7. Other Toxic Effects

7.1 Toxic and other adverse effects

7.1.1 Humans

Most of the available data on the safety of carotenoids concern β-carotene. Studies of toxicity in experimental animals have shown that β-carotene is not mutagenic or teratogenic. Doses of 20–180 mg/d β-carotene given for many years have been used to treat patients with erythropoietic protoporphyria, with no evidence of toxicity and without the development of abnormally elevated blood vitamin A concentrations (Mathews-Roth, 1986; Meyers *et al.*, 1996).

The conversion of β-carotene and other provitamin A carotenoids is regulated by the vitamin A status of individuals. Thus, high intakes of carotenoids do not lead to abnormally high vitamin A concentrations or symptoms of hypervitaminosis (Olson, 1994). Hyper-carotenaemia, or high serum concentrations of carotene, may occur when people take supplements containing 20 mg or more of β-carotene for extended periods. Hypercarotenaemia has also been seen in people who consume large quantities of food rich in β-carotene. People who take high concentrations of β-carotene supplements or who consume large quantities of carotene-rich foods may, in addition to having higher serum concentrations of β-carotene, develop yellow palms and soles, a condition technically known as hypercarotenodermia. This condition can be clearly differentiated from jaundice because the whites of the eyes (sclera) are yellow only in patients with jaundice. The change disappears with discontinuation of increased intake (Lascari, 1981). Hyper-carotenaemia can also be caused by a rare genetic inability to convert β-carotene to vitamin A (McLaren & Zekian, 1971; Monk, 1982) and is sometimes seen in association with hypothyroidism, diabetes mellitus and hepatic and renal disease. The hypercarotenaemia is a secondary condition and is not the cause of these diseases (Meyers *et al.*, 1996).

In the ATBC study, 11% more total cardiovascular deaths, including deaths from ischaemic heart disease, all types of stroke and other cardiovascular disease, were seen in the men taking β-carotene (Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group, 1994b). When the analyses were restricted to the 1862 participants who had previously had a myocardial infarct, men who received β-carotene alone had relative risks of 1.75 (95% CI, 1.16–2.64) for fatal coronary heart disease and 3.44 (95% CI, 1.70–6.94) for fatal myocardial infarct (Rapola *et al.*, 1997). The corresponding relative risks for those who received β-carotene plus α-tocopherol were 1.58 (95% CI, 1.05–2.40) and 2.67 (95% CI, 1.30–5.46). Similarly, an increased number of deaths from cardiovascular disease was seen in the CARET among men taking supplemental β-carotene plus retinol: the relative risk was 1.26 (95% CI, 0.99–1.61; Omenn *et al.*, 1996b).

Limited data exist on the toxicity of other carotenoids. Canthaxanthin is an approved food colour additive, but it has been used without regulatory approval for attaining a skin colour similar to a suntan. Excessive intake produces discoloured plasma and faeces, which probably has no physiological significance; however, crystalline deposits occurred in the retinas of all subjects ingesting 60 mg, and a change in retinal function after long-term treatment was observed in a few persons with such deposits (Weber *et al.*, 1992). The changes in retinal function are relatively minor, however, and are corrected over a period of months or years as the canthaxanthin crystals disappear (Arden & Barker, 1991; Weber *et al.*, 1992). Similar canthaxanthin retinopathy induced in monkeys was not associated with retinol

dysfunction or abnormal morphology (Goralczyk *et al.*, 1997).

In contrast, the retinas of patients with erythropoietic protoporphyria given high doses of β-carotene for up to 10 years showed no crystalline deposits. The dose of canthaxanthin required for this ocular response appears to be more than 30 mg/d (Köpcke *et al.*, 1995). The retinas of 26 patients with protoporphyria who received treatment with β-carotene (200 30-mg capsules per month for several months) for periods of 1–10 years were not found to have crystalline deposits; however, asymptomatic retinopathy, consisting of multiple, bright yellow, glistening crystalline deposits in and around the maculae, were observed in 6 of 50 patients who had ingested more than 200 30-mg doses of canthaxanthine as a photoprotectant and systemic skin colourant (Poh-Fitzpatrick & Barbera, 1984).

Lycopene is a more intensely coloured pigment than β-carotene. High lycopene intake also produces hypercarotenodermia; however, a deeper orange is usually observed than with β-carotene (Lascari, 1981).

There have been several other isolated reports of carotenoid-related toxicity; however, the findings are associated with very large intakes of foods containing β-carotene, among other constituents, or a genetic defect in the metabolism of carotenoids (Bendich, 1988; Ahmed *et al.*, 1994; Diplock, 1995; Olmedilla *et al.*, 1995; Svensson & Vahlquist, 1995).

7.1.2 Experimental animals

Few experimental studies of the toxicity of β-carotene *in vivo* have been reported. Many are quite dated and generally do not provide details of experimental outcomes. They are reviewed here as extensively as possible.

The toxicity of β-carotene, lycopene and bixin was studied in rats, rabbits and dogs given 7–1000 mg/d orally or intramuscularly by various schedules. No side-effects were reported; however, the number of animals was small and the histological examination rather simple (Zbinden & Studer, 1958). Similarly, in a multigeration study of rats receiving 0.1% β-carotene in the diet and in a study of toxicity in dogs receiving 0, 1, 10 or 100 mg/kg for five days per week for 13 weeks, no side-effects were seen (Bagdon *et al.*, 1960).

The results of a two-year study in dogs, a study of toxicity and tumorigenicity in rats and a study of carcinogenicity in mice were reported in an abridged paper. The livers of treated dogs and mice had some histological changes, with vacuolated cells, but these were considered to be fat storage cells and of no major toxicological importance (Heywood *et al.*, 1985). [The Working Group did not have access to numerical results or statistical analyses.]

Certain beadlet formulations of β-carotene may potentiate the hepatotoxicity of alcohol. Rats were given vitamin A and β-carotene for two months, with or without beadlets or corresponding amounts of beadlets without β-carotene. Ethanol enhanced the concentrations of hepatic β-carotene in the presence of beadlets and to a lesser extent in their absence. As the configurations of the beadlets differed for the various β-carotene preparations, further studies may be needed (Leo *et al.*, 1997).

7.2 Reproductive and developmental effects

Exposure of many laboratory species and humans during pregnancy to high doses of preformed vitamin A induces a variety of malformations (reviewed by Schardein, 1993; Friedman & Polifka, 1994). In contrast, β-carotene consumption has not been associated with teratogenicity in humans (Anon., 1987; Pinnock & Alderman, 1992; Nau *et al.*, 1994).

7.2.1 Humans

In a review of reports of elevated β-carotene concentrations and adverse birth outcome, it was noted that no malformations were mentioned in one study of 149 infants delivered to women with exceptionally high concentrations of carotene in blood, and several other studies of hypercarotenaemic women did not mention malformations in the offspring or noted that the infants were themselves carotenaemic; a few showed reversible desquamation of the skin. The author concluded that no abnormalities in humans were attributable to the carotenoid molecule (Matthews-Roth, 1988). A similar conclusion was reached in a review of the

literature on experimental animals and humans (Bendich, 1988) and in another, partial review of the same literature (Diplock, 1995).

No association was found between the β-carotene concentrations in the sera of women infected with the human immunodeficiency virus and vertical transmission of the virus to the fetus. The concentrations of β-carotene were 9.5 μg/dl in transmitters and 11.4 μg/dl in non-transmitters (Burger *et al.*, 1997).

The cryptoxanthin concentrations in the cord blood of human fetuses remained relatively constant, at 50 ng/ml, throughout gestation; however, the concentrations in cord blood were significantly higher in 18 infants with intrauterine growth retardation than in 65 with normal weight (Moji *et al.*, 1995).

No mention of any reproductive effects of lycopene in humans or experimental animals was included in recent reviews of the biochemistry and biophysics (Stahl & Sies, 1996) or potential human health effects (Gerster, 1997) of this carotenoid. No association was found between the lycopene concentrations in the sera of women infected with the human immunodeficiency virus and vertical transmission of the virus to the fetus. The concentrations of lycopene were 9.5 μg/dl in transmitters and 11.4 μg/dl in non-transmitters (Burger *et al.*, 1997)

7.2.2 Experimental animals

In one of the earliest studies of the toxicity of β-carotene, groups of 2–16 Wistar rats of each sex were exposed from 30–40 days of age to 0.1% in the diet for four generations. The offspring did not show signs of hypervitaminosis A syndrome (Bagdon *et al.*, 1960). [The Working Group noted the small sample sizes and the incomplete reporting of the results.]

In a review of studies of the effect of nutrient status on the reproductive efficiency of livestock, 12 studies of dairy cows supplemented with β-carotene showed positive effects, 10 studies showed no effect, and one study reported a detrimental effect (Hurley & Doane, 1989).

In a multigeneration test, groups of 15 male and 15 female rats were given β-carotene as 0.1% dietary mixture for 100 weeks. Three additional generations were produced; equal numbers of rats were maintained as controls. The sizes of the groups declined throughout the study due both to deaths unrelated to treatment and to sampling. No effects were observed on growth, food consumption, haematopoietic tissues or reproduction (Bagdon *et al.*, 1960).

β-Carotene was administered in beadlets (containing approximately 11.5%) in the diet of Sprague-Dawley rats through three generations. A total of 210 males and 420 females were assigned to one of six doses (0, 100, 250, 500 or 1000 mg/kg per day); one control group received the diet only and another received placebo beadlets. The dietary concentrations were reduced to 3.5% in nursing females from day 12 *post partum*, continuing through weaning, to avoid excess dosage of the growing young. The pregnancy rates, pregnancy performance, gestation periods and litter parameters were measured, and organ weights and the results of histopathological examinations were recorded for selected offspring of the F_{3b} pups on day 21. The authors stated that β-carotene had no effect on reproductive function (Heywood *et al.*, 1985). [The Working Group noted that the publication did not present actual data.] The same group reported that studies of teratogenicity in albino rabbits given daily oral doses of 0, 100, 200 or 400 mg/kg β-carotene by gavage on days 7–19 of gestation and in albino rats given daily doses of 0, 250, 500 or 1000 mg/kg on days 7–16 of gestation showed no evidence of embryotoxicity or teratogenicity in term fetuses. [The Working Group again noted the lack of data.]

β-Carotene was administered to groups of pregnant Sprague-Dawley rats as a 0.2% supplementation in the diet (about 125 mg/kg bw) in order to evaluate its potential to reduce the effects of of cigarette smoke (2 h per day throughout pregnancy) on fetal weight. Thus, rats underwent whole-body exposure to control air and to cigarette smoke, with or without β-carotene supplementation, although none received only β-carotene. The concentrations of retinol in the liver, but not in plasma, were higher in rats exposed to both smoke and β-carotene than in rats given placebo, and the

plasma and liver concentrations of β-carotene were 0.46 μmol/L and 0.10 μmol/g, respectively; only trace concentrations were found in the other groups. β-Carotene did not prevent retardation of growth due to cigarette smoke, nor did it appear to accentuate the adverse effects on fetal development (Leichter & Dunn, 1992).

Canthaxanthin was microencapsulated in water-soluble beadlets and administered in the diet to four groups of rats during three generations at concentrations of 0, 250, 500 or 1000 mg/kg. No effects on reproductive function were reported, but reduced food consumption and growth and increased serum transaminase and alkaline phosphatase activity and increased cholesterol concentrations were seen in adult females at all doses. In the weanlings, the organ:body weight ratios were increased for the liver and decreased for the adrenals at all doses. At the two highest doses, some histopathological changes were evident in the livers of F_2 females (Mantovani, 1992). [The Working Group noted that experimental details and results were generally lacking from this brief report, which summarized a report from WHO (1990).]

7.3 Genetic and related effects

7.3.1 Humans

In a cross-sectional study, the concentrations of urinary aflatoxin B_1–DNA adducts in 85 healthy Taiwanese men were positively associated with their plasma concentrations of α- and β-carotene and inversely related to their plasma concentrations of lycopene (Yu *et al.*, 1997).

7.3.2 Experimental systems

7.3.2.1 In vitro

Several studies have been reported on the mutagenicity of carotenoids in Ames' *Salmonella*/microsome test. As most of the results were generated in studies of the ability of these compounds to modulate the mutagenic response (see section 4.2.2.2), the results are limited to one or two strains of *S. typhimurium*. As shown in Table 57, all of the reported results are negative, with one exception. β-Carotene was not mutagenic in strains TA1535, TA1538, TA98 or TA100, either in the presence or absence of exogenous metabolic activation, but significant enhancement of 'spontaneous' revertants in strain TA104 was reported in one study (Han, 1992). Canthaxanthin, 8'-apo-β-carotenal and 8'-apo-β-carotene methyl ester were not mutagenic in strain TA98 or TA100, either in the presence or absence of exogenous metabolic activation. Cryptoxanthin extracted from orange juice, carrot carotenoids containing 51% β-carotene, 32% α-carotene and 17% other carotenoids and lycopene extracted from tomato paste were not mutagenic in strain TA98 or TA100 in the presence of an exogenous metabolic system. β-Carotene also did not induce differential toxicity in *Bacillus subtilis* or *Escherichia coli rec* strains or reversion in strain W2P uvrA of *E. coli*, either in the absence of metabolic systems or in the presence of rat liver microsomes or caecal extracts.

In cultured mammalian cells, β-carotene did not affect the frequencies of sister chromatid exchange in cultured Balb/c mouse mammary glands, of sister chromatid exchange, chromosomal aberrations or micronucleus formation in Chinese hamster ovary cells or of micronuclei in metabolically competent Hep G2 human hepatoma cells.

7.3.2.2 In vivo

β-Carotene did not affect the frequency of 6-thioguanine-resistant T lymphocytes extracted from the spleens of Fischer 344 rats given 0.15% in drinking-water for two, four, six or eight weeks. Although at each time the frequency of these mutations was consistently higher than in untreated rats, the differences were not statistically significant (Aidoo *et al.*, 1995). No significant variation was observed in the frequency of micronuclei in bone-marrow polychromatic erythrocytes of Swiss mice receiving 2.5 mg β-carotene in drinking-water daily for 15 days (Lahiri *et al.*, 1993) or of hybrid $B6C3F_1$ mice receiving 100 mg/kg β-carotene in the diet for one week (Raj & Katz, 1985). β-Carotene also did not induce chromosomal aberrations in bone-marrow cells of Balb/c mice receiving β-carotene dissolved in corn oil by gavage at a concentration of 200 mg/kg bw for five days (Salvadori *et al.*, 1992a,b). Two additional studies yielded

Table 57. Genetic and related effects of carotenoids in short-term tests in vitro and in vivo

End-point	Code	Test system	Result		HID[a]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
β-Carotene						
D	BSD	*Bacillus subtilis rec* strains, differential toxicity	-	0	1 mg/ml	Kada *et al.* (1972)
D	ERD	*Escherichia coli* rec strains, differential toxicity			100 µg/ml	Haveland-Smith (1981)
G	ECW	*Escherichia coli* WP2 *uvr* A, reverse mutation	-	-	100 µg/ml	Haveland-Smith (1981)
G	SAO	*Salmonella typhimumrium* TA100, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SAO	*Salmonella typhimurium* TA100, reverse mutation	–	–	800 nmol/plate [?][c]	Azuine *et al.* (1992)
G	SAO	*Salmonella typhimurium* TA100, reverse mutation	–	0	10 µmol/plate	Camoirano *et al.* (1994)
G	SA5	*Salmonella typhimurium* TA1535, reverse mutation	–	–	200 µg/plate	Belisario *et al.* (1985)
G	SA8	*Salmonella typhimurium* TA1538, reverse mutation	–	–	100 µg/ml	Haveland-Smith (1981)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	500 µg/plate	Terwel & van der Hoeven (1985)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	0.86 µmol/plate	Whong *et al.* (1988)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	-	–	3.45 µmol/plate	Ong *et al.* (1989)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	–	–	800 nmol/plate [?][c]	Azuine *et al.* (1992)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	10 µmol/plate	Camoirano *et al.* (1994)
G	SAS	*Salmonella typhimurium* (other miscellaneous strains), reverse mutation)	–	–	4151 µg/plate	Heywood *et al.* (1985)
G	SAS	*Salmonella typhimurium* (other miscellaneous strains), reverse mutation	–	0	10 µmol/L	Han (1994)
S	SIM	Sister chromatid exchanges, mouse cells *in vitro*	–	0	1 µmol/L	Manoharan & Banerjee (1985)
S	SIS	Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	0	10 µmol/L	Cozzi *et al.* (1997)

Table 57 (contd)

End-point	Code	Test system	Result		HID[a]	Reference
			Without exogenous metabolic system	With exogenous metabolic system		
β-Carotene (contd)						
M	MIA	Micronucleus induction, Chinese hamster ovary cells *in vitro*	–	0	6 μmol/L	Salvadori *et al.* (1994)
M	MIA	Micronucleus induction, Chinese hamster ovary cells *in vitro*	–	0	0.035 μmol/L	Stich & Dunn (1986)
C	CIS	Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	0	0.035 μmol/L	Stich & Dunn (1986)
C	CIS	Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	0	1 μmol/L	Cozzi *et al.* (1997)
M	MIH	Micronucleus induction, Hep G2 human hepatoma cells	–	0	6 μmol/L	Salvadori *et al.* (1993)
C	GVA	Gene mutation, rat T lymphocytes *in vivo*	0	–	0.15% in drinking-water for 8 weeks	Aidoo *et al.* (1995)
M	MVM	Micronucleus induction, mouse bone-marrow cells *in vivo*	0	–	100 mg/kg food for 1 week	Raj & Katz (1985)
M	MVM	*Micronucleus induction, mouse bone-marrow cells in vivo*	0	+	27 mg/kg bw orally for 7 days	Mukherjee *et al.* (1991)
M	MVM	Micronucleus induction, mouse bone-marrow cells *in vivo*	0	#	#	Umegaki *et al.* (1994a)
M	MVM	Micronucleus induction, mouse bone-marrow cells *in vivo*	0	–	2.5 mg/mouse in drinking-water for 15 days	Lahiri *et al.* (1993)
C	CBA	Chromosomal aberrations, mouse bone-marrow cells *in vivo*	0	+	27 mg/kg bw orally for 7 days	Mukherjee *et al.* (1991)
C	CBA	Chromosomal aberrrations, mouse bone-marrow cells *in vivo*	0	–	200 mg/kg bw by gavage for 5 days	Salvadori *et al.* (1992a,b)

Table 57 (contd)

End-point	Code	Test system	Result			Reference
			Without exogenous metabolic system	With exogenous metabolic system	HID[a]	
Canthaxanthin						
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SAO	Salmonella typhimurium TA100, reverse mutation	–	–	800 nmol plate [?][c]	Azuine *et al.* (1992)
G	SA9	*Salmonella typhimurium* , TA98 reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	–	–	800 nmol plate[?]	Azuine *et al.* (1992)
8′-Apo-β-carotenal methylester						
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	–	–	800 nmol/plate [?] [c]	Azuine *et al.* (1992)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	–	–	800 nmol/plate [?] [a]	Azuine *et al.* (1992)
Carrot carotenoids (51% β-carotene, 32% α–carotene, 17% other carotenoids						
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	–	–	800 nmol/plate [?] [c]	Azuine *et al.* (1992)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	–	–	800 nmol/plate [?] [c]	Azuine *et al.* (1992)
Cryptoxanthin (extracted from orange juice)						
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
Lycopene (extracted from tomato paste)						
G	SA0	*Salmonella typhimurium* TA100, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)
G	SA9	*Salmonella typhimurium* TA98, reverse mutation	0	–	100 µg/plate	He & Campbell (1990)

[a] Result: + , positive; –, considered to be negative; 0, not tested; # 'spontaneous' frequency of micronuclei was decreased by either β-carotene or a diet containing β-carotene (see Table 55).
[b] Highest ineffective dose
[c] Presumably nmol/plate

conflicting results. In one study (Mukherjee *et al.*, 1991), oral administration of 27 mg/kg bw β-carotene significantly enhanced the frequencies of both micronuclei and chromosomal aberrations in bone-marrow cells of Swiss albino mice over that in controls receiving the solvent (olive oil) only. In contrast, another study (Umegaki *et al.*, 1994a) showed that β-carotene given by gavage or incorporated into a *Dunaliella bardawil* diet reduced the 'spontaneous' frequency of micronuclei in peripheral blood reticulocytes of ICR mice.