

Opinion On A Request For The Use Of Algal Beta-Carotene As A Food Colour (Expressed On 13th June 1997)

Terms of reference

To advise on the safety in use as a food additive of a beta-carotene preparation produced from *Dunaliella salina*

Background

A first petition has been introduced for the addition to the list of food colours authorized in the E.U. of beta-carotene produced in a bio-reactor by an uni-cellular alga, *Dunaliella salina*. The Committee had given a first opinion on the 10 December 1987 (21 st series of reports) in which it asked more information about the composition of the product and on the process used for its preparation. In 1991, the additional information provided were not considered satisfactory to judge on the safety of the product and this first petition was withdrawn. In 1992, a new request was made by another petitioner for the same "natural beta-carotene" also produced by *Dunaliella salina*, but grown in large saline lakes located in Whyalla, South Australia. By 1997 the information available was considered sufficient by the Committee to give the following opinion.

Safety evaluation

1. Composition

The beta-carotene preparation is a dispersion in a soya bean oil (containing 0.3% natural tocopherol) at a concentration of 20-30%. It is composed of all-trans and of cis- (mainly 9- but also 13- and 15-) isomers. The respective concentrations of trans- and cis- isomers has been found in the range of 50/50 - 71/29. The other major components are alpha-carotene and oxygen containing carotenoids. It also contains minor constituents such as sterols, hydrocarbons, free fatty acids, oxidized carotenoids and chlorophyll. 0.5% of the product before the addition of soya oil remains unidentified. The heavy metals content is very low (10 ppm expressed as Pb).

Microbiological contamination could not be found in the samples analyzed (total count, E. coli, yeast and mould not detected in 0.1g, Salmonella not detected in 10g).

2. Stability

The Committee was satisfied that the beta-carotene preparation was stable

3. Production process

Dunaliella salina grows in 30% salt concentration, coupled with high light intensity and high temperature. The Committee was informed that beta-carotene content of the culture, pH and brine density are measured on a daily basis. Weekly controls include chemical analysis of the algal cells and of the brine as well as a microscopic examination of the cells. The data provided demonstrate a batch to batch consistency. Harvesting of the algal cells and concentration of the beta-carotene from the cellular components involve only physical processes. Isolation of the beta-carotene is obtained after several steps; breakdown of protein and carbohydrate into soluble components by food-grade enzymes, extraction of the beta-carotene using an essential oil, saponification, partial crystallisation without isolation of carotene crystals and finally oil dispersion of the beta-carotene material and adjustment of its concentration.

4. Toxicological data

Dunaliella salina belongs to the Chlorophyceae. It is considered that this uni-cellular alga, like 25 other species of Chlorophyceae which are classified as food sources, does not produce toxins

Acute toxicity : LD50 of a preparation containing 30% of beta-carotene is higher than 20g/kg b.w. in the mouse.

Short-term toxicity : several studies have been conducted during 2 to 8 weeks on rat and chicken with a powder of the alga *Dunaliella bardawil* which the Committee has been informed is identical to the species *Dunaliella salina*, without signs of toxicity up to the equivalent of 0.1% of beta-carotene in the diet.

Multigeneration reproduction study : no toxicity has been assigned to a powder of *Dunaliella bardawil* ingested by the rat up to 10% in the diet (equivalent to 0.2% of beta-carotene).

Genotoxicity : the algal beta-carotene was unable to induce gene mutation or chromosomal aberrations in two Ames tests and one chromosome aberration assay in human lymphocytes.

Data obtained on the algal powder provide reassurance of the absence of toxicity of the components other than beta-carotene. This is important for the safety evaluation of the beta-carotene preparation in the absence of a total crystallisation of beta-carotene.

Conclusion

On the basis of the information provided, the Committee consider that the use of a dispersion of beta-carotene produced by the alga *Dunaliella salina* growing in large, shallow saline lakes in Whyalla, South Australia, is acceptable as a food additive. This opinion is expressed on the basis of a maximum use level of this preparation around 50ppm (equivalent to around 10ppm of beta-carotene).

This conclusion is valid only for the beta-carotene produced in the conditions and at the sites described in the dossier provided by the petitioner which provided reassurance on the composition and relative purity of the material. A specification should be developed which complies with all the above information and recommendations.