Opinion on Canthaxanthin (expressed on 13 June 1997)

Terms of Reference

To re-evaluate the safety in use of canthaxanthin as a food additive in the light of additional information received.

Background

Canthaxanthin was considered by the Committee for the first time during its comprehensive review of the Colouring Matters in Food in 1983 (1), when the Committee established an ADI of 0-25 mg/kg b.w./day. Subsequently new data, derived from studies on individuals taking canthaxanthin for therapeutic, dermatological or cosmetic purposes (e.g. as sun-tan producing agent), showed that the high doses employed for these purposes could induce the formation of crystalline deposits in the retina, eventually identified as canthaxanthin. These data pointed to a lowest effect level in man of 30 mg/day (0.5 mg/kg b.w./day). Consequently, in 1989, the Committee changed the existing ADI to a temporary ADI of 0-0.05 mg/kg b.w./day, using a safety factor of 10 because of the availability of human data (2). The Committee also requested the provision of certain additional information during the next 5 years to elucidate more precisely the pharmacokinetics underlying this deposition of canthaxanthin in the retina of both animals and man and to establish, whether any functional visual effects were associated with these findings. Further information supplied by the manufacturer to a national advisory committee together with their assessment of this information was later communicated to the Committee. Following its own assessment in 1992 of this new information, the Committee decided to leave unchanged the temporary ADI of 0-0.05 mg/kg b.w./day (3), the validity of which it had limited in 1990 to a period of 5 years. Additional information has now been submitted to support a reconsideration of the CommitteeÂ's 1992 assessment. The same information was reviewed by JECFA in 1995, which established an ADI of 0.03 mg/kg b.w (4).

Information submitted since the 1989 review

Animal studies

Long-term feeding studies in rats, extending over 104 weeks, were carried out recently as two separate studies in males and females using dose levels ranging from 5-250 mg/kg b.w./day (5). Earlier studies in the mouse extended over 98 weeks (6) and in the dog over 52 weeks (7). None of these studies showed any evidence of crystal deposition in the retina of the test animals or any other ocular pathology. The rat studies showed increased liver weights both in males and females with associated biochemical changes in the males and histological changes in the liver of males and females interpreted as evidence of a possible hepatotoxic effect. The NOAEL in these rat studies was 5 mg/kg b.w./day. No specific adverse effects were seen in the mouse apart from pigment deposition in hepatic sinusoidal cells. No changes in tumour incidence were noted even at 1000 mg/kg b.w., the highest dose level tested. The dog study showed only that the adipose tissue contained the highest canthaxanthin concentration, followed by the adrenals and the liver.

A three year feeding study in cynomolgus monkeys, using doses ranging from 0.2 to 1000 mg/kg b.w./day, revealed no treatment-related effects on body weight gain, food consumption, haematology, clinical biochemical parameters and urinalysis. The only treatment-related macroscopic changes were orange-red discolourations of the gastrointestinal mucosa, the adipose and the connective tissues. No unusual findings were noted in organ weights and the histopathology of all organs examined showed no evidence of any systemic target organ toxicity. However, examination of the retinae showed birefringent inclusions especially in the peripheral regions. At the lowest dose of 0.2 mg/kg b.w./day no inclusions were seen. Canthaxanthin concentrations in the retina correlated with the presence of birefringent inclusions. These and the microscopic crystalline inclusions in the liver were shown by chemical analysis to be associated with canthaxanthin. No functional visual impairment was associated with these inclusions. The NOAEL for systemic toxicity was therefore 1000 mg/kg b.w./day and for observable inclusions in the retina it was 0.2 mg/kg b.w./day (8).

Recent pharmacokinetic and metabolism studies in the cynomolgus monkey, using 14C-labelled canthaxanthin showed plasma concentrations to be higher than those in the blood with peak values occurring in males within 4 hours and females within 6 hours. Faecal excretion was the major route of elimination amounting to 85%-90%, urinary excetion accounted for 1.6%-3.6%, while tissue retention ranged from 1.6% to 4.6% and occurred preferentially in the adrenals. Monkeys therefore appeared to absorb from 3%-8% of orally administered. canthaxanthin. (9).

In a study comparing the pharmacokinetics in rats and monkeys under steady state conditions the urinary excretion was faster in the rat, some 4.6% being found in the rat urine after 96 h compared to 2.1% in the monkey urine over the same period. Some 91.4% of the administered dose was recovered from the rat faeces after 96 h compared to 87.2% after the same period in monkey faeces. Overall tissue residues after 96 h amounted in the rat to about 0.8%, accumulating mostly in the speen and liver. whilst the equivalent figure for the monkey after 96 h was 5.1% with accumulation being highest in the adrenals. Excretion and metabolism were thus faster in the rat than in the monkey. In the rat the urinary metabolites contained some very polar compounds which were present only in trace amounts in monkeys, while monkey urine contained some less polar compounds absent from rat urine. In the monkey retina 4Â'-OH-echinenone and isozeaxanthin were identified as metabolites. The urinary metabolites have not been identified so far in both species. The mean plasma level of canthaxanthin in the monkey at the NOAEL of 0.2 mg/kg b.w. was 156 µg/L (10).

In a search for appropriate animal model species to study crystal deposition of canthaxanthin in the retina, various other species were exposed to this colourant. Thus, the ferret was found to be unsuitable as no canthaxanthin residues could be detected in any of its tissues (11). 5 different mouse strains were examined but little deposition in the retina could be detected and the distribution differed from that found in humans. However some functional ERG effects were noted which were however reversible (12). Birefringent crystals accumulated in a dose-related manner in the retina of broiler chicken exposed to canthaxanthin, correlating also with the plasma concentrations (13). Guineapigs accumulated the colourant in their retina after some 10 months treatment (14).

Canthaxanthin administered to rats at a dose of 300 mg/kg diet (15 mg/kg b.w.) for 15 days increased the concentration of hepatic P450 and thereby the activities of the hepatic isoenzymes P4501A1 and P4501A2. There was co-induction of the microsomal Phase II enzyme activities of p-nitrophenol- and 4-hydroxybiphenyl-UDP glucuronyl transferases as well as quinone reductase, an effect reminiscent of 3-methylcholanthrene inducing activity (16).

In vitro studies

Neuronal retina reaggregate cultures, derived from chick embryos, were found to be a suitable system to study cellular crystal deposition. Red-brown birefringent entities formed in these cells in proportion to the amount of canthaxanthin in the culture medium. No accumulation occurred at 120 µg/L medium but crystals appeared at 1200 µg/L medium (15).

Human ophthalmological data

A retrospective biostatistical analysis of 411 cases of canthaxanthin treatment either for cosmetic or therapeutic reasons, for which sufficient relevant information was available, indicated that 95 individuals had a crystal retinopathy. This finding occurred only when high doses had been ingested over long periods and a dose-response relationship was discernible. A dose of 30 mg (0.5 mg/kg b.w.)/day or a cumulative intake over a prolonged period of 3 g/person appeared to be the lowest effective dose (LEL) causing crystal deposition in the retina. No such deposits were reported with exposures of less than 30 mg/day. Doses > 105 mg (1.75 mg/kg b.w.)/day led to crystal deposition in the retina in 50% of cases (!7).

Some reversibility of the deposits of canthaxanthin in the retina was noticed in patients who, over 12 years, had accumulated these deposits and had thereafter discontinued treatment for at least 5 years (19).

No hepatotoxicity was detected in 11 patients treated for up to 12 years for erythropoeitic protoporphyria with cumulative doses up to 150 g (20).

An earlier review of the ocular toxicity of canthaxanthin in persons with a high intake of canthaxanthin and deposits in

their retina had demonstrated, that this phenomenon was not associated clinically with any significant adverse functional visual defects. However, a reproducible and reversible small decline in the scotopic ERG b wave amplitude at higher light intensities without any change in peak latency was detected. This small functional effect was:

not detectable by psychophysical tests;

always associated with normal visual acuity and colour vision but occasionally with minimally reduced threshold static perimetry and mild reversible delayed dark adaptation;

not due to retinopathy with loss of retinal sensitivity because the ERG kinetics and the ERG a waves (parameter for photoreceptor activity) were unaffected and weak stimuli did not induce any ERG b waves (parameter for general retinal damage);

due to a depolarization of the glial Müller cells, because crystals were found near the end feet of the Müller cells near the inner surface of the retina. These crystals were eventually voided into vacuoles and either phagocytosed by cells of the retinal pigment epithelium or disappeared very slowly from the retinal surface due to the absence of an active removal process

These changes were not considered to be of pathological significance or indicative of significant functional damage to the retina. In this review the LEL for effects on the ERG b wave was estimated to lie between 0.25 - 1 mg/kg b.w (18).

Human intake estimates

According to Directive 94/36/EEC the use of canthaxanthin as a food additive is restricted to the colouring of Saucisse de Strasbourg. The Committee is aware that it is also used as an animal feed additive for poultry and fish and that residues may therefore be present in egg yolks and farmed trout and salmon. Canthaxanthin also occurs naturally in certain wild mushrooms. Reliable intake estimates are not available at present but data suggest that 0.2 mg/egg and 0.1 mg/100g fish are representative residue levels. In view of the low ADI it is particularly important that intakes from all sources should be taken into account in any future risk assessment.

Conclusion

The lowest effect level for ERG b wave changes in man was 0.25 mg/kg b.w./day but in view of the fact that these changes were not of pathological significance or indicative of significant functional damage to the retina, a safety factor of 10 is considered appropriate. This is supported by the finding of a one order of magnitude difference between the plasma level (156 µg/L) at the NEL in monkeys and the *in vitro* concentration (1200 µg/L medium) first showing the presence of cellular microcrystal formation in neuronal retina reaggregate cultures. An ADI of 0.025 mg/kg b.w., rounded up to 0.03 mg/kg b.w., can therefore be established

The Committee considers that up-to-date information should be obtained on human intake from the use of canthaxanthin in animal feeds to give assurance that total exposure by this route would not exceed the ADI.

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