does not describe this study as a published study, an article that describes this study is currently in press and available on the Internet.

(3) A reversible condition associated with a yellowish discoloration of the skin.

(4) A reversible condition associated with a deep orange discoloration of the skin.

(5)The Secretary of the Department of Health and Human Services (DHSS). The Secretary of DHSS has delegated the authority for this provision of the FFDC A to FDA.

(6)We note that section 721(b)(4) of the FFDC A provides that a color additive shall be deemed to be safe and suitable for the purpose of listing under section 721(b) of the FFDC A while there is in effect a published finding of the Secretary declaring that the substance is exempt from the definition of "food additive" because of its being generally recognized by qualified experts as safe for its intended use as provided in section 201(s) of the FFDC A. Importantly, FDA's response to GRN 000119 does not constitute a "finding of the Secretary" within the meaning of section 721(b)(4) of the FFDC A.

Food Ingredients and Packaging Summary of all GRAS Notices

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Department of Urology

David L. McCullough, M.D.
*The William H. Boyce
Professor and Chair
Prostate Disease and
Urologic Oncology*

William H. Boyce, M.D.
Professor Emeritus

Lloyd H. Harrison, M.D.
Professor Emeritus

R. Lawrence Kroovand, M.D.
Professor Emeritus

Dean G. Assimos, M.D.
Endourology/Laparoscopy

Ojas D. Shah, M.D.
Fellow, Endourology/Laparoscopy

M. Craig Hall, M.D.
Urologic Oncology

Peter E. Clark, M.D.
Urologic Oncology

Dominick J. Carbone, Jr., M.D.
Infertility/Impotence

Elizabeth A. Albertson, M.D.
*Female Urology
Neurourology/Incontinence*

Joel C. Hutcherson, M.D.
Pediatric Urology

Ross P. Holmes, Ph.D.
Urology Research Lab

Scott Cramer, Ph.D.
Urology Research

Clinical
Joyce Brown, R.N.
Nurse Manager
Telephone: (336) 716-4131
Fax: (336) 716-9042

Academic
Kitty Daniel Shoaf
Planning Coordinator
Telephone: (336) 716-5690
Fax: (336) 716-5711

April 9, 2003

Dr. Zohar Nir
VP Sales and Marketing
LycoRed Natural Products Industries, Ltd.
P.O. B. 320
Beer-Sheva 84102 ISRAEL

Dear Dr. Nir:

I wanted to give you an update regarding our study entitled "A dose escalating phase II trial of lycopene for biochemical relapse of prostate cancer following definitive local therapy." As you know, in this dose escalation study, we have treated six patients at 6 dose levels between 15 mg per day and 120 mg per day with lycopene (Lyco-o-Mato® Oleoresin). We have now completed enrollment of all 36 patients. The final results of this study, however, will not be available until December 2003 or January 2004.

As you know, patients are treated on this study a total of 12 months. As part of this clinical trial, we have monitored toxicity and tolerability. This is done at their monthly evaluations and also by way of laboratory studies including electrolytes, liver function studies, and CBC performed every 3 months. To date, we have found that even at the higher dose ranges, the product is well tolerated. I know of only one patient who was taken off study for a presumed treatment related adverse event. This patient developed loose stools/diarrhea that was felt to be potentially related to the study medication. Otherwise, we have not noted any significant toxicity, and it appears to be well tolerated.

Sincerely,



M. Craig Hall, M.D.
Associate Professor of Surgery (Urology)

MCH/cm

cc: J. Craig Rowlands, Ph.D.
Diplomat, American Board of Toxicology
Burdock Group
Toxicology & Risk Assessment
780 US Highway One, Suite 300
Vero Beach, FL 32962-1660

Wake Forest University Health Sciences

Medical Center Boulevard • Winston-Salem, North Carolina 27157-1094