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OPINION ON SYNTHETIC LYCOPENE AS A COLOURING MATTER FOR USE IN FOODSTUFFS

(expressed on 2 December 1999)

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Opinion on synthetic lycopene as a colouring matter for use in foodstuffs

(expressed on 2 December 1999)

Terms of Reference
To advise the Commission on the safety in use of synthetic lycopene as a food colour having particular regard to purity criteria in accordance with Commission Directive 95/45/EC (1).

Background
Lycopene is authorized as a food colour in the EC and listed as E 160d in Directive 94/36/EC (2). The present application (3) seeks an extension of the specifications laid down in Directive 95/45/EC, that is not less than 5% total colouring matters for lycopene obtained by solvent extraction from tomatoes, to include lycopene produced by chemical synthesis containing not less than 96% lycopene.

The Committee has previously accepted the use of lycopene prepared from natural sources by physical processes, provided that the amount consumed does not differ significantly from the amount consumed through the relevant foodstuffs. An ADI, however, could not be derived. If the use of lycopene is widened or the substance can be produced by chemical synthesis, an evaluation on the basis of a modern toxicological data base was considered necessary (4, 5).

Intake
On the basis of the proposed use of synthetic lycopene in soft drinks, confectionery and ice cream with maximum levels of 25 ppm, 50 ppm and 50 ppm, respectively, the petitioner estimated an average intake of about 0.5 mg/day lying in the same range as the intake from the normal diet. The Committee considers that this could be a considerable underestimate, should consumers select products containing this colour.

Chemical aspects
Lycopene (C.I. No. 75121) is practically insoluble in water and most organic solvents and sensitive to oxygen and light. In contrast to the material prepared from tomatoes which has a low lycopene content, namely not less than 5% of total colouring matters, and contains other pigments, oils, fats, waxes, and naturally occurring flavour components, the synthetic lycopene is at least 96% pure. It can contain, however, up to 0.3% of the by-product lycopene-C_{25}-aldehyde. The synthetic lycopene also differs in the composition of geometrical isomers. While lycopene in tomatoes consists of 94-96% all-trans, 3-5% 5-cis, 0-1% 9-cis, 1% 13-cis, and <1% other cis isomers, the composition of the synthetic lycopene is at least 70% all-trans, at most 25% 5-cis, 1% 9-cis, 1% 13-cis, and 3% other cis isomers.

Synthetic lycopene is stabilized by the preparation of a special beadlet formulation, containing about 10% lycopene, 1.5% and 5.0%, respectively, of the antioxidants dl-α-tocopherol and ascorbyl palmitate as well as a number of carrier substances (peanut oil, fish gelatin, sucrose and corn starch). Due to the presence of high concentrations of antioxidants, this formulation is stable over 36 months at 5 and 25 ^{o}C. For the same reason, synthetic lycopene in beverages

coloured with the beadlet formulation is relatively stable if the beverages are stored under normal conditions (7% loss after 12 months). However, storage of beverages under intensive light conditions results in a substantial loss of the initial lycopene content (22% after 4 weeks).

Toxicological aspects

The available toxicity data on lycopene include data from acute toxicity studies in mice using an unspecified material that produced no relevant effects after oral, i.p. and s.c. administration of 3000 mg/kg b.w. (6).

In a 4-week feeding study with rats the beadlet formulation was tolerated at a daily dose of 1000 mg lycopene/kg bw without relevant adverse effects. A red discolouration of the faeces and a brown-orange discolouration of the liver were observed, the latter being due to deposition of a brown-yellow fine granulated pigment in hepatocytes. Pigment deposition was more pronounced in females but not associated with morphological alterations. Slight effects on food consumption and body weight development were not considered substance-related. The contaminant lycopene-C_{25}-aldehyde neither produced additional effects nor did it influence the lycopene effects when tested in a diet achieving a dose of 20 mg/kg b.w./day together with 1000 mg lycopene/kg b.w./day.

The beadlet formulation was well tolerated and did not produce relevant toxic effects in a 14-week feeding study with rats up to the highest dose of 500 mg lycopene/kg b.w./day. Deposition of lycopene or a metabolite resulted in a dose-related orange-red discolouration of the liver and adipose tissue, again more pronounced in females, with only limited elimination of these deposits after a recovery period of 5 weeks. Slight variations in haematological and clinical chemical parameters were observed in the high and occasionally the mid dose (150 mg/kg b.w./day). Changes in the relative weight of the thyroid and brain at the high dose were found in one sex only. They were not associated with morphological alterations and did not persist beyond the recovery period.

A series of special studies on pigment deposition in rat liver over up to two years confirmed the absence of gross and microscopic liver changes associated with pigment deposition. After 13 weeks of depletion, the liver deposits disappeared completely.

Pigment deposition in the liver was also found in older feeding studies with rats and one dog and in a human individual who had consumed very high doses of lycopene through natural tomato foodstuffs over a long period of time (7, 8).

In a study on embryotoxicity and teratogenicity in rats, the beadlet formulation at a dose of 1000 mg lycopene/kg bw/day, administered from day 6-18 of gestation, induced no relevant effects apart from an increase in the number of complete additional thoracic ribs (14th rib) in the pups. Lycopene-C_{25}-aldehyde co-administered at a dose of 20 mg/kg b.w./day did not induce substance-related effects. The study, however, was designed to allow just a rough risk estimation of the aldehyde in lycopene and for this reason was not performed in accordance with the OECD- and EU-guidelines concerning the number of pregnant animals/group.
Non-GLP in vitro examinations in rat limb bud cell cultures revealed a "marginal teratogenic activity" of the metabolic degradation products of lycopene-C_{25}-aldehyde and a "weak positive reaction" of the lycopene-C_{25}-acid. In a nuclear retinoid receptor binding and activation assay with the aldehyde and the acid, no activity was observed.

In the mammalian-microsome reverse mutation assay with Salmonella (Ames Test) and E. coli, synthetic lycopene of a high degree of purity of >99% was negative or showed at most only a marginal positive trend, whereas lycopene of 96% purity gave clearly positive results that were most pronounced in strains TA100 and TA97 without metabolic activation (S9 mix). Additional examinations suggest that mutagenic degradation products are formed by oxidative processes during storage of the unformulated product.

The beadlet formulation was negative in the Ames test, in a gene mutation assay with E. coli, in the mouse lymphoma assay, in a test on chromosome aberrations with human peripheral blood lymphocytes, in the mouse bone-marrow micronucleus test and in a study on unscheduled DNA-synthesis in rat liver cells after in vivo administration. In addition, no mutagenicity was observed in an Ames test with 0.19% lycopene-C_{25}-aldehyde in a beadlet preparation of synthetic lycopene and in a micronucleus test in peripheral blood of mice who received a softdrink containing 25 or 50 ppm synthetic lycopene.

In toxicokinetic studies, less than 10% of an orally administered dose of radiolabelled lycopene was absorbed in rats and monkeys. Most of the absorbed material was rapidly eliminated and only small amounts of residual radioactivity were found in several organs and tissues, most of it in the liver. Accumulation of lycopene in the liver was higher in rats fed beadlet lycopene compared to those fed tomato preparations which could be due to a higher bioavailability of synthetic lycopene compared to natural lycopene. In ferrets, cis-isomers of lycopene are more bioavailable than trans-lycopene (9). In humans, relatively high tissue concentrations of lycopene were reported for the adrenal glands, testes, liver and prostate (10).
The ratio of cis/trans isomers in human plasma was different from that of synthetic lycopene but similar to that in cooked tomato based foodstuffs.

Conclusion
The proposed specification ‘not less than 96%’ lycopene is not acceptable because highly concentrated lycopene is sensitive to oxygen and light, forms degradation products with mutagenic activity, and is not identical with the beadlet formulation that has been tested toxicologically. Furthermore, the toxicological data provided on the beadlet formulation are insufficient. The embryotoxicity study is regarded as inadequate as, in the words of the petitioner, it was designed to allow only a rough risk estimation and not to meet international guidelines. A teratogenicity study on a second species as well as a multigeneration and chronic toxicity/carcinogenicity studies are also lacking. In accordance with its earlier opinion, the Committee reitarates that synthetic lycopene can only be evaluated on the basis of a modern toxicological database. Therefore the Committee is not able to allocate an ADI and considers its use in food is unacceptable at present.

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