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Opinion of the Scientific Committee on Food on the safety of use of beta carotene from all dietary sources

(Opinion adopted by the SCF on 7 September 2000)

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Terms of reference

The Committee was requested by the European Commission to deliver an opinion on the safety of use of ß-carotene from all dietary sources.

1. BACKGROUND

The Committee has been asked to assess the safety of β-carotene from all dietary sources. This opinion sets out the scientific data relevant to all sources but the conclusions relate only to food additive uses. An opinion on the setting of a Tolerable Upper Intake Level (UL) for all dietary sources will follow at a later date.

In 1975, the SCF established a Group ADI of 0-5 mg/kg b.w. (79) for the carotenoids now designated under the colours Directive 94/36/EC (23) as E 160a: carotenes (mixed carotenes and ß-carotene); E 160e: ß-apo-8'-carotenal; and E 160f: ethyl ester of ß-apo-8'-carotenal. Carotenes can be used as colours at *quantum satis* levels in a wide variety of foods, while ßapo-8'-carotenal and its ethyl ester may be used as colours in designated foods up to specified levels, as set out in Annex V, Part 2, of Directive 94/36/EC.

The Group ADI of 0-5 mg/kg b.w. established by the SCF was an endorsement of the ADI set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for a similar group of carotenoids in 1974 (99). This ADI was based on results from a range of animal studies, the pivotal one being a 4-generation study in rats fed 1000 ppm in the diet, equivalent to 50 mg/kg b.w./day, in which no adverse effects were seen. At that time, because of the low toxicity of carotenoids in animal studies and because of their natural occurrence in the human diet, a lower safety factor of 10 was used to derive the ADI. However, since that time, knowledge of the behaviour of β-carotene in animal models has increased and new findings from intervention studies in humans on synthetic β-carotene have emerged, suggesting that the ADI is no longer appropriate. At its 107th meeting, the Committee indicated that it considered the ADI was too high and would review its scientific basis (81).

A number of reviews, monographs and comments on the safety of ß-carotene have been published during the last decade (e.g. 8, 38, 41, 59, 73, 82, 100). A dossier has been received by the Secretariat of the SCF on a synthetic ß-carotene (74). In particular, on 19-March 1998 the SCF adopted a report based on the observed effects of ß-carotene supplementation in combination with tocopherol and ascorbate in clinical and chemopreventive human trials (82). In clinical trials doses up to 180 mg/day and in chemopreventive trials up to 50 mg/day were given. Most of the chemopreventive trials with ß-carotene alone and in combination with tocopherol, retinol or ascorbate in well nourished population groups showed no protective effects against malignant neoplasms, cardiovascular disease or death from all causes. On the contrary, an increase of lung

cancer incidence (18-28%) and more overall deaths (8-17%) were seen in smokers ingesting over a long period of time (4-8 years) supplements of 20 mg ß-carotene per day.

The SCF could not identify any specific explanation for these unexpected findings and therefore reconfirmed its earlier concern expressed in June 1997 (81) regarding the use of ß-carotene supplements. The SCF recommended that research is initiated to resolve the issue as a matter of urgency which would allow an upper safe limit to be established for ß-carotene intake, both alone and in combination with other antioxidants, to be used for the general public and for special population groups at risk (82).

2. INTRODUCTION

ß-carotene and carotenoids in general are isoprenoid compounds which are not synthesized in animals but biosynthesised by plants and micro-organisms. About 700 naturally occurring carotenoids have been identified so far. About 10% of them can be found in the human diet and about 20 of these compounds have been found in plasma and tissues of the mammal. The predominant carotenoids observed in the plasma are ß-carotene, lycopene, lutein, ß-cryptoxanthin and α-carotene, accounting for more than 90% of the circulating carotenoids in humans (see 73 for specific references).

Some dietary carotenoids, such as ß-carotene, serve as an important source of vitamin A, which is the major known function of carotenoids in humans. ß-carotene is a hydrocarbon $C_{40}H_{56}$ that has a ß-ionone structure as the terminal ring system at each side of the poliene chain. Carotenoids containing at least one unsubstituted ß-ionone ring and a poliene chain are potential precursors of vitamin A. The preformed vitamin A is only present in animal products (e.g. liver, eggs, milk products) thus, in countries where the intake of animal products is low, carotenoids have to meet (i.e. by 80 % or more in Asia and Africa) the vitamin A requirements. Even in developed countries carotenoids usually contribute to vitamin A supply by more than 40% (see 100).

The best-characterised natural functions of carotenoids are to serve as light-absorbing pigments during photosynthesis and protection of cells against photosensitization. Carotenoids provide considerable coloration and identification for many species, from vegetables to animals. In addition, carotenoids serve several other functions, such as radical quenching, antioxidant and anticarcinogenic activities in different animal sites and are regulators of cell function (see below).

3. SOURCES OF INTAKE

In the majority of industrialised countries, fruits and vegetables provide an estimated 2-3 mg/day of provitamin A carotenoids, of which β-carotene is the principal component (29). An approximate β-carotene intake of 1.8mg/day in a randomly-selected population of women in the USA has been reported (18), the main dietary sources being carrots, orange juice, oranges, tomatoes and dark green leafy vegetables. The average intake in the German National Food Consumption Survey was 1.81mg/day (64), mainly from carrots. An average β-carotene intake of 1.7-2.1 mg/day has been reported in Finland (36), and of 3.0 mg/day in the Netherlands (27). Levels of fruit and vegetable consumption, however, vary greatly between individuals and β-carotene intake may be much higher than average in people who regularly consume substantial amounts of foods

such as carrots (32, 83). Some authors have reported that β-carotene intake varies according to seasonal factors, perhaps due to the differing availabilities of specific fruits and vegetables, or because of factors such as light and heat that may affect the carotenoid content of foods (58, 68, 90). The SCF has not recommended the consumption of any specific amount of β-carotene, or carotenoids in general, beyond what is needed to supply vitamin A (80).

The Committee has not received comprehensive information on how much the use of ß-carotene as an additive contributes to the overall intake. However, unpublished information from Danish and Austrian surveys showed that present use levels of β-carotene or related carotenoids in most cases lie below 5 mg/litre for beverages and below 10 mg/ kg for solid food. Combined with the knowledge of the eating habits of the population, the estimated average exposure from ß-carotene and related carotenoids, used as a food additive, is around 1-2 mg/person/day (22, 81).

The most common use of β-carotene as a nutritional supplement is to correct vitamin A deficiency. It is listed as a permitted form of vitamin A in the Infant Formula and Follow-on Formula Regulations 1995 and the Processed Cereal-based foods and Baby Foods for Infants and Young Children Regulations 1997. Both these Regulations specify a maximum level of 180 μg retinol equivalents (RE)/100 kcal. The Foods intended for Use in Energy Restricted Diets for Weight Reduction Regulations specify that a whole meal replacement must provide at leats 700 μg RE/day and a meal replacement 210 μg per meal. The Margarine Regulations make it compulsory for manufacturers to add 800-1000 μg RE/100g of margarine.

As a medicine, β-carotene is given orally in doses up to 300 mg/day for the reduction of photosensitivity in individuals with erythropoietic protoporphyria (51).

4. ABSORPTION AND METABOLISM

The general mechanism of intestinal ß-carotene absorption in mammals is by passive diffusion of mixed micelles, which are formed during fat digestion in the presence of bile acids. In general, the types and amounts of carotenoids in the plasma reflect those in the diet. Depending on specific conditions, the extent of absorption for ß-carotene reported in the literature varies between 10% and 90% (see 100). Absorption appears to be linear up to intakes of 20-30 mg, but becomes limited at higher intakes. Limiting factors are dependent on the formulation or food matrix, the amount and type of fat coingested with the carotenoid and the presence of bile acids. Release from the food matrix into the lipid phase and solubilization within mixed micelles appear to be the most critical steps in ß-carotene absorption. Dietary fibre and other meal components, together with a number of metabolic factors and subject characteristics (see 73) may also affect ß-carotene absorption. Important differences in the rates of absorption and intestinal cleavages have been demonstrated between man and laboratory animals (see below).

The main site of carotenoid metabolism is the intestinal mucosa, at least in rodents, but peripheral tissues such as lung, kidney, liver and fat of several mammals, including humans and rodents, can also convert ß-carotene to retinoic acid (RA) (see 69 and 93). About 15% of ß-carotene is cleaved into retinal in the intestine of poorly nourished humans; that percentage decreases as the amounts of ß-carotene in the diet increases, so that even massive doses of ß-carotene do not yield enough intestine-derived retinol to cause hypervitaminosis (see 59).

ß-carotene can be cleaved in mammalian tissues mainly at the central double bond (C-15,15') yielding two molecules of retinal which may either be reduced to retinol (vitamin A) or further oxidised to RA; an alternative pathway (which can also yields RA, with or without the involvement of intermediate retinal) is the non-central (eccentric) cleavage at eccentric double bonds (e.g. C-13',14', C-11'.12', C-9',10' and C-7'-8') (see 44, 93 and 94) to form retinoids and apo-ß-carotenoids, which have structures that are similar to retinoids, the function of these being largely unknown.

Carotenoids are known to exist in different forms (*cis*- and *trans*-isomers) which may be interconverted by light, thermal energy or chemical reactions. For example, the cooking of vegetables promotes isomerization of carotenoids from the *trans* to the *cis* forms. Interest in the *cis* isomers of the carotenoids has been stimulated by the recognition of isomer-specific biological functions that clearly exist for the retinoids and may exist for the carotenoids. Synthetic ß-carotene is almost entirely in the trans-isomeric form. There is isomerization of dietary carotenoids and the accumulation of these isomers occurs *in vivo*. In serum, most of the ß-carotene is present as the all-*trans* isomer, in spite of significant intakes of the 9-*cis* isomer, whereas the liver and adrenal tissue contained more of the 9-*cis* and 13-*cis* isomers of ß-carotene (73, 100).

Carotenoids are transported in association with the lipoproteins, with a distribution highly correlated to that of cholesterol. After absorption retinyl esters formed in the enterocyte are incorporated into chylomicrons, before they are secreted into the intestinal lymph and move into the blood stream. In the fasted state about 75% of the ß-carotene is bound to LDL and about 25% to HDL and VLDL. Circulating carotenoid concentrations are found to be lower in smokers than in non smokers, due in part to the depletion of these compounds by components of cigarette smoke (35). Tissue distribution of ß-carotene roughly parallels LDL receptor density on the plasma membranes of the cells. Liver and adipose tissue are the main sites of carotenoid deposition.

Species differences in ß-carotene metabolism

Most laboratory animals break down ß-carotene in their intestine and thus absorb almost none intact. Hence, rodents have low serum carotenoid levels (about 1/1000 of human levels) that are not related to dietary intake due to very active dioxygenase cleavage to retinal. In man, roughly 20-75% of the ß-carotene is absorbed intact (73, 93).

Studies have thus indicated that the rat and mouse are not suitable models for studying the uptake of β-carotene into the plasma, with the possible exception of experiments using very high doses or a non-oral way of administration. Similarly, studies in hamsters showed that β-carotene concentrations remained low in animals given dietary β-carotene supplementation, although retinol levels increased, indicating that hamsters are also efficient converters of β-carotene to retinol (*cited in* 41). Rabbits do not appear to absorb β-carotene well and these animals when fed a carotenoid-rich diet showed no carotenoids in the blood and only small increases in liver vitamin A concentrations (*cited in* 41). Strict carnivores obtain a diet rich in pre-formed vitamin A and thus do not depend on provision via carotenoids in the diet. Indeed, cats reportedly lack the enzyme, β-carotene-15, 15'-dioxygenase and, thus, have a requirement for pre-formed vitamin A in the diet (8).

Ferrets (33, 73, 93, 94, 96), the pre-ruminant calf (66) and the Mongolian gerbil (44, 51), have been proposed as useful models for human ß-carotene absorption and cleavage as these animals also absorb and release intact ß-carotene from the enterocyte. The ferret studies are particularly relevant to the present report. This animal model mimics the absorption and tissue metabolism of ß-carotene in humans, it has been used for studies of tobacco smoking and inhalation toxicology (85) and also it has been used to test the hazard associated with a high dose of ß-carotene and tobacco smoking on lung (93, 94). Although serum β-carotene levels are normally very low in these animals, dietary supplementation has been shown to increase concentrations to levels similar to those detected in human serum, and also to increase levels in the liver, adipose and other tissues (33, 70, 71, 72, 94, 95, 96). It has to be recognised that no single species provides a good model for studying all aspects of β-carotene in humans (41, 92) but ferrets are particularly interesting as an example which allows reproducing (to some extent) the problem in the particular tissue (the lungs) pointed out by human trials.

5. OTHER BIOLOGICAL ACTIVITIES

Activity of retinoids as antineoplastic agents has been demonstrated in several *in vivo* experimental carcinogen models (mainly for skin, respiratory tract, urinary bladder, breast, digestive tract) (see section 6.1.2). Many of the effects of ß-carotene can be mediated by the formation of retinoic acid (RA) that has a key function as a regulator of gene expression, morphogenesis, and growth in vertebrate embryos.

Cellular responses to retinoids are generally mediated by two families of nuclear receptors (RARs and RXRs) that belong to the steroid-thyroid hormone (or nuclear) receptor superfamily and behave as ligand-activated transcription factors that bind as dimers to the *cis*-acting response elements of target genes (16). The retinoic acid (RA) receptors (RARs) are activated by both all-trans and 9-cis-RA, whereas the retinoid-X-receptors (RXRs) only show binding affinity for 9-cis-RA. So far, three main isotypes of RAR (α, β, γ) and RXR (α, β, γ) have been recognised in mammalian tissues. RAR/RXR heterodimers appear to preferentially transduce the retinoid signal *in vivo* (16).

Different retinoic acid receptors isotypes display a characteristic pattern of tissue distribution, RARα being the most ubiquitously distributed (16). RARß plays an important role in lung development and has been proposed to have a tumour suppresser function in lung (39). Primary lung tumours and lung cancer cell lines lack RARß expression, and such loss of expression may be an early event in lung carcinogenesis. RARß2 is the most abundant isoform in normal human lung tissue and restoration of RARß2 in a RARß-negative lung cancer cell line has been reported to inhibit tumorigenicity in nude mice (see references in 94).

Carotenoids act as antioxidants and scavengers of reactive oxygen species. (24, 91 and see 41 and 59). The antioxidant properties of carotenoids are related to their extended system of conjugated double bonds. *In vitro*, carotenoids efficiently quench excited molecules such as singlet oxygen and can scavenge peroxyl radicals; interactions with several other radicals have also been reported. The role *in vivo* and in humans is less clear (see 41, 46, 62).

Antioxidant nutrients are known to interact. A synergistic antioxidant protection by carotenoids with vitamins E and C has been shown (21). In general, compounds with

antioxidant properties may, depending on the presence and levels of all reactants, behave as pro-oxidants. The switch from antioxidant to pro-oxidant behaviour can be, for example, a function of oxygen concentration (21, 62). The pro-oxidant activity of ß-carotene has been demonstrated at a high partial pressure of oxygen; because this is highest in the outermost cells of the lung, these cells might be particularly subject to the pro-oxidant effect of ß-carotene (cited in 63).

Other effects of carotenoids which can be related to cancer prevention are the enhancement of the immune response observed in some experimental models, which may be due to production of tumour specific antigens (41). In addition, carotenoids have been reported to modulate cytochrome P450 metabolism, inhibit arachidonic acid metabolism, inhibit chromosome instability and chromosome damage, influence apoptosis, and affect several other biological processes (see 41 and text later on).

6. HAZARD IDENTIFICATION: TOXICOLOGICAL STUDIES

A number of epidemiological studies in humans and several animal studies developed during the last third of the past century support the idea that ß-carotene can prevent cancer, cardiovascular diseases and other diseases in humans. However, human chemoprevention trials developed the last decade (see section 6.2) have shown that ß-carotene actually increases both lung-cancer incidence and mortality in human smokers and, more recently, mechanisms which offer likely explanations of these adverse effects have been derived from experimental studies in appropriate animal models (see section 6.3).

6.1. Animal studies6.1.1. Standard toxicological studies

In summary, no adverse effects of high-dose oral β-carotene supplementation have been observed in several standard toxicological studies in various experimental animals (rat, mice, rabbits)(see 41 and 100). The studies included acute toxicity up to 5000 mg/kg bw/day in Sprague Dawley rats (100) and up to 2000 mg/kg bw/day in Wistar rats (12, 89), and chronic toxicity/carcinogenicity up to 1000 mg/kg bw/day for life in rats (38, 40) or mice (13, 38), teratogenicity and reproductive toxicity (up to 1000 mg/kg bw/day for 3 generations, or during days 7 to 16 of gestation, in rats; up to 400 mg/kg bw/day during days 7 to 19 of gestation in rabbits)(Komatsu 1971 cited in 14, 38, 42 and 100).

In beagle dogs (15, 38) no toxic effects (up to 250 mg/kg bw/day for 2 years) were observed. However, this study, in addition to the problem of using a hydrosoluble formula, had a non-explained episode, at week 88 of the study (15), when a dramatic weight loss in dogs after withdrawing ß-carotene was observed.

However, the above studies were not aimed at investigating specific effects in the lung, which now we know appears to be the more sensitive tissue. In addition, species used are particularly unsuitable for oral studies, due to the high efficiency of conversion to vitamin A, such that no significant levels of unaltered β-carotene are absorbed and incorporated into the systemic circulation.

6.1.2. Carcinogenicity

A large number of studies have been carried out to assess the potential inhibitory effects of β-carotene therapy on experimentally-induced carcinogenesis in animal models and the vast majority of these studies have indeed shown either preventive or no effects. These data have been reviewed in detail by the International Agency for Research on Cancer (IARC) (41) and TNO Nutrition and Food Research Institute (100). An association of supplementation with cancer preventive effects has been reported for tumours of the skin (mice), liver (rats), colon (rats, mice), pancreas (rats, hamsters), forestomach (mice), bladder (mice), salivary gland (rats) and adenocarcinomas and nephroblastomas (rats). Other studies have shown no effect of β-carotene supplementation on the incidence of tumours of the skin (mice), liver (mice, rats), colon (rats), pancreas (rats, hamsters), glandular stomach (rats), bladder (mice, rats), small intestine (rats), salivary gland (rats) and respiratory tract (mice, hamsters).

One study, carried out to test the effects of β-carotene supplementation on chemically-induced skin carcinogenesis in mice, showed a significant increase in (benign) papilloma formation in a high dose β-carotene group compared with a low dose group, but the incidence of skin carcinomas was significantly lower in the high dose group (17).

In contrast to studies relating to tumours at other sites, only one report has described an inhibitory effect of β-carotene supplementation on carcinogen-induced respiratory tract tumourigenesis. Supplementation of the diet with β-carotene at levels up to 0.25% (approximately 250 mg/kg bw/day), for 12 weeks, resulted in a significant reduction of the incidence of benign respiratory tract changes (hyperplasia and papillomas) in hamsters exposed to cigarette smoke (26). The majority of studies have shown no effect of β-carotene supplementation on experimentally-induced respiratory tract tumourigenesis in mice (56, 57, 102), or hamsters (9, 54, 97).

Three reports (9, 97, 94) describe potential stimulatory effects of β-carotene therapy on experimentally-induced carcinogenesis in animal models, although statistically significant increases in the incidences of malignant tumours have not been reported. These reports, indicating some ß-carotene enhancement of chemically-induced respiratory tract tumourigenesis in hamsters and the effects of ß-carotene and smoking in ferrets are described below.

Studies in hamsters

Wolterbeek et al. (97) investigated the effects of high-dose, dietary β-carotene supplementation on benzo[*a*]pyrene (B[*a*]P) (a cigarette-smoke carcinogen)-induced tumourigenesis in Syrian golden hamsters. The test animals consisted of two groups of 50 males, fed a pelleted diet containing 4000 IU/kg retinyl palmitate (the "normal" level of vitamin A), either with or without 1% (w/w) (approximately 990mg/kg bw/day) β-carotene. After one month, all animals received 10 intratracheal instillations of 8mg B[*a*]P + 8mg ferric oxide in saline, for 12 weeks. The controls, two groups of 20 males, received identical treatment except that intratracheal instillations contained ferric oxide only (no B[*a*]P). The incidences of pre-neoplastic and neoplastic changes (hyperplasia, squamous metaplasia, papillomas, adenoma, squamous-cell and adeno-carcinoma) in the larynx, trachea and lungs were almost twice as high in the β-carotene group (15/41, 37%) than the non-β-carotene test group (8/39, 21%), although the difference was not statistically significant. No respiratory tumours occurred in the control groups.

Beems et al. (9) investigated the effects of dietary β-carotene supplementation in association with B[*a*]P on the incidence of respiratory tumours in the Syrian golden hamster. A group of 40 male and 40 female hamsters received a diet containing 56 mg/kg β-carotene (beadlet formulation, as a feed admixture, approximately 5.6 mg/kg bw/day) for 374 days (females) or 429 days (males). The control group, comprising 60 male and 60 female hamsters, were fed the same diet minus the β-carotene supplement. After 30 days on this diet, all hamsters were treated, by intratracheal instillation, with 8 mg B[*a*]P attached to 8 mg ferric oxide particles, in saline, once every 2 weeks for 16 weeks. Respiratory tract tumour incidences (epidermoid carcinoma, adenocarcinoma and papilloma in the trachea and bronchi; epidermoid carcinoma in the larynx; adenoma in the lungs) were 34/57 and 37/57 in the male and female control groups, respectively, 26/38 and 25/36 in the male and female β-carotene groups, respectively. Statistical analysis showed a positive trend towards increased tumour incidence in the β-carotene group (not significant) and a statistically significant increase in the incidences of (benign) epidermoid papillomas in the trachea, bronchi and larynx, and also of the overall incidence of tracheal tumours in these animals. The development of pre-neoplastic changes in the respiratory tract was not affected by β-carotene supplementation.

Study in ferrets

Wang et al. (94) used a ferret model to assess the single or combined effects of cigarette smoke and/or β-carotene supplementation on lung histopathology/biochemistry.

To mimic human trials, by correcting for species differences in ß-carotene absorption, Wang et al (94) fed ferrets with 2.4 mg ß-carotene/kg per day (15 times higher than the 0.16 mg of ß-carotene/kg per day for the control group fed a low ß-carotene diet). This dose mimics an intake equivalent to 30 mg of ß-carotene per day in a 70-kg human. It was shown that: 1) Using the above supplementation doses, ß-carotene in the plasma of the ferrets increased (17-22 fold) similar to that observed in human trials (see section 6.2.2); 2) Tissue levels of ß-carotene, retinol and RA in control ferrets were within the range found in the normal human, although this was not the case for the higher plasma levels of retinyl esters in the ferret; 3) The lung architecture and formation of oxidative metabolites from ß-carotene were considered similar in both species (references listed in reference 94); 4) The concentration of urinary cotinine equivalents in the smoke-exposed ferrets was similar to that found in humans smoking 1.5 packs of cigarettes per day.

Four groups of 6 males were treated with either 2.4 mg/kg bw/day β-carotene supplementation (in corn oil, fed orally), cigarette smoke exposure (smoke from 10 cigarettes, in a chamber, for 30 minutes, twice daily), both, or neither, for a period of 6 months, at which point they were killed. Histopathological analysis revealed that all β-carotene treated animals showed an increase in cell proliferation and squamous metaplasia in lung tissue, and this was further enhanced in the animals that were also exposed to cigarette smoke. Animals exposed to cigarette smoke alone did not show these changes. The assessed histopathological endpoint, squamous metaplasia, may not be directly related to carcinogenesis, but this study did reveal interestingly related molecular/biochemical changes in the lungs of the animals tested which are discussed later in this report (see section 6.3.2).

6.1.3. Genotoxicity and modulation of genotoxic effects

ß-carotene was not mutagenic in several strains of *S. typhimurium* (Ames Test), either in the absence or in the presence of exogenous metabolic activation (S9)(27, 75).The IARC review (41) summarised results regarding the Ames test for β-carotene as follows:- "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, the results are limited to one or two strains of *S. typhimurium*, being all of the reported results 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 [34]." The IARC (41) also reported that "β-carotene 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 vitro studies have shown that ß-carotene does not affect the frequencies of sister chromatid exchanges (SCEs) in cultured Balb/c mouse mammary gland cells (50), of chromosomal aberrations or micronuclei in Chinese Hamster Ovary cells (20, 75, 88) or micronuclei in metabolically competent HepG2 human hepatoma cells (76). Recently it has been reported that relatively high concentrations of ß-carotene enhance hydrogen peroxide-induced DNA damage in human hepatocellular HepG2 and HT29 cell lines (49, 98). Actually, in HT29 cells (49) both lycopene and ß-carotene protect against oxidative damage at low concentration but rapidly lose this capacity at higher doses.

Additionally, one report described a significant, though equivocal, increase in micronucleus formation in human lymphocytes induced by synthetic all-trans β-carotene at levels of 1-30 μg/ml (approximately 2–56 μmol/l) (101). Natural β-carotene (containing a proportion of the *9-cis* isomer) had no effect on micronucleus formation in the same study.

In vivo studies

ß-carotene was unable to induce gene mutations (thioguanine-resistance) in T lymphocytes extracted from the spleens of Fischer 344 rats given 0.15% in drinking water for two, four, six or eight weeks (2).

No increase in micronuclei was observed in bone-marrow cells of Swiss Albino mice receiving 2.5 mg ß-carotene (of unspecified origin) in drinking-water daily for 15 days (45) or for hybrid B6C3F1 mice receiving 100 mg/kg β-carotene in the diet for one week (67).

In studies designed to detect the protective effect of β-carotene, no induction of chromosomal aberrations was observed in bone-marrow cells of Balb/c mice receiving the substance in distilled water or corn oil by gavage at concentrations of 200 mg/kg bw for five days (77, 78).

Two additional studies yielded conflicting results. Within a study on the anticlastogenic activity of synthetic β-carotene, the substance dissolved in oil was orally administered for 7 days at 2.7 and 27 mg/kg bw to male Swiss Albino mice. Significant increases of both structural chromosomal aberrations and micronuclei were observed at the highest dose (55). These results were not confirmed in a subsequent study by the same authors (1).

The data presently available are insufficient for an overall, adequate evaluation of the genotoxic potential of ß-carotene. Most studies were not designed to specifically address the genotoxicity of ß-carotene 'per se', rather its antimutagenic and anticlastogenic activity. Because of serious limitations in the protocols used, the results of *in vivo* assays in particular must be considered inconclusive. Positive results obtained in *in vitro* micronucleus test with synthetic ß-carotene or with ß-carotene on *S. typhimurium* strain TA104 might be due to indirect mechanisms in view of the well known pro-oxidant activity of ß-carotene.

6.1.4. Remarks from animal studies

1) The adverse effects shown only at higher doses can not be dismissed, because of the problems of poor absorption by the intestine of the animals used and the use of particular formulations.

2) There are no good experimental studies especially addressing the genotoxicity of ß-carotene "in vivo". Negative findings in studies designed to asses the anticlastogenic activity of ß-carotene do not provide conclusive evidence on the lack genotoxicity *in vivo*. Positive results obtained in a limited study with synthetic ß-carotene (101) should be evaluated with caution but not dismissed, in view of the pro-oxidant activity of ß-carotene and the evidence of micronucleus induction *in vitro* by synthetic ß-carotene. The latter study also suggests that the genotoxicity *in vitro* of ß-carotene formulations can be modulated by their relative stereoisomer composition. This findings should be taken into account also in the evaluation of studies *in vivo*, which used samples of ß-carotene of different and/or unspecified composition. In summary, the data available are insufficient for an adequate evaluation of the genotoxicity of ß-carotene *in vivo*.

3) Most of the studies reporting negative findings have been done in animal models (namely rat and mice) which are now recognized as unsuitable models for analyzing the adverse effects of ß-carotene as described in human chemopreventive trials. Only by using high doses of ß-carotene and looking at target tissues such lung, which appears to be particularly sensitive, could adverse effects have been detected. This was not the aim in those previously considered studies, probably because they were designed before the results of the ATBC and CARET chemopreventive trials were published.

4) More recently, using rats, Paolini et al. (63) investigated the effect of a high dose of ß-carotene in rats, looking at the lung tissue. ß-carotene mediated induction of key carcinogen bioactivating enzymes was shown. Using ferrets (94), a dose of ß-carotene (2.4 mg/kg bw/day) comparable to that administered in the critical human trials was associated with the development of squamous cell metaplasia in the lungs. These two recent studies are considered further in section 6.3.

6.2. Human studies

In humans, doses of 20-180 mg/d ß-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 (51, 53).

A substantial amount of epidemiological information linking higher carotenoid intake with lower cancer incidence was accumulated in the 1970s and 1980s. Also noted was the apparent lack of toxicity of ß-carotene in high-dose clinical use against

erythropoietic photoporphyria. Thus, these facts, together with the known biological properties of ß-carotene (see above), combined to justify large-scale, cancer prevention trials in humans. However, these trials did not confirm the positive expectations.

6.2.1. Epidemiological studies

6.2.1.1. ß-carotene and incidence of cardiovascular disease

A number of descriptive, cohort and case-control studies have been reviewed suggesting that carotenoid and/or ß-carotene rich diets may prevent cardiovascular disease (see 41, 100).

Recently, the Rotterdam 1999-Study in the elderly (43) confirmed a protective association. It involved 4802 participants aged 55-95 y who were free of myocardial infarct (MI) at baseline and that were followed during a 4-year period. Risk of MI for the highest compared with the lowest tertile of ß-carotene intake was 0.55 (95% CI 0.34-0.83), adjusted for age, sex, body mass index, smoking, income, education, alcohol intake, energy adjusted intakes of vitamins C and E, and use of antioxidative vitamin supplements. Association between vitamin C or vitamin E and MI was not observed. The results support the hypothesis that high dietary ß-carotene intakes may protect against cardiovascular disease. However, the finding in numerous observational studies that increased intake of carotenoid-containing diets and higher blood concentrations of carotenoids are associated with reduced risks for cardiovascular disease cannot be interpreted as a specific protective effect of ß-carotene or other carotenoids *per se*.

The results of the ATBC and CARET human chemopreventive trials (see below) with regard to cardiovascular disease strongly suggest a possible harmful role of supplemental ß-carotene. In the ATBC study (6), 11% more total cardiovascular death were seen in men taking ß-carotene. When the analysis was restricted to the 1862 participants who had previously had an MI, men who received ß-carotene alone had relative risks of 1.75 for fatal coronary heart disease and 3.44 for fatal MI. Similarly an increased number of deaths from cardiovascular disease was seen in the CARET study (60, and see 59) among men taking supplemental ß-carotene plus retinol (relative risk of 1.26).

6.2.1.2. ß-carotene and cancer incidence

A number of reviews published around 1990 have summarised the research on diet and lung cancer during the preceding 25 year period (see 104 and 87). The consensus was that observational studies of diet and lung cancer, whether prospective or retrospective, consistently demonstrated reduced risk with increased intake of carotenoids from vegetables and fruits. Further, high levels of ß-carotene in the blood were consistently associated with reduced incidence of lung cancer in prospective studies. The simplest explanation of the epidemiology was that ß-carotene was protective although other carotenoids or other compounds from vegetables and fruits, and associated dietary patterns had not been adequately explored.

The observational data suggesting cancer preventive effects are most consistent for some types - lung, oral, pharyngeal and stomach - (see 100 and 41) of cancer, the incidence of which tends to be inversely related to ß-carotene intake or blood concentrations. A review (87) that summarised results from 206 human epidemiological

studies confirmed the evidence for a protective effect of greater vegetable and fruit consumption against cancers of the stomach, oesophagus, lung, oral cavity and pharynx, endometrium, pancreas and colon. The types of vegetables or fruit that most often appeared were raw vegetables, allium vegetables, carrots, green vegetables, cruciferous vegetables and tomatoes. A number of interesting substances present in these foods include dithiolthiones, isothiocyanates, indol-3-carbinol, allium compounds, isoflavones, protease inhibitors, saponins, phytosterols, inositol hexaphosphate, vitamin C, D-limonene, lutein, folic acid, ß-carotene, lycopene, selenium, vitamin E, flavonoids and dietary fibre (87).

The associations of retinoids and carotenoids with breast cancer in a case-control study (103) using concentrations of these nutrients in breast adipose tissue was conducted among women attending a breast clinic in the Boston area in 1989-1992. They observed inverse associations between some (retinyl palmitate, ß-carotene, lycopene, and lutein/zeaxantin) although not all of these compounds and risk of breast cancer (103).

A recent case-control study in Greece (11) involved 820 women with histologically confirmed breast cancer who were compared with 1548 control women. Among post menopausal women there were no associations between any of the micronutrients evaluated and risk of breast cancer. Among premenopausal women, ß-carotene, vitamin C and vitamin E were each inversely associated with breast cancer, but after mutual adjustment among the three nutrients only ß-carotene remained significant.

In conclusion, the general assumption is confirmed that individuals who eat more fruits and vegetables, rich in carotenoids, and/or have high levels of serum ß-carotene, have a lower risk for cancer and cardiovascular diseases. However, a possibility could be that ß-carotene may be only a marker of the intake of other beneficial substances in fruits and vegetables, or perhaps other life-style habits. Actually (see below) no clinical trial of ß-carotene as a single agent, has shown a reduction in the risk of cancer at any specific site. On the contrary there is evidence of an increase in the risk for lung cancer among smokers and asbestos workers receiving ß-carotene supplements at high doses, which resulted in blood concentrations an average of 10-15 times higher than normal.

6.2.1.3. ß-carotene and other diseases

Erythropoietic protoporphyria (EP) (51) is a genetic disease of porphyrin metabolism, characterised by abnormally elevated concentrations of protoporphyrin, which acts as an endogenous photosensitizer. As carotenoids can interact and quench photosensitizer triplet states and single oxygen, their efficacy in this disorder appears to be a consequence of the quenching of excited species. Most patients with EP or other photosensitivity diseases benefit from recommended doses for adults of about 180 mg/d, with no serious side-effects and no long-term toxicity reported. These photosensitivity diseases are the only current therapeutic use of carotenoids.

Dietary carotenoids have been suggested to reduce the risk of age-related macular degeneration (84, and see 19), the most common cause of irreversible blindness in people over age 65 in western countries.

Senile cataract is another ocular condition potentially related to oxidation, and ß-carotene has been studied for a possible role in the prevention of this disorder. However the available results are somewhat inconsistent. Carotenoids have also been

suggested to be of benefit for several other health outcomes (such us ageing, impaired cognition, rheumatoid arthritis and cystic fibrosis), however the data are scant (41).

6.2.2. Prevention trials in humans with ß-carotene supplementation

Six major prevention trials with ß-carotene supplementation have been completed so far (6, 10, 30, 31, 37, 60). Short-term trials using sputum as a presumed intermediate endpoint were conducted as well with some preliminary promising results (see 59). However, results from the majority of clinical trials reported are not in support of using ß-carotene supplementation as a means to reduce cancer and cardiovascular disease rates.

The first study (31) showed that supplementation with 50 mg ß-carotene/day for 5 years had no effect on the occurrence of new basal-cell or squamous-cell carcinoma in well nourished patients who had skin cancer previously. However, a 12-year latency period for these cancers diminished the present value of these results.

In a second study (30), ß-carotene (25 mg/day), with or without vitamin C (1 g/day) and α-tocopherol (400 mg/day) for 5-8 years, was not found to reduce the occurrence of colorectal adenoma in patients who had a prior history of adenomas.

A lack of effect of long term supplementation with ß-carotene on the incidence of malignant neoplasms and cardiovascular disease was reported in 1996 (37).

The Tyler asbestos cohort studied 755 randomised asbestos workers (52) at Tyler (Texas), receiving 50 mg of ß-carotene together with 25,000 IU retinol/day or placebos. There was no difference in the two groups by criteria of sputum atypia. The ß-carotene was obtained from BASF and it is thought that the 50 mg dosage is almost equivalent to 30 mg of Roche-ß-carotene.

6.2.2.1. The Alpha-Tocopherol/Beta-Carotene (ATBC) Trial in Finland (6).

The ATBC trial (6) involved 29,133 male smokers (age 50-59) with a smoking history averaging one pack/day for 36 years. The 2x2 factorial design evaluated 20 mg ß-carotene (from Roche) and/or 50 IU alpha-tocopherol (vitamin E) daily for 6.5 years. These doses represents a 10-fold and 5-fold excess over the median intake of ß-carotene and α-tocopherol, respectively in this population. After 2 years of treatment, median serum ß-carotene levels had increased 17.5-fold in the ß-carotene treatment groups.

Vitamin E supplementation did not reduce the incidence of lung cancer (relative risk (RR) was 0.98). Participants receiving ß-carotene alone or in combination, had significantly higher lung cancer incidence (RR 1.18; 95%CI 1.03-1.36) and higher mortality (RR 1.08; CI 1.01-1.16) than subjects receiving placebo. These results were unexpected.

The excess lung cancer incidence was not apparent in the initial 18 months, but the incidence curves significantly diverged thereafter. Subsequent subgroup analysis (see 3) revealed a higher risk in heavy smokers (20 or more cigarettes/day) (RR 1.25, CI 1.07-1.46) than in light smokers (5-19 cigarettes/day) (RR 0.97, CI 0.76-1.23). Also associations with alcohol intake and with non-small-cell histology were noted. The risk was confined to the heavier drinkers (more than 11 g ethanol per day).

Interestingly, in agreement with earlier observational studies, both dietary intake and serum ß-carotene levels at baseline (before treatment) were found to be inversely related to risk of lung cancer during the trial (3).

6.2.2.2. The Beta-Carotene and Retinol Efficacy Trial (CARET) in the USA (60, also 59, 61)

CARET (60) successfully randomised 18,314 participants. 30 mg ß-carotene and 25,000 IU vitamin A (retinyl palmitate) were administered daily to 14254 smokers and former smokers (45% female) age 50-59 at enrolment, and to 4060 asbestos exposed males (age 45-74). After five years of study the median serum ß-carotene levels in the active treatment group was increased by 12-fold (170 ng/ml versus 2100 ng/ml).

A total of 388 new cases of lung cancer were diagnosed during the 73,135 person-years of follow- up (mean 4.0 years). The active treatment group had a RR of lung cancer of 1.28 (CI 1.04-1.57) compared with the placebo group. The differences (significant from 24 months of treatment onwards) were greater as the intervention progressed. There were no statistically significant differences in the risks of other types of cancers.

In the active group the RR of death from any cause was 1.17, of death from lung cancer 1.46, and of death from cardiovascular disease 1.26.

As in further analysis from ATBC published in the same issue (3), there was an association (less clear trend than in ATBC study) of the excess lung cancer incidence between treatment groups with the highest quartile of alcohol intake, but no association with baseline serum ß-carotene concentrations.

In the CARET study it is not possible to distinguish the ß-carotene effects from those of the vitamin A, since the two compounds were administered in combination.

6.2.2.3 Physicians Health Study

This trial was to test the effect of aspirin on cardiovascular disease incidence (86). β-carotene was added in a 2x2 design, using 50 mg BASF ß-carotene on alternate days. 22,071 male physicians were followed for a mean of 12.5 years. Those assigned to receive ß-carotene had significantly higher serum concentrations than those given placebo (2240 nmol/l vs. 560 nmol/l)(4-fold). It has to be noted that this increase is lower compared with that obtained in the two previously considered trials, a situation that could be related to higher basal levels in the PHYS population and/or to a lower bioavailability of ß-carotene compared with the other trials.

In this healthy population, with 50% never-smokers and only 11% current smokers, 170 lung cancers were accumulated over the follow up period. The relative risks were 1.02 (CI 0.93-1.11) for overall mortality, 0.98 (CI 0.91-1.06) for all malignant neoplasms, and 0.93 for lung cancer.

6.2.2.4 The Linxian Trials

Two notable trials (10, 47) were conducted which were very complex in design and difficult to compare with the findings in western populations. In the first study (10) the population (29584 men and women from the general population, age 40-69) had low

serum concentrations of several micronutrients (including ß-carotene, retinol, riboflavin, vitamin C and vitamin E). The primary endpoint was incidence of oesophageal and gastric cancers, which were of high incidence in the site (Linxian County, China).The combinations were: A (retinol plus Zn), B (riboflavin plus niacin), C (vitamin C plus molybdenum) and D (15 mg ß-carotene, 50 µg selenium and 30 mg alpha-tocopherol). At the end of five years none of the combinations produced a significant reduction in oesophageal and gastric cancer incidence. However, the group D showed a RR of 0.87 (CI 0.75-1.00) for total cancer deaths, 0.91 (CI 0.84-0.99) for total deaths, 0.96 (CI 0.78-1.18) for oesophageal cancer deaths, and 0.79 (CI 0.64-0.99) for gastric cancer deaths.

This is the only large scale population trial that demonstrated positive benefits for ß-carotene supplementation, and it could be argued that the 15 mg per day ß-carotene dose could be regarded as safe. However, there is a key difference from the other major trials (i.e. consistently low population nutrient intake). Furthermore, the observed benefits cannot be directly attributed to ß-carotene as a combined supplementation was given.

The second trial (47) in 3318 Linxian residents at high risk for oesophageal cancer (diagnosed with oesophageal dysplasia), applied a single supplement containing 26 vitamins and minerals, plus a capsule with 15 mg ß-carotene (58). After six years the corresponding relative risks for deaths from cancer were 0.92 (CI 0.67-1.28) for oesophagus plus gastric cardia, 0.84 (CI 0.54-1.29) for oesophagus alone, 0.93 (CI0.75-1.16) for total mortality, and 0.96 (CI 0.71-1.20) for cancer mortality in the supplemented group. Stomach cancer mortality (which was statistically reduced in the first Linxian trial) was higher (RR 1.18, CI 0.76-1.85).

6.3 Mechanisms

In light of the adverse findings in human intervention trials, in which β-carotene supplementation was associated with a promotional effect on lung tumourigenesis in smokers, studies in animals have been carried out to elucidate potential mechanisms by which these effects may have occurred.

A number of mechanisms have been proposed, which are related to effects in the same target tissue, the lungs, where adverse effects have been observed in humans.

6.3.1. Effects on P450-related activities

Perocco et al. (65) first reported that β-carotene enhanced the transforming effect of benzo(a)pyrene (B[*a*]P) and cigarette-smoke condensate (tar) on mouse BALB/c 3T3 cells in an *in vitro* cell transformation assay, although β-carotene alone was not transforming in this system. The authors suggested that β-carotene may exert its effects by inducing P450 activities (in particular CYP 1A1/2), with a consequent increase in the metabolism of cigarette smoke constituents. Interestingly, however, β-carotene showed no capacity to enhance the transforming activity of 3-methylcholanthrene (3-MCA), which also requires metabolic activation by CYP1A1.

The same group, Paolini et al. (63) found that dietary supplementation of rats with 500mg/kg bw/day β-carotene, for 5 days, significantly increased lung enzyme activities

associated with CYP1A1 and 1A2 (activating aromatic amines, polychlorinated biphenyls, dioxins and PAHs), CYP2A (activating butadiene, hexamethyl phosphoramide and nitrosamines), CYP2B1 (activating olefins and halogenated hydrocarbons) and CYP3A (activating aflatoxins, 1-nitropyrene and PAHs). The authors postulated that these powerful booster (stimulating) effect on phase I carcinogen-bioactivating enzymes might explain why ß-carotene supplementation increases the risk of lung cancer in smokers, probably due to the co-carcinogenic properties of β-carotene and its capacity to generate oxidative stress.

Other studies (4, 5, 7, 28) showed no ß-carotene enhanced effects on phase I or phase II xenobiotic-metabolising enzymes but measurements were made in the liver and not in lung tissue.

Astorg and colleagues reported that lower levels of β-carotene supplementation in rats, either 300 mg/kg diet (approximately 12 mg/kg bw/day) for 15 days, or 10 mg/kg bw by intraperitoneal injection (to bypass the effect of high-level conversion to vitamin A during absorption) every other day for 15 days, had no effect on any measured phase I or phase II xenobiotic-metabolising enzymes in the liver (including markers for CYP1A1, 1A2, 2B1, 2B2, 2E1, 3A1/2, 4A) (5, 28), although dietary supplementation with 300 mg/kg diet β-apo-8'-carotenal for 15 days significantly induced liver CYP1A1 and 1A2 levels (28).

Studies in mice have not shown β-carotene-stimulated induction of hepatic P4 activities. Basu et al. (7) reported that supplementation of mice with 20-500 mg/kg diet (approximately 2.5-62.5 mg/kg bw/day) β-carotene for 14 days induced a significant decrease in hepatic P450 content, as measured by spectroscopy and hydroxylase activity using biphenyl substrate. Astorg et al. (4, 5) found that supplementation with 300 mg/kg diet (approximately 37.5 mg/kg bw/day) β-carotene or β-apo-8'-carotenal for 15 days had no effect on any phase I or phase II xenobiotic-metabolising enzymes in the liver (including markers for CYP1A1, 1A2, 2B1/2, 1A/2B/3A, 2E1 and 3A), although CYP1A-dependent activities were significantly upregulated by equivalent levels of the carotenoid canthaxanthin.

6.3.2. Altered retinoid signalling

The results of a recent study (94) may explain why high-dose ß-carotene supplements unexpectedly increased lung cancer rates in the two cancer prevention trials. When ferrets (animals that metabolise ß-carotene in much the same way as humans) were given ß-carotene doses equivalents to those used in the clinical trials, changes in ß-carotene metabolism were induced that may promote rather than inhibit tumorigenesis.

Ferrets were given a ß-carotene supplement, exposed to cigarette smoke, or both for 6 months (see section 6.1.2). Cell proliferation and squamous metaplasia in lung tissue were assessed by examination of proliferating cell nuclear antigen expression and histopathological examination, respectively. ß-carotene and retinoid concentration in lung tissue and plasma were analysed. Expression of genes for retinoic acid receptors (RARs) and activator protein-1 (encoded by c-jun and c-fos genes) in lung tissue specimens was examined. The results clearly showed that a strong proliferative response in lung tissue was observed in <u>all</u> ß-carotene-supplemented animals, and this response was enhanced by exposure to tobacco smoke. The treatment groups had statistically significant lower levels of retinoic acid in lung tissue, and they exhibited 18%-73%

reductions in RARß-gene expression, without reduction of RARα and RARγ. Ferrets given a ß-carotene supplement and exposed to tobacco smoke had threefold to fourfold elevated expression of the c-jun and c-fos genes.

According to Wang et al (94, see also 48), decreased lung concentration of retinoic acid may cause diminished retinoic signalling, enhanced lung cell proliferation, and potential tumour formation. Results showed that localised keratinized squamous metaplasia (a precancerous lesion) was observed in all individual ferrets in the high-dose of ß-carotene, with or without exposure to smoke. Retinoic acid levels are lowered in lung tissue as a result of ß-carotene supplementation, in spite of having increased levels of ß-carotene (by 300 fold). The possibility that some of the eccentric cleavage products of ß-carotene could act as a ligand and interfere with RA requires further investigation. Thus it is possible that ß-carotene supplementation in itself might modify ß-carotene metabolism. Reduction of retinoic signalling could occur after induction of cytochrome P450 enzymes (see section 6.3.1), perhaps by the ß-apo-8'-carotenal (increased by 2.5-fold by smoke exposure).

It can be deduced from the preceding study that diminished retinoid signalling, resulting from suppression of RARß gene expression and overexpression of activator protein-1 could be a mechanism to enhance lung tumorigenesis after high dose ß-carotene supplementation and exposure to tobacco smoke. However a relationship between the endpoint studied (squamous metaplasia) and lung cancer has not been demonstrated, and the dose-response relationships was not studied. Nevetheless, lung carcinogenesis is associated with an alteration in retinoid signalling involving the AP-1 complex, which mediates the signal from growth factors, inflammatory peptides, oncogenes, and tumour promoters, usually resulting in cell proliferation. AP-1 (c-fos, c-jun) transcriptional activity can be inhibited by RA treatment, thus contributing to the suppression of human bronchial epithelial squamous metaplasia.

In contrast to what occurs with high doses of ß-carotene (even more when smoking), if low levels of ß-carotene are ingested, eccentric cleavage products are produced by the cells (as would be the case when one consumes ß-carotene from a carotenoid enriched diet). This form of carotenoid intake could be beneficial by giving rise to some RA.

Adverse effects of high dose supplemental ß-carotene (alone) cannot be ruled out. The intervention trial (37) which did not include many smokers, and that did not reveal any increase in incidence of cancer or death, can not be considered conclusive, because precancerous lesions (analogous to those observed in ferrets under high ß-carotene intake) were not considered. They should be further analysed to deduce more definitive conclusions.

6.3.3 Pro-oxidant activity

The pro-oxidant activity of β-carotene (see section 5) may also play a role in lung toxicity, but this hypothetical mechanism has not been directly derived from studies in the lung. Antioxidant activity of carotenoids may shift into prooxidant activity, depending on the biological environment (oxygen tension, carotenoid concentration, interaction with other antioxidants) in which they act (62). It has been suggested that the relatively high partial oxygen presure in the lung combined with reactive oxygen species derived from tobacco smoke or induced by asbestos is conducive for β-carotene auto-oxidation and that the oxidative metabolites can act as propagators of free-radical

formation in smokers' lungs (see 48). When an inappropriate prooxidant activity develops in normal cells, this could generate oxidative damage, which may depress cell integrity or induce neoplastic transformation. However, prooxidant activity can induce either beneficial or harmful effects in biologic systems (62). Further studies of the prooxidant role of carotenoids *in vivo* and *in vitro* will help in testing hypotheses relating to the influence of these compounds in the development of human chronic diseases, together with a better understanding of carotenoid-carotenoid or other interactions.

7. DOSE-RESPONSE ASSESSMENT

No dose/response relationship for ß-carotene effects is available from the intervention trials in humans, as single doses were used in each study, and the conditions were different in the different studies.

The study in ferrets also used a single daily dose. Further studies in ferrets using a range of different ß-carotene doses and a wider range of selected parameters would be appropriate to assist in future toxicological evaluation.

It can be presumed that the effects of ß-carotene are dependent on the specific source of exposure, and that differences will not be unexpected with different matrices or different formulations containing ß-carotene, depending on the composition of accompanying antioxidants and of other components, and also depending on the relative proportion of isomers of ß-carotene. Natural ß-carotene preparations differ from synthetic all-*trans*-ß-carotene in the relative proportion of *trans/cis*- isomers. From preliminary studies (see section 6.1.3) the isomeric form appears important in the genotoxicity and antigenotoxicity of ß-carotene. However, at present, there is insufficient information to establish the role of all these factors.

Three general ß-carotene sources can be considered (81): a) natural food sources, b) food additives, and c) supplements. Natural food sources may contribute in Europe an average of around 2 mg/person/day and up to 5 mg/person/day in high consumers of carotenoid-rich foods, while food additives contribute 1-2 mg/person/day. The combination of these two sources represents about 3-7 mg/day (or up to 10 mg/day depending on seasonal and regional variations) of β-carotene exposure. Moreover, β-carotene from natural sources may confer health benefits (81). Thus, there may be a very small difference between the levels which may produce adverse effects in smokers in the general population (20 mg/d in the ATBC study) and those which may confer health benefits (up to 10 mg/d, mainly from natural sources, according to previous statements by the SCF). In this situation it seems that the use of ß-carotene as a supplement, in doses representing additional intakes and of synthetic origin, should be regarded cautiously.

8. CONCLUSIONS

1. A number of reviews have summarised the research on diet and lung cancer in humans during the past 30 years. Observational studies of diet and lung cancer, whether prospective or retrospective, have consistently demonstrated reduced risk with increased intake of vegetables and fruits rich in carotenoids. Further, high levels of ß-carotene in the blood were consistently associated with reduced incidence of lung cancer in

prospective studies. However, the general assumption that individuals who eat more fruits and vegetables, rich in carotenoids, and/or have high levels of serum ß-carotene have a lower risk for cancer and cardiovascular diseases cannot be extended to synthetic pure ß-carotene or to specific formulations containing ß-carotene, because the role of other carotenoids or other compounds from vegetables and fruits, and associated dietary or life style patterns, has not been adequately explored in the epidemiological studies.

2. Current evidence from human trials indicates that supplemental ß-carotene (20 mg/day or more) is contraindicated for use in current, heavy smokers.

3. Studies using animal models have been carried out to investigate potential mechanisms by which β-carotene supplementation could have enhanced the incidence of lung cancer in two major intervention trials involving human smokers. A number of mechanisms have been suggested: (a) alterations in retinoic acid signalling pathways; (b) induction of specific cytochrome P450 xenobiotic-metabolising enzymes, with a consequent enhancement of the metabolism of cigarette smoke precarcinogens; and (c) pro-oxidant activity. The lung appears to be the sensitive tissue and ferrets might be an appropriate model for studying the role of oral ß-carotene in lung carcinogenesis.

4. The Committee considered food additive uses of ß-carotene and its related carotenoids, in the context of the overall intake of β-carotene from natural sources and food additives. Natural food sources may contribute in Europe an average of around 2 mg/person/day and up to 5 mg/person/day in high consumers of carotenoid-rich foods., while food additives contribute 1-2 mg/person/day. The combination of these two sources represents about 3-7 mg/day (or up to 10 mg/day depending on seasonal and regional variations) of β-carotene exposure. The Committee decided to withdraw the Group ADI for ß-carotene, mixed carotenes, ß-apo-8´-carotenal and its ethyl ester of 0-5 mg/kg b.w., which was based on rodent studies, because of the lack of relevance of these studies for human risk assessment and the adverse findings in human studies on smokers taking supplements of 20 mg/day or more of synthetic β-carotene, amounts that are much lower than the previously established ADI. However, there are no indications that intakes of 1-2 mg/day consumed as food additives, in the context of the overall dietary intake of ß-carotene are harmful. The Committee therefore finds currently permitted food additive uses of ß-carotene and related carotenoids temporarily acceptable from a health point of view at the levels of intake quoted above. At present, there is insufficient scientific basis, either from human or experimental studies, on which to set a new ADI for ß-carotene and related carotenoids.

5. The Committee is aware of ongoing studies on ß-carotene and will wish to review the above advice within the next 3 years.

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