SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of β-apo-8'-carotenal (E 160e) as a food additive[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of β-apo-8'-carotenal (E 160e) as a food additive in the EU. β-Apo-8'-carotenal was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 and the EU Scientific Committee for Food (SCF) in 1975 and 2000. Both committees established an Acceptable Daily Intake (ADI) of 0-5 mg/kg bw/day, which was withdrawn by the SCF in 2000. The Panel concluded that the available in vitro and in vivo genotoxicity studies do not give reason for concern with respect to genotoxicity. Upon a public call for data two subchronic toxicity studies in rats performed according to OECD guidelines and under GLP became available for evaluation. Based on the 13-week study the Panel established that based on increased incidence of eosinophilic droplets in the kidneys the LOAEL was 10 mg β-apo-8'-carotenal active ingredient/kg bw/day. Upon a public call for data two additional studies on reproductive and developmental toxicity became available revealing a NOAEL of 500 mg/kg bw/day, the highest dose level tested. Overall, the Panel concluded that the present database on β-apo-8'-carotenal provides a basis to revise the ADI. The Panel concluded that based on the LOAEL of 10 mg/kg bw/day from the 13 week study in rats and an uncertainty factor of 200, an ADI for β-apo-8'-carotenal of 0.05 mg/kg bw/day can be established. Exposure estimates at Tier 3 indicate that the newly set ADI is reached for adults on average and exceeded by adults at the 95th percentile and by children on average and at the 95th/97.5th percentile.

KEY WORDS

β-apo-8'-carotenal, E 160e, CAS Registry Number 1107-26-2, food colour.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Panel on Food Additives and Nutrient Sources added to Food was asked to deliver a scientific opinion re-evaluating the safety of β-apo-8'-carotenal (E 160e) when used as food colour.

β-Apo-8'-carotenal (E 160e) is allowed as food additive in the EU and was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 and the EU Scientific Committee for Food (SCF) in 1975 and 2000. The SCF and JECFA both established an Acceptable Daily Intake (ADI) of 0-5 mg/kg bw/day, which was withdrawn by the SCF in 2000.

JECFA defined the ADI for the sum of the carotenoids β-apo-8'-carotenal, β-carotene, β-carotenoic acid methyl ester and β-carotenoic acid ethyl ester. However, in 2000 the SCF withdrew the entire group ADI based on a recent evaluation of β-carotene. The SCF decided that there was insufficient scientific basis, either from human or experimental studies, on which to set a new ADI for β-carotene and related carotenoids, but was nonetheless of the opinion that currently permitted food additive uses of β-carotene and related carotenoids are temporarily acceptable from a health point of view at the estimated levels of intake.

At present only β-apo-8'-carotenal and β-apo-8'-carotenoic acid ethyl ester are specifically defined in Commission Directive 2008/128/EC and JECFA. According to specifications, β-apo-8'-carotenal has to comprise at least 96% of the final product in commercial β-apo-8'-carotenal products. The sum of all subsidiary colouring matters is less than 3% and these subsidiary colouring matters are related carotenoids, mainly β-carotene. The Panel noted that the specifications should be updated to more clearly define the purity of the material.

The Panel concluded that the available data indicate that after oral administration absorption of β-apo-8'-carotenal and/or its metabolites is at least 15%. The absorbed β-apo-8'-carotenal is metabolically converted to β-apo-8'-carotenoic acid as well as to its ethyl and methyl esters in rats.

The Panel noted that the SCF has questioned the suitability of rodents as a test species for evaluating the bioavailability and effects of β-carotene in human, since rodents were considered to convert β-carotene to vitamin A much more efficiently than humans. Most laboratory animals were reported to degrade β-carotene in their intestines and absorb almost no β-carotene intact, due to high dioxygenase activity converting β-carotene to retinal, which, according to SCF, is in contrast to humans where β-carotene is mainly (20-75%) absorbed intact. Therefore rodent studies were considered to lack relevance for human risk assessment, and the Panel considered that such limitations may also apply to β-apo-8'-carotenal evaluated in the present opinion. Therefore, the Panel carefully evaluated the ADME characteristics for β-apo-8'-carotenal in both rodent and human focusing on possible differences.

An overview of the major results from an ADME study with β-apo-8'-carotenal in rats compared to those from a human study was made. According to the metabolite pattern in plasma the uptake and metabolism of β-apo-8'-carotenal seems qualitatively similar in rats and humans. Comparison of the plasma kinetic data from the rat with the data from the human study indicates also quantitative similarities between the two species.

Based on the metabolite pattern in blood plasma and based on the plasma kinetics the Panel concluded that for β-apo-8'-carotenal rats are a suitable model for humans concerning uptake and systemic exposure to β-apo-8'-carotenal and metabolites.

A direct comparison of the formation of vitamin A from β-apo-8'-carotenal in rats and humans is more difficult, since the experimental setup of the two studies was not identical. Results obtained reveal that in rats as well as in humans vitamin A is formed from β-apo-8'-carotenal. From the existing data a quantitative comparison can not be made.

No acute oral toxicity of β-apo-8'-carotenal was observed at relatively high doses.

Studies on genotoxicity provided upon a public call for data revealed generally negative results in *Salmonella typhimurium/Escherichia coli* reverse mutation assays, an in vitro genotoxicity assay using Chinese hamster ovary (CHO) cells and an in vivo rat bone marrow micronucleus test.

A few more recent studies have addressed the genotoxicity of β-carotene cleavage products. In a study with primary rat hepatocytes, statistically significant increases of micronuclei and chromosomal aberrations were observed when cells were treated with a mixture of β-carotene cleavage products or apo-8'-carotenal at concentrations from 0.01 to 10 μM. In the same experimental conditions, a dose related increase of sister chromatid exchanges, which attained statistical significance at top dose, was also observed. β-Carotene, tested in the same dose range, induced neither significant cytotoxic nor genotoxic effects. Concerning the significance of these experimental findings, the Panel noted that there is limited experience with cytogenetic assays in primary rat hepatocytes, which show a very high spontaneous incidence of both micronuclei and chromosomal aberrations. Moreover the increases in the frequency of micronuclei and chromosomal aberrations observed in presence of β-carotene cleavage products, and micronuclei in the presence of apo-8'-carotenal, were not dose-related over a 10 000-fold concentration range. The statistically significant increases in the frequency of micronuclei are only 20 and 11% (at 0.1 and 1 μM, respectively) over control incidence, which is within the range of experimental variation for the end-points studied, and thus has limited or no biological significance. The frequencies of chromosomal aberrations in the presence of apo-8'-carotenal show an apparent treatment-related increase, but data are unreliable since they are based on only 20 metaphases/culture. The increase in sister chromatid exchanges observed in presence of both β-carotene cleavage products and apo-8'-carotenal is more credible, but the biological significance of this indicative assay, in relation to genotoxicity is indirect.

In another study the genotoxic effects of the β-carotene breakdown product β-apo-8-carotenal in Human Retinal Pigment Epithelial Cells (ARPE-19) was investigated using the Comet assay. The authors concluded that their results suggest that β-apo-8-carotenal, when applied at partially toxic doses, is genotoxic inducing DNA single strand breaks, prevented by high levels of GSH. The authors however also stated that the mechanism of genotoxicity of β-apo-8-carotenal, i.e. whether direct via DNA damage and apoptosis, or indirect through plasma membrane damage and necrosis, could not be disclosed. Thus no firm conclusion on the genotoxic potential of the β-apo-8-carotenal can be drawn from this study.

The Panel concluded that the genotoxicity studies of the β-carotene breakdown products in primary rat hepatocytes and in ARPE-19 cells in the studies quoted above, provide very limited evidence of genotoxicity.

The induction of DNA strand breaks by β-apo-8'-carotenal, but not by β-carotene, was reported in another in vitro Comet assay on A549 cells.

Overall the Panel concluded that the available in vitro genotoxicity studies and the in vivo micronucleus study with β-apo-8'-carotenal do not give reason for concern with respect to genotoxicity.

Upon a public call for data two subchronic toxicity studies in rats performed according to OECD guidelines and under GLP became available for evaluation, including a 4 weeks toxicity study in rats and a 13 weeks study in rats both using β-apo-8'-carotenal 10% WS/N. Five groups of 10 male and 10 female Sprague-Dawley rats were dosed continuously by diet for 13 consecutive weeks at levels of 0, 0 (placebo), 10, 30 and 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. There were no signs of neurotoxicity, body weight or food consumption effects that could be attributed to administration of β-apo-8'-carotenal 10% WS/N. Statistically significant increases in white blood cells and some of the associated parameters were seen in males receiving 30 and 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. Minor increases were seen in AST and ALT levels in females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. These differences were not evident after the recovery phase. Histopathologically, administration of β-apo-8'-carotenal 10% WS/N in the diet was associated with eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above, multinucleate hepatocytes in the liver of females at 30 mg β-apo-8'-carotenal

active ingredient/kg bw/day and above, and with increased numbers of inflammatory cell foci in the liver of females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day.

The finding of eosinophilic droplets at 10 and 30 mg β-apo-8'-carotenal active ingredient/kg bw/day was minimal in degree in all affected animals and, in the absence of other changes in the kidney, was considered by the authors not to be an adverse effect.

Overall, the authors concluded in first instance that the No Observed Adverse Effect Level (NOAEL) was considered to be 10 mg β-apo-8'-carotenal active ingredient/kg bw/day.

An amendment to this first description of the study was also submitted. In this report it was stated that it was realized that the originally defined NOAEL in the 13-week rat study for β-apo-8'-carotenal (Edwards et al., 2007) may be open to question. Specifically the histopathological change of multinucleated hepatocytes (MNHs) was reported as an adverse change whereas there are literature references to this change being adaptive and not adverse (Williams and Iatropoulos, 2002). Accordingly, a limited peer review was organized. Based on the peer review, expert opinion and also taking into account published information on the long half life of multinucleated hepatocytes (MNHs), the study pathologist and the peer review pathologist came to the mutually agreed-upon conclusion that the change of MNHs as observed in this study is not adverse. Accordingly the overall interpretation of the NOAEL in this study has been reviewed.

The amendment indicates that histopathologically, administration of β-apo-8'-carotenal 10% WS/N in the diet was associated with eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above, for which there was no NOAEL. The finding of eosinophilic droplets at 10 and 30 mg β-apo-8'-carotenal active ingredient/kg bw/day was minimal in degree in all affected animals and, in the absence of other changes in the kidney, was considered in the amendment not to be an adverse effect.

Administration of β-apo-8'-carotenal 10% WS/N was also associated with increased numbers of inflammatory cell foci in the liver of females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day, for which the NOAEL was 30 mg β-apo-8'-carotenal active ingredient/kg bw/day. Increased incidence of multinucleate hepatocytes in the liver was seen in females at 30 mg β-apo-8'-carotenal active ingredient/kg bw/day and above, for which the NOAEL was 10 mg β-apo-8'-carotenal active ingredient/kg bw/day.

The amendment report concluded that overall, for males the NOAEL was 100 mg β-apo-8'-carotenal active ingredient/kg bw/day and for females it was 30 mg β-apo-8'-carotenal active ingredient/kg bw/day (based on increased hepatic inflammatory cell foci).

The Panel closely evaluated the data on the eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above. The Panel concluded that the data were not suitable for a BMD analysis, but that a LOAEL of 10 mg/kg bw/day could be identified for these histopathological changes in the kidneys.

Two studies on reproductive and developmental toxicity have been conducted well before the introduction of (OECD) GLP guidelines, both in rats (Anonymous, 1966). Although on the basis of these two studies, there appears to be no direct reason for concern, it should be considered that the available studies were conducted well before the introduction of (OECD) GLP guidelines, and that in addition, the studies have been described in very little detail in an evaluation conducted over three decades ago.

Upon a public call for data two additional studies on reproductive and developmental toxicity became available for evaluation; a range-finding study and a subsequent study investigating the effects of β-apo-8'-carotenal 10% WS/N on embryonic and fetal development of the rat when administered by the oral route (dietary admixture). These studies were performed according to OECD guidelines and under GLP. The NOAEL for maternal toxicity was considered by the authors to be the dietary concentration of 2064 mg/kg diet (20 mg β-apo-8'-carotenal/kg bw/day). The Panel noted that the reduction in mean body weight gain was observed during the first 9 days of treatment (days 6 to 15 of gestation) in the 100 and 500 mg/kg bw/day groups compared with the vehicle control group and that this effect

attained statistical significance only between days 6 and 11 of gestation. As a consequence, net mean body weight gain during the treatment period (days 6 to 20 of gestation) was slightly but not statistically significantly lower in these groups. The Panel therefore considered the slight reduction in mean body weight and food intake not as adverse and concluded that the NOAEL of this study was 500 mg/kg bw/day.

Long term carcinogenicity studies on β-apo-8'-carotenal were made available to the Panel upon a public call for data and were performed well before the introduction of (OECD) GLP guidelines. A summary of these chronic toxicity studies with β-apo-8'-carotenal was also provided. β-Apo-8'-carotenal was administered at 0.1% in the diet to the first generation of Wistar rats and their offspring (second generation) for a period of 2 years and to a third generation for a period of 1 year. The average intake of β-apo-8'-carotenal was about 40 mg/kg bw/day. The spontaneous tumours were distributed evenly over both groups (11/147 treated rats and 10/122 untreated rats) and consequently the authors concluded that their occurrence was not connected with the application of β-apo-8'-carotenal. Based on these results the Panel considered that there were no adverse effects in this chronic toxicity study at the single dose level tested amounting to about 40 mg/kg bw/day.

Overall, the Panel concluded that the present database on β-apo-8'-carotenal provides a basis to revise the ADI of 5 mg/kg bw/day.

The Panel concluded that based on the LOAEL of 10 mg/kg bw/day from the 13 week study in rats showing an increased incidence of eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and an uncertainty factor of 200, an ADI for β-apo-8'-carotenal of 0.05 mg/kg bw/day can be established. The Panel considered an uncertainty factor of 200 to derive the ADI from the LOAEL sufficient, given the fact that the increase in the eosinophilic droplets in the kidneys at the LOAEL was minimal.

The Panel also considered whether this carotenoid could be included in a group ADI with β-carotene.

However, since the re-evaluation of β-carotene concluded that an ADI for β-carotene could not be established the Panel concluded that a group ADI including β-apo-8'-carotenal and β-carotene can not be established.

Exposure estimates based on Tier 2 using maximum permitted levels would result in exposures to β-apo-8´-carotenal of 0.9 mg/kg bw/day for adults on average and of 3.3 mg/kg bw/day at the 95th percentile. For children, Tier 2 estimates would result in exposures to the colour in the range of 0.5-3.4 mg/kg bw/day on average and in the range of 1.2-7.2 mg/kg bw/day at the 95th/97.5th percentile.

It was indicated by food industry however that the colour is relatively rarely used due to its comparability to β-carotene. Based on this information and the maximum reported use levels provided, Tier 3 estimates lead to exposure to β-apo-8´-carotenal of adults of 0.05 mg/kg bw/day on average and of 0.19 mg/kg bw/day at the 97.5th percentile with non-alcoholic flavoured drinks being the main contributor (92%). Exposure estimates at Tier 3 for children were calculated across European countries in the range of 0.02-0.22 mg/kg bw/day on average and in the range of 0.09-0.71 at the 95th/97.5th percentile. Main contributors for children´s exposure to β-apo-8´-carotenal were non-alcoholic flavoured drinks (50-91%) and fine bakery wares (11-50%).

The Panel concluded that exposure estimates at Tier 3 using the maximum reported use levels for the few food categories where food industry reported the use of β-apo-8´-carotenal are at the level of the ADI of 0.05 mg/kg bw for adults on average and exceed this ADI for adults at the 95th percentile and for children at both average and 95th/97.5th percentiles for all European countries.

The Panel noted that the specifications should be updated to more clearly define the purity of the material.

The Panel noted that specifications should be extended to include maximum residue limits for residual solvents.

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[4] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010[5]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[6] of 2001. The report "Food additives in Europe 2000[7]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] OJ L 354, 31.12.2008, p. 16.
[5] OJ L 80, 26.03.2010, p19
[6] COM(2001) 542 final.
[7] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of β-apo-8'-carotenal (E 160e) when used as a food colour.

β-Apo-8'-carotenal (E 160e) is authorised as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 and the EU Scientific Committee for Food (SCF) in 1975 and 2000.

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Chemistry

β-Apo-8'-carotenal (E 160e) is a carotenoid food colour with the chemical name (2E, 4E, 6E, 8E, 10E, 12E, 14E, 16E)-2,4,6,8,10,12,14,16-heptadecaoctaenal, 2,6,11,15-tetramethyl-17-(2,6,6-trimethyl-1-cyclohexen-1-yl)-. The CAS Registry Number is 1107-26-2 the EINECS number is 214-171-6, and its colour index number is 40820. The molecular formula is C30H40O, its molecular weight is 416.65 g/mol and its structural formula is presented in Figure 1.

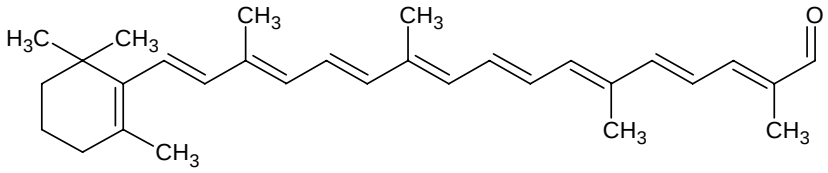

Figure 1: Structural formula of β-apo-8'-carotenal

β-Apo-8'-carotenal consists of dark violet crystals with metallic lustre or is a crystalline powder. The Panel noted that the compound is essentially insoluble in water (US EPA, Syracuse Research Corporation), and that the compound is slightly soluble in ethanol and soluble in lipids. Synonyms include: CI Food Orange 6, C Orange 16.

2.2. Specifications

β-Apo-8'-carotenal is specifically defined in Commission Directive 2008/128/EC[8] and by JECFA (JECFA, 2011). In the JECFA and in the EU specifications, it is indicated that the specifications apply to predominantly the all-trans (E) isomer of β-apo-8'-carotenal together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from ß-apo-8'-carotenal meeting these specifications, and include solutions or suspensions of β-apo-8'-carotenal in edible fats or oils, emulsions and water dispersible powders; these preparations may have different cis/trans isomer ratios (JECFA, 2011; Directive 2008/128/EC).

[8] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20-63

The β-apo-8'-carotenal content in the additive should be not less than 96% of the total colouring matters.

Table 1 presents the specifications for β-apo-8'-carotenal according to Commission Directive 2008/128/EC and JECFA (JECFA, 2011).

Table 1: Specifications for β-apo-8'-carotenal according to Commission Directive 2008/128/EC and JECFA (JECFA, 2011)

	Commission Directive 2008/128/EC	**JECFA (2011)**
Assay	Not less than 96% of total colouring matters $E_{1\ cm}^{1\%}$ 2 640 at ca 460-462 nm in cyclohexane	Not less than 96% of total colouring matters
Identification	Maximum in cyclohexane at 460-462 nm	Determine the absorbance of the sample solution (See Method of Assay) at 461 nm and 488 nm. The ratio A_{488}/A_{461} is between 0.80 and 0.84
Purity		
Subsidiary colouring matters	≤ 3.0%f total colouring matters may be carotenoids other than β-apo-8'-carotenal	≤ 3% of total colouring matters may be carotenoids other than β-apo-8'-carotenal
Sulphated ash	≤ 0.1%	≤ 0.1%
Arsenic	≤ 3 mg/kg	-
Lead	≤ 10 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	-
Cadmium	≤ 1 mg/kg	-
Heavy metals (as Pb)	≤ 40 mg/kg	-

According to the specifications and the purity criteria, β-apo-8'-carotenal should comprise at least 96% of total colouring matters in commercial products. As, however, the amount of total colouring matters is not quantified, the amount of β-apo-8'-carotenal as well as the major part of the commercial product, remain undefined in the present specifications. Upon request EFSA was informed that the pure, crystalline β-apo-8'-carotenal final product complies with this criterion of "not less than 96%". According to EU Directive 2008/128/EC this is determined by absorption (E1cm, 1% 2640 at ca 460 – 462 nm) measured in cyclohexane. The sum of all subsidiary colouring matters is less than 3% and these subsidiary colouring matters are related carotenoids, mainly β-carotene. The Panel noted that the specifications should be updated to more clearly define the purity of the material.

The Panel noted that the JECFA specification for lead is ≤ 2 mg/kg whereas the EC specification is ≤ 10 mg/kg.

The Panel noted that specifications should be extended to include maximum residue limits for residual solvents.

2.3. Manufacturing process

β-Apo-8'-carotenal is produced by chemically synthesis. The detailed production process description for β-apo-8'-carotenal was made available to EFSA. The solvent employed in the production process are methanol, iso-propanol, acetone and water and do not give reason for concern provided that specifications are extended to include residue limits for these solvents. The pure, crystalline β-apo-8'-carotenal final product complies with the criterion of "not less than 96%".

2.4. Methods of analysis in food

Several methods for the determination of β-apo-8'-carotenal in foods are described in public literature, of which variations of High Performance Liquid Chromatography (HPLC) appear to be most generally employed (Oliver and Palou 2000). In addition, according to JECFA (JECFA, 2011) β-apo-8'-carotenal can be analyzed by means of spectrophotometry. The analytical methods described for the parent colour are not necessarily suitable for the determination of impurities in the stabilized forms.

EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety of use of colouring agents, including β-apo-8'-carotenal, in animal nutrition (EFSA, 2009). In this opinion it is indicated that the CRL (European Union Reference Laboratory for GM Food and Feed formerly known as Community Reference Laboratory) reports that no ISO and CEN methods could be found as the official analytical method for the determination of β-apo-8'-carotenal in feeding stuffs or other relevant matrices, and that HPLC is currently the method of choice for carotenoid analysis since it gives the most accurate, sensitive and reproducible quantitative analyses of carotenoid content and composition. β-Apo-8'carotenal was analyzed by using thin layer chromatography (TLC) and HPLC with UV detection from red pepper (Minguez-Mosquera et al., 1995). The opinion also indicated that β-apo-8'-carotenal was analysed from retail foods and beverages by using HPLC and photodiode array (Scotter et al., 2003). The limit of detection (LOD) and the limit of quantification (LOQ) of β-apo-8'-carotenal in their method were 0.01 and 0.1 mg/kg, respectively. The response was linear over the range of 0–50 mg/l. β-Apo-8'-carotenal was well separated from the *trans* and *cis* isomers of β-carotene.

2.5. Reaction and fate in food

No data were available on the reaction and fate in food of β-apo-8'-carotenal. However, in general the majority of colour additives are unstable in combination with oxidising and reducing agents in food. Since colour depends on the existence of a conjugated unsaturated system within the dye molecule, any substance which modifies this system (e.g. oxidising or reducing agents, sugars, acids, and salts) will affect the colour (Scotter and Castle, 2004).

2.6. Case of need and proposed uses

Authorised use levels have been defined in the EU legislation (Directive 94/36/EC)9.

Currently, β-apo-8'-carotenal is an authorised food colour in the EU with maximal permitted levels of 50 to 500 mg/kg food for various foodstuffs (individually or in combination with other food colours). β-Apo-8'-carotenal is also allowed in beverages at levels up to 200 mg/l (individually or in combination with other food colours). Table 2 summarizes those beverages and foodstuffs that are permitted to contain β-apo-8'-carotenal up to specified maximum permitted levels (MPLs) set by EC legislation (Council Directive 94/36/EC).

[9] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.09.1994, p.13-29.

Table 2: Maximum permitted levels (MPLs) of β-apo-8'-carotenal in beverages and foodstuffs according to Council Directive 94/36/EC and maximum reported use levels

Beverages	**Maximum Permitted Level** (mg/l)	**Maximum reported use levels for β-apo-8´-carotenal** (mg/l)
Non-alcoholic flavoured drinks	100	11.8
Spirituous beverages	200	0
Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails		0
Fruit wines, cider and perry		0
Foodstuffs	**Maximum Permitted Level** (mg/kg)	**Maximum reported use levels (mg/kg)**
Complete formulae for weight control intended to replace total daily food intake or an individual meal	50	0
Complete formulae and nutritional supplements for use under medical supervision		0
Soups		0
Flavoured processed cheese	100	0
Fish paste and crustaceans paste		0
Smoked fish		0
Savoury snack products and savoury coated nuts		0
Liquid food supplements/dietary integrators		0
Meat and fish analogues based on vegetable proteins		0
Edible ices	150	0
Desserts including flavoured milk products		0
Fine bakery wares	200	7.5
Candied fruit and vegetables, Mostarda di frutta		0
Preserves of red fruits		0
Extruded or expanded savoury snack products		0
Pre-cooked crustaceans	250	0
Confectionery	300	8.26
Mustard		0
Fish roe		0
Solid food supplements/dietary integrators		0
Decorations and coatings	500	0
Sauces, seasonings, pickles, relishes, chutney and piccalilli		0
Salmon substitutes		0
Surimi		0
Edible cheese rind and edible casings	*Quantum satis*	0

2.7. Information on existing authorisations and evaluations

Apo-8'-carotenal is authorised as a food additive in the EU under Directive 94/36/EC.

Based on the JECFA evaluation of 1975, a group Acceptable Daily Intake (ADI) of 0-5 mg/kg bw/day was estimated for the sum of the carotenoids β-carotene, β-apo-8'-carotenal, and β-apo-8'-carotenoic acid methyl and ethyl ester. Results from a four-generation rat study with β-carotene (Bagdon et al., 1960) were used for calculation of the group ADI. In this study, no adverse effects were seen at 50 mg β-carotene/kg bw/day, and an uncertainty factor of 10 instead of 100 was used because of the natural occurrence of carotenoids in the human diet and the low toxicity of carotenoids in animal studies.

In 1975, the SCF endorsed the ADI established by JECFA of 0-5 mg/kg bw/day as the sum of the carotenoids β-carotene, β-apo-8'-carotenal and β-apo-8'-carotenoic acid methyl and ethyl ester. The SCF in an accompanying comment (1975) mentions that only the ethyl ester is listed in the Community Directive and that this was the only compound considered. Consequently, the ADI was expressed as the sum of β-carotene, β-apo-8'-carotenal and the ethyl ester of β-apo-8'-carotenoic acid alone.

When however β-carotene was re-evaluated (SCF, 2000), the SCF decided to withdraw the group ADI of 5 mg/kg bw/day for β-apo-8´-carotenal, β-carotene and the ethyl ester of β-apo-8'-carotenoic acid. The ADI was withdrawn for two main reasons. First, the ADI was based on rodent studies, and since rodents were considered to convert β-carotene to vitamin A much more efficiently than humans, these studies were considered to lack relevance for human risk assessment. The second reason was the adverse findings in human smokers receiving β-carotene supplements at 20 mg/person/day or more, amounts that are much lower than the previously established ADI. The SCF considered the scientific basis to be insufficient to set a new ADI.

As, however, there were no indications that daily intakes of about 1-2 mg β-carotene and/or related carotenoids, as food additives, are harmful in the context of the overall dietary intake of these substances, and as, in addition, the scientific basis was considered to be insufficient to set a new ADI, the Committee decided that currently permitted food additive uses of β-carotene and related carotenoids would be temporarily acceptable (SCF, 2000).

An additional evaluation can be found in a report released by the Nordic Council of Ministers (TemaNord, 2002) who has taken into account the literature published until 2000.

Data on specifications and permitted levels have been defined in the EU legislation (in particular Directives 2008/128/EC and 94/36/EC) and by JECFA (JECFA, 2011).

EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety of use of colouring agents, including β-apo-8'-carotenal, in animal nutrition (EFSA, 2009). It was stated that data on the safety of β-apo-8'-carotenal for the target animals are not available, but that given the natural occurrence of the compound and considering the molecular structure of the carotenoid, the FEEDAP Panel does not see reasons for concern. It was also concluded that several in vitro studies, performed with β-apo-8'-carotenal in both prokaryotic and eukaryotic test systems, do not give rise to safety concerns with respect to the genotoxicity of the compound. The FEEDAP Panel also concluded that there are no safety concerns for the consumer from the consumption of eggs from hens fed β-apo-8'-carotenal supplemented diets and that although data to estimate human exposure from poultry tissues are not available, safety concerns are not likely.

2.8. Exposure

The Panel followed the principles of the stepwise approach, which were used in the report of the scientific cooperation (SCOOP) Task 4.2 (EC, 1997), to estimate intakes of food additives. For each successive Tier, this involved a further refinement of intake estimates. The approach progresses from the conservative estimates that form the first Tier of screening, to more realistic estimates that form the second and third Tiers. In the tiered approach, Tier 1 is based on theoretical food consumption data and maximum permitted levels (MPLs) for additives as permitted by relevant Community legislation. The second and third Tiers refer to assessment at the level of individual Member States, combining national data on food consumption with the maximum permitted levels for the food additive (Tier 2) and with its actual usage patterns (Tier 3).

2.8.1. Crude estimates (Budget Method) for β-apo-8'-carotenal

The dietary exposure to β-apo-8'-carotenal from the maximum permitted levels was estimated using the Budget method (Tier 1), which is based on the fact that there is a physiological upper limit to the amount of food and drink (for beverages 100 ml/kg bw and for solids 25 g/kg bw), and thus of food additives, that can be consumed each day. A further assumption is that only a certain proportion of the

diet is likely to contain food additives (25%). Full details on the budget method are described in the report of the SCOOP Task 4.2 (EC, 1997).

In the case of β-apo-8'-carotenal, the maximum permitted level in beverages was 200 mg/l (Directive 94/36/EC). The maximum permitted level in solid foods was 500 mg/kg.

The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. In fact, even though β-apo-8'-carotenal may be used in a variety of solid foods and beverages that could represent more than 25% of processed foods, it is unlikely that a person would systematically choose all processed foods with the same colour added even considering brand loyalty. This assumes that a typical adult weighing 60 kg consumes daily 1.5 liters of beverage and 375 grams of solid foods containing β-apo-8'-carotenal .

The overall theoretical maximum daily exposure to β-apo-8'-carotenal for adults would therefore be:

(200 x 0.1 x 0.25) + (500 x 0.025 x 0.25) = 5 + 3.12 = 8.1 mg/kg bw/day.

For children, the level of β-apo-8'8'-carotenal considered in beverages was 100 mg/l (after exclusion of alcoholic drinks) and in solid food was 500 mg/kg. As recommended by SCOOP task 4.2 (EC, 1997) for children, it is assumed that 100% of beverages contain the additive. This conclusion was derived from UK data on consumption of soft drinks by children aged under 5 years, where the 97.5th percentile of consumption was between 70 and 80 ml/kg bw/day. This assumes that a typical 3 year-old child weighing 15 kg consumes daily 1.5 litres of beverages and 94 g of solid foods containing β-apo-8'-carotenal.

The overall theoretical maximum daily exposure to β-apo-8'-carotenal in children would therefore be:

(100 x 0.1 x 1) + (500 x 0.025 x 0.25) = 10 + 3.12 = 13.1 mg/kg bw/day.

It was noted that β-apo-8'-carotenal may be used *quantum satis* in edible cheese rind and edible casings. As this is a very specific food category, which is unlikely to be consumed in high amounts on a daily basis, if at all, it was excluded from the Budget method calculation, since it is not expected to influence the outcome of this exposure calculation to any relevant extent.

2.8.2. Refined estimates for β-apo-8'-carotenal

Refined exposure estimates have been performed for Tier 2 using MPLs presented in Table 2, and for Tier 3 using the maximum reported use levels presented in Table 2, both combined with national consumption data for children and the adult population.

The Panel noted that its estimates could be considered as being conservative as it is assumed that all processed foods and beverages contain β-apo-8'-carotenal added at the maximum reported use levels.

For adults, the Panel calculated the exposure based on the UK consumption survey, as the UK population is considered to be one of the highest consumers of soft drinks in Europe, and also because detailed individual food consumption data (UK NDNS, 2000-2001) were available from the UNESDA report (Tennant et al., 2006) and the Natural Food Colours Association (NATCOL) report (Tennant 2007).

Exposure estimates for children (1-10 years old) have been performed by the Panel based on detailed individual food consumption data from eleven European countries (Belgium, France, the Netherlands, Spain, Italy, Finland, Greece, Cyprus, Czech Republic, Sweden and Germany) provided by the EXPOCHI ("Individual food consumption data and exposure assessment studies for children") consortium (Huybrechts et al., 2010). As the UK is not part of the EXPOCHI consortium, estimates for UK children (aged 1.5 - 4.5 years) were made by the Panel with the use of detailed individual food consumption data (UK NDNS, 1992-1993) available from the UNESDA report (Tennant et al., 2006) and the NATCOL report (Tennant, 2007). Table 3 summarises the anticipated exposure of children and adults to β-apo-8'-carotenal.

Tier 2

In the case of β-apo-8'-carotenal, when considering MPLs (Tier 2), estimates reported for the UK adult population give a mean dietary exposure to β-apo-8'-carotenal ranging from 0.9 to 3.3 mg/kg bw/day for high level (97.5th percentile) consumers. The main contributors to the total anticipated mean exposure to β-apo-8'-carotenal (>10%) were non-alcoholic flavoured drinks (47%).

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg) considered on the basis of the EXPOCHI data and UK children ranged from 0.5 to 3.4 mg/kg bw/day, and from 1.2 to 7.2 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to β-apo-8'-carotenal (>10% in all countries), were non-alcoholic flavoured drinks (14-55%), fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (13-46%), desserts, including flavoured milk products (12-50%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (11- 44%).

Tier 3

Further data suggest that reported use levels of β-apo-8'-carotenal in some food categories are lower than the MPLs. Therefore, it was decided that concentration data made available to the Panel by the food industry (Tennant 2008, updated in February 2011) would be used to refine the estimate of dietary exposure to β-apo-8'-carotenal (Tier 3).

When considering the maximum reported use levels from Table 2, estimates reported for the UK adult population give a dietary exposure to β-apo-8'-carotenal ranging from 0.05 mg/kg bw/day on average to 0.19 mg/kg bw/day for high level (97.5th percentile) consumers. The main contributors to the total anticipated mean exposure to β-apo-8'-carotenal (>10%) were non-alcoholic flavoured drinks (92%).

The mean dietary exposure of European children (aged 1-10 years and weighing 16-29 kg), considered on the basis of the EXPOCHI data and UK children, ranged from 0.02 to 0.22 mg/kg bw/day, and from 0.09 to 0.71 mg/kg bw/day at the 95th percentile. The main contributors to the total anticipated mean exposure to β-apo-8'-carotenal (>10% in all countries), were non-alcoholic flavoured drinks (50-91%), and fine bakery wares (e.g. Viennoiserie, biscuits, cakes, wafer) (11-50%).

Table 3: Summary of anticipated exposure to β-apo-8'-carotenal. using the tiered approach (EC, 2001) in children and the adult population

	Adult UK population (>18 years old) mg/kg bw/day	**Children UK & EXPOCHI population** (1-10 years old, 15.8-29 kg body weight) mg/kg bw/day
Tier 1. Budget method	8.1	13.1
Tier 2. Maximum Permitted Level		
• Mean exposure	0.9	0.5 – 3.4
• Exposure 95th*or 97.5th percentile*	3.3	1.2 – 7.2
Tier 3. Maximum reported use levels		
• Mean exposure	0.05	0.02 – 0.22
• Exposure 95th*or 97.5th percentile*	0.19	0.09 – 0.71

* For the UK population, estimates are based on the UNESDA report which gives the 97.5th percentile (Tennant, 2006).

3. Biological and toxicological data

β-Apo-8'-carotenal (E 160e) has been previously evaluated by JECFA in 1974, the SCF in 1975 and 2000, and TemaNord in 2002. The present opinion briefly reports the major studies evaluated in these reports and describes additional newly reported literature data in some more detail.

The studies used for the JECFA and SCF evaluations were performed in the 1960s when Good Laboratory Practice (GLP) guidelines were not yet implemented. OECD GLP guidelines were not promulgated before 1981. It is unclear whether these reported studies comply with OECD and GLP guidelines.

In the new ADME and toxicity studies provided the β-apo-8'-carotenal tested was typically a standard commercial product form of beadlets, such as β-apo-8'-carotenal 10% WS/N with WS denoting water soluble. β-Apo-8'-carotenal 10% WS/N consists of free flowing particles (beadlets), containing β-apo-8'-carotenal dispersed in a cornstarch-coated matrix of gelatin, sucrose and corn oil.

In the ADME study radio-labeled material needed to be used, and thus no commercial formulation could be tested. However, the investigators of the ADME study in report no 2500426 used a preparation denoted as "simulated beadlets". The investigators of the study intended to express with the term "simulated beadlet" that they endeavoured as best as possible to formulate and test a material in the ADME study that was as similar as possible to the commercial product form, being the WS/N form of β-apo-8'-carotenal, a beadlet formulation.

3.1. Absorption, distribution, metabolism and excretion

JECFA in its evaluation in 1975 describes several studies on the toxicokinetic aspects of β-apo-8'-carotenal and the ethyl and methyl esters of β-apo-8'-carotenoic acid.

After dietary administration of β-apo-8'-carotenal to rats, some β-apo-8'-carotenal accumulates in the liver together with vitamin A (= retinol) and β-apo-8'-carotenoic acid (no further details provided) (Thommen, 1962; Brubacher et al., 1960).

When large doses (100 and 500 mg/kg bw /day) of a mixture containing β-apo-8'-carotenal and β-apo-8'-carotenoic acid methyl ester are given to rats, only a minor fraction is absorbed and most (76-104%) is excreted in the faeces. β-Apo-8'-carotenoic acid as well as its ethyl and methyl esters are normal metabolites of β-apo-8'-carotenal (Wiss and Thommen, 1963).

After dietary exposure of vitamin A-deficient rats to β-apo-8'-carotenal, only 4% is converted to vitamin A in the gut (compared to 10% after exposure to β-carotene). Carotenals are easily oxidized to carotenoic acids and less readily reduced to alcohols in vivo. This finding points to metabolic pathways other than β-oxidation (Wiss and Thommen, 1963; Glover, 1960).

In dogs, β-apo-8'-carotenal is poorly absorbed from the gastrointestinal tract and excreted in the urine together with β-apo-8'-carotenoic acid. No hypervitaminosis A was noted (Bagdon et al., 1960).

Dogs (2-4/group/sex) received 0, 100 or 1000 mg β-apo-8'-carotenal/animal/day for 14 weeks (no detail on route of administration). The level of β-apo-8'-carotenal in serum was elevated in the group receiving 1000 mg and there was an occasional trace in the group receiving 100 mg. The level of vitamin A in the kidney was increased three to five-fold compared to controls. Values of β-apo-8'-carotenal levels in other tissue were variable. Microscopic examination revealed pigmentation of the adipose tissue, kidney and adrenal cortex (Bagdon et al., 1962). The Panel noted that although the authors concluded that the content of vitamin A in the kidneys of β-apo-8'-carotenal treated dogs was 3 to 5-fold higher than in the kidneys of the control animals, close evaluation of the study results does not seem to support this conclusion. The control animal kidneys had a mean vitamin A concentration of 104 IU/g, with a range from 45 – 165 IU/g. The kidneys from the animals dosed with 100 mg β-apo-8'-carotenal/day had a mean concentration of 359 (204 – 580) IU/g and the dogs treated with 1000 mg β-apo-8'-carotenal/day a mean concentration of 227 IU/g with a range of 46 – 440 IU vitamin A/g. From this it appears that there was no dose dependency in the kidney vitamin A concentration, and the mean concentration from the high dose treatment appears to be even lower than that from the low dose treatment. The variations in the animals within a dose group are high and two from six animals from the high dose groups have kidney concentrations similar to the control animals.

β-Apo-8'-carotenal has been associated with hypercholerolemic activity (Wood, 1963).

The ester of β-apo-8'-carotenoic acid (not further specified as ethyl or methyl) was eliminated from the blood of human infants rapidly and proportionally to the blood concentration (Kübler, 1963).

TemaNord (2002) mentions an additional study which was allegedly derived from the JECFA evaluation. In this study in humans, a single oral dose of β-apo-8'-carotenal was extensively metabolised, mainly to the corresponding acid, alcohol, and palmitate ester (no specific reference given).

The following new study on absorption, distribution, metabolism and excretion has been provided upon a public call for data.

Five male rats received a single oral dose of [6,7-^{14}C]-β-apo-8'-carotenal by gavage formulated as "simulated beadlets" (for an explanation of this material see above at the introduction for section 3) at a dose of 1.3 mg/kg bw (Rümbeli et al., 2007). One animal was sacrificed 3 hours post dose and terminal blood was collected. From the other four animals multiple blood samples were taken at 1, 2, 4, 6, 8, 10 hours and 24 hours post dose. Urine and faeces were collected from 0-24 hours and 24-48 hours post dose. Those animals were sacrificed 48 hours post dose and the total radioactivity in excreta, plasma, liver, kidneys, fat, intestinal tract and remaining carcass were determined. After dosage of [^{14}C]-β-apo-8'-carotenal the total radioactivity in plasma reached a maximum concentration of 342 ng β-apo-8'-carotenal equivalents/g plasma after 10 hours and the radioactivity was eliminated with a half life of 21 hours.

The major part of the radioactivity applied was excreted within 0-48 hours via faeces (49%) and urine (15%) and 10% remained in the gastrointestinal tract. The concentration of radioactive residues was highest in liver (4.4% of dose), corresponding to 1.6 µg β-apo-8'-carotenal equivalents/g. The concentration in kidney, fat and blood was 1.1, 0.1 and 0.05 µg β-apo-8'-carotenal equivalents/g, respectively. Urine was analyzed by HPLC with radioactivity detection. At least 13 radioactive polar metabolite fractions were characterized each representing $\leq$ 2% of the dose applied. Faeces were extracted and the extracts were analyzed by HPLC with radioactivity and UV/VIS detection. The major radioactive residue eliminated via faeces was identified as β-apo-8'-carotenal, representing 18% of the dose applied. Additional metabolites identified in faeces were 8% β-apo-8'-carotenoic acid and 1% β-apo-8'-carotenol.

Liver was extracted which released 83% of the liver radioactivity. The extract was fractionated and the apolar fraction (67%) was analyzed by HPLC. The major radioactive residues were identified as retinol (16%), retinyl-palmitate (16%) and two other retinyl-fatty acid conjugates (17% and 10%, respectively). No β-apo-8'-carotenal, β-apo-8'-carotenol or β-apo-8'-carotenoic acid was found in liver.

Blood plasma from one animal sacrificed 3 hours post dose was extracted and the extract was analyzed by HPLC. Besides minor amounts of β-apo-8'-carotenal, significant amounts of β-apo-8'-carotenol and β-apo-8'-carotenoic acid were characterized.

The authors concluded that, based on the metabolite patterns in urine, plasma and liver the metabolic pathway of β-apo-8'-carotenal proceeds via oxidation to β-apo-8'-carotenoic acid or reduction to β-apo-8'-carotenol, cleavage at the 15-16 position followed by reduction to retinol, conjugation with fatty acids and excretion as polar metabolites via urine.

The Panel concluded that after oral administration, absorption of β-apo-8'-carotenal and/or its metabolites is at least 15%.

The Panel also noted that the SCF (2000) has questioned the suitability of rodents as a test species for evaluating the bioavailability and effects of β-carotene in human, since rodents were considered to convert β-carotene to vitamin A much more efficiently than humans. Most laboratory animals were reported to degrade β-carotene in their intestines and absorb almost no β-carotene intact, due to high dioxygenase activity converting β-carotene to retinal, which, according to SCF, is in contrast to humans where β-carotene is mainly (20-75%) absorbed intact. Therefore rodent studies were considered to lack relevance for human risk assessment, and the Panel considered that such limitations may also apply to β-apo-8'-carotenal evaluated in the present opinion. Therefore, the Panel carefully

evaluated the ADME characteristics for β-apo-8'-carotenal in both rodent and human focusing on possible differences.

Table 4 presents an overview of the major results from an ADME study with β-apo-8'-carotenal in rats (Rümbeli et al., 2007) compared to those from a human study (Zeng et al., 1992). The rats were dosed with a single dose of 3.1 µmol/kg bw ^{14}C- β-apo-8'-carotenal and the human subjects received a single dose of 1.6 µmol/kg bw. In both species β-apo-8'-carotenal was identified only as a minor component in blood plasma or serum but major metabolites in humans and rats were β-apo-8'-carotenol and fatty acid conjugates of β-apo-8'-carotenol. Additionally, significant amounts of β-apo-8'-carotenoic acid were found in rat plasma, which was only a minor metabolite in human plasma. According to the metabolite pattern in plasma the uptake and metabolism of β-apo-8'-carotenal seems qualitatively similar in rats and humans.

Table 4. Comparison of ADME data from β-apo-8'-carotenal in rat and human.

Species	**Rat**	**Human**
Study	Rümbeli R et al., DSM Nutritional Products Ltd Report, 2007, No. 2500426	Zeng S et al., Am. J. Clin. Nutr. 1992, 56, 433-439
Number of subjects	4	6
Dose	Single oral dose 3.1 µmol/kg bw ^{14}C-β-Apo-8'-carotenal	Single oral dose 1.6 µmol/kg bw β-Apo-8'-carotenal
Major plasma metabolites	β-Apo-8'-carotenol β-Apo-8'-carotenoic acid Fatty acid conjugates	β-Apo-8'-carotenol β-Apo-8'-carotenyl palmitate
Minor plasma metabolites	β-Apo-8'-carotenal	β-Apo-8'-carotenal β-Apo-8'-carotenoic acid
Tmax [h]	6 (total radioactivity) 10 (total radioactivity)	5.5 (β-Apo-8'-carotenol) 10.9 (β-Apo-8'-carotenyl palmitate)
Cmax [µmol/kg] or [µmol/l]	0.71 (total radioactivity) 0.82 (total radioactivity)	0.23 (β-Apo-8'-carotenol) 0.29 (β-Apo-8'-carotenyl palmitate)
AUC [µmol/h x kg] or [µmol/h x l]	19.7 (total radioactivity)	15.6 (β-Apo-8'-carotenol) 4.2 (β-Apo-8'-carotenyl palmitate)

Comparison of the plasma kinetic data from the rat with the data from the human study (see Table 4) indicates also quantitative similarities between the two species. In the human study a plasma maximum (Tmax) of 5.5 hours was found for β-apo-8'-carotenol and one of 10.9 hours for β-apo-8'-carotenyl palmitate. In the rat study the total radioactivity also showed two maxima, one at 6 hours and one at 10 hours post dose (mean plasma curve from all animals). The plasma maxima (Cmax) of the radioactivity in rat plasma corresponded to 0.71 and 0.82 µmol β-apo-8'-carotenal equivalents/l plasma and the human serum maxima corresponded to 0.23 and 0.29 µmol/l based on β-apo-8'-carotenol and β-apo-8'-carotenyl palmitate, respectively. Taking into account that the total radioactivity measurements in rats include additional minor metabolites besides the major metabolites β-apo-8'-carotenol and β-apo-8'-carotenyl palmitate as quantified in the human serum, it can be concluded, that the Cmax in rat plasma is roughly twice as high as the Cmax in human serum. This correlates with the dosing which was twice as high for the rats as for the humans.

A direct comparison of the formation of vitamin A from β-apo-8'-carotenal in rats and humans is more difficult, since the experimental setup of the two studies was not identical. In the human study only blood serum was analyzed for vitamin A and it was stated, that retinyl palmitate was slightly but significantly elevated in all subjects at 12-32 hours after dosing with β-apo-8'-carotenal, indicating that formation of vitamin A in humans can only be detected in blood at a relatively late time point

after dosing of β-apo-8'-carotenal. In the rat study blood plasma was analyzed at a very early time point post dose (2 hours after dosing β-apo-8'-carotenal) – and no retinol or metabolites of retinol were identified. It might be that analysis of the rat plasma at a later time point would also show the presence of vitamin A. Analysis of the radioactive rat liver metabolites showed that retinol and retinyl fatty acid conjugates were the major metabolites. This indicates that in rats as well as in humans vitamin A is formed from β-apo-8'-carotenal. From the existing data a quantitative comparison can not be made.

Also of importance is that literature suggests that β-apo-8'-carotenal can serve as a substrate for the cleavage enzyme β-carotene-15,15`-monooxygenase, but that in vitro studies show a lower enzyme activity though for the substrate β-apo-8'-carotenal as compared to β-carotene. Bachmann et al. (2002) in their feedback regulation study of the β-carotene-15,15`-monooxygenase used the liver storage assay to assess the amount of newly formed retinol in the liver of vitamin A depleted rats as well as retinol plasma levels. Even though not a physiological approach, these data show an increase of vitamin A in the liver of 1.5 – 3.5% of the administered dose after a single dose of β-apo-8'- carotenal. Serum retinol could be restored to approx. 80% compared to β-carotene.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA gives the results of acute toxicity tests with both β-apo-8'-carotenal in mice. In this study the LD50 was found to be >10,000 mg/kg bw (Anonymous, 1966).

The following additional studies on oral acute toxicity have been provided upon a public call for data.

A series of acute oral toxicity studies reported LD50 values of β-apo-8'-carotenal in rats of > 20,000 mg/kg bw (Bächthold, 1972; Bächthold, 1975; Bächthold, 1976), oral LD50 values > 10,000 mg β -apo-8'-carotenal/kg bw in rats (Bächthold, 1973; Bächthold, 1977; Bächthold, 1980; BASF 1972), and oral LD50 values > 10,000 mg β-apo-8'-carotenal/kg bw in mice (Bächthold, 1977).

In another study (Loget and Arcelin, 2006a) two groups, each of three female HanRoc:WIST rats were treated with β-apo-8'-carotenal 10% WS/N by oral gavage at a dose of 2000 mg/kg bw, corresponding to 232 mg β-apo-8'-carotenal/kg bw. The LD50 was higher than 2000 mg/kg bw, corresponding to 232 mg β-apo-8'-carotenal/kg bw.

A similar study with crystalline β-apo-8'-carotenal revealed an LD50 of higher than 2000 mg β-apo-8'-carotenal/kg bw (Loget and Arcelin, 2006b).

3.2.2. Short-term and subchronic toxicity

JECFA has evaluated two subchronic studies with β-apo-8'-carotenal.

Groups of male rats (16/group) were given β-apo-8'-carotenal by gavage at doses of 0, 100 or 500 mg/kg bw/day, 5 days/week, for 34 weeks. Testicular weight was significantly lower in the high-dose group compared to controls. Microscopic examination revealed granular pigment deposition in the liver and kidneys of treated animals. Fertility, as shown by monthly mating of four females, was not affected. No adverse effects were seen on body weight gain, general health, survival, liver and kidney function, or organ weights.

In an identical experiment with β-apo-8'-carotenoic acid methyl ester the results were similar to those described above. No adverse effects on mortality or weight gain were noted, but the male rats receiving 500 mg/kg bw/day showed reduced testicular weights compared with controls and granular pigment deposits in the liver and kidney. No deleterious effect on spermatogenesis was noted (Anonymous, 1962; Anonymous, 1966). According to TemaNord, these studies were performed with β-apo-8'-carotenal and β-apo-8'-carotenoic acid ethyl ester instead of β-apo-8'-carotenoic acid methyl ester.

Groups of dogs (2-4/group/sex) received 0, 100 or 1000 mg β-apo-8'-carotenal/animal/day for 14 weeks (no detail on route of administration). Microscopic examination revealed pigmentation of the adipose tissue, kidney and adrenal cortex. No significant compound-related effects were noted regarding general health, post mortem pathological lesions, peripheral blood picture, liver function, serum enzymes, blood urea levels or organ weights (Bagdon et al., 1962).

The SCF (2000) refers to two short-term studies with β-apo-8'-carotenal.

In mice, supplementation with 300 mg β-apo-8'-carotenal/kg diet (supposedly equal to 37.5 mg/kg bw/day) for 15 days, had no effect on any phase I or phase II xenobiotic-metabolising enzymes in the liver (Astorg et al., 1994, 1997).

In a study in rats, animals were given 300 mg β-apo-8'-carotenal/kg diet (equivalent to 15 mg/kg bw/day) for 15 days. A significant induction on liver CYP-1A1 and -1A2 levels was observed (Gradelet et al., 1996).

The following new studies on subacute and subchronic toxicity have been provided upon a public call for data.

3.2.2.1. Rats

A 13 week rat toxicity study of β-apo-8'-carotenal and degraded β-apo-8'-carotenal was performed (Bagdon, 1964). The latter consisted of 75% β-apo-8'-carotenal and 25% degradation products. Four groups of 20 rats each (10 males and 10 females except for one group (pure β-apo-8'-carotenal) that contained 11 males and 9 females) were given diets containing 0 (control), 1% degraded β-apo-8'-carotenal, 0.5% degraded β-apo-8'-carotenal or 1% pure β-apo-8'-carotenal. The animals were weighed at weekly intervals and food consumption was measured. Complete blood counts were taken during the 2nd, 4th, 9th and 13th weeks. Measurement of liver function was ascertained after 6 and 12 weeks of treatment by determination of alanine aminotransferase (ALT) (SGP-T). Urinalysis was also performed. Upon sacrifice the animals were examined for gross pathological changes. Sections of several tissues (including brain, peripheral nerve, heart, lungs, liver, spleen, pancreas, stomach, small and large intestines, thyroids, adrenals, gonads, urinary bladder, kidneys, bone marrow, prostate, seminal vesicles or uterus) were examined microscopically. The study report stated that the results obtained indicated that rats tolerated 1% of β-apo-8'-carotenal administered in the diet and 1% degraded β-apo-8'-carotenal, 0.5% degraded β-apo-8'-carotenal (consisting of 75% β-apo-8'-carotenal and 25% degradation products) without toxic manifestations. The Panel established a No Observed Adverse Effect Level (NOAEL) of 1% β-apo-8'-carotenal in the diet equivalent to about 500 mg/kg bw/day.

In another study the subacute toxicity of β-apo-8'-carotenal and β-apo-8'-carotinic acid (C30)-methylester were investigated (Schärer et al. 1961). β-Apo-8'-carotenal and β-apo-8'-carotinic acid (C30)-methylester were administered by stomach tube to young male rats (24 animals per treatment group with the control group consisting of 12 animals) in daily doses of 1 g/kg bw 5 days a week for 4 weeks. These dosages were well tolerated. Weight development was not impaired. Liver function (bromsulphalein test) proved normal after 4 weeks. No effects on the testes were observed. Pigments deposits were observed in the kidneys of treated animals. No pigment deposits were observed in the liver although this organ was somewhat heavier in treated animals than in controls. Histopathological examination was performed on liver, kidney, spleen, testis, heart, lung, small intestine, large intestine and adrenals, and revealed no adverse effects.

In a more recent study four groups of 5 male and 5 female Sprague Dawley rats were dosed continuously by diet for at least 4 consecutive weeks with β-apo-8'-carotenal 10% WS/N, at levels of 0, 20, 100 and 500 mg β-apo-8'-carotenal active ingredient/kg bw/day (Loget and Morgan 2006). β-Apo-8'-carotenal 10% WS/N consists of free flowing particles (beadlets), containing β-apo-8'-carotenal dispersed in a cornstarch-coated matrix of gelatin, sucrose and corn oil. An additional group of 5 male and 5 female Sprague-Dawley rats received placebo in their diet over a similar treatment period. The animals were regularly monitored for any signs of ill health or reaction to treatment. Body

weights and food consumption were recorded once during pretrial, then twice weekly during treatment. Urine and blood samples were collected for laboratory investigations during week 4 of the study.

On completion of 28 days of treatment, all animals were killed and subjected to necropsy where pre-specified organs were weighed and/or placed in fixative. During necropsy, plasma and liver samples were obtained from each animal for analysis. Histopathological evaluation was performed on all animals receiving 0 or 500 mg β-apo-8'-carotenal active ingredient/kg bw/day.

Consistent with the nature of the test item, dietary administration with β-apo-8'-carotenal 10% WS/N for at least 4 consecutive weeks resulted in orange/red coloured faeces and skin in animals treated at 100 or 500 mg/kg bw/day. A slight reduction in body weight gain and food consumption was also recorded in animals treated at 500 mg/kg bw/day. There were no premature decedent animals in this study.

Increases in ALT and aspartate aminotransferase (AST) levels were noted in all treated groups along with increases in creatinine and total bilirubin levels in treated female animals. Increases in liver weight were also noted in all treated female groups. However, the authors indicated that toxicological significance of these changes was unclear.

The changes in AST were as follows: 87 $\pm$ 10 in male control rats, 87 $\pm$ 3 in male placebo control rats, 102 $\pm$ 4 ($p<0.05$) in male rats at a dose of 20 mg/kg bw/day, 96 $\pm$ 5 (not significant) in male rats at 100 mg/kg bw/day and 110 $\pm$ 14 ($p<0.001$) in male rats at 500 mg/kg bw/day, and 74 $\pm$ 3 in female control rats, 80 $\pm$ 8 in female placebo control rats, 77 $\pm$ 7 (not significant) in female rats at a dose of 20 mg/kg bw/day, 96 $\pm$ 27 ($p<0.05$) in female rats at 100 mg/kg bw/day and 92 $\pm$ 5 ($p<0.01$) in female rats at 500 mg/kg bw/day.

The changes in ALT were as follows: 77 $\pm$ 11 in male control rats, 77 $\pm$ 11 in male placebo control rats, 107 $\pm$ 14 ($p<0.01$) in male rats at a dose of 20 mg/kg bw/day, 125 $\pm$ 15 ($p<0.001$) in male rats at 100 mg/kg bw/day and 118 $\pm$ 12 ($p<0.001$) in male rats at 500 mg/kg bw/day, and 54 $\pm$ 2 in female control rats, 54 $\pm$ 16 in female placebo control rats, 62 $\pm$ 13 (not significant) in females rats at a dose of 20 mg/kg bw/day, 82 $\pm$ 21 ($p<0.01$) in female rats at 100 mg/kg bw/day and 77 $\pm$ 18 ($p<0.05$) in female rats at 500 mg/kg bw/day.

There were no other in-life findings that could be attributed to administration with β-apo-8'-carotenal 10% WS/N. Yellow or orange discolouration, abnormal colour or staining of the skin and body tissues was noted at necropsy in the majority of animals treated with β-apo-8'-carotenal 10% WS/N. One male and all female animals given the highest dose of β-apo-8'-carotenal 10% WS/N (500 mg/kg bw/day) exhibited minimal eosinophilic droplet formation in the tubular epithelial cells of the outer renal cortices and this was correlated with the increases in creatinine levels noted in the treated female groups. However in this study the incidence of eosinophilic droplets is unusual in that the females are more affected than the males. For this reason the Panel concluded that the effect should not be disregarded. The authors indicated that the severity of the finding is very minimal, and that therefore at this point, the finding is considered to be of equivocal toxicological significance. The Panel does not agree with this conclusion.

The authors concluded that administration of β-apo-8'-carotenal 10% WS/N in the diet at doses up to 500 mg/kg bw/day was associated with discolouration of the tissues and faeces of treated animals which is an expected effect of the test item. A slight reduction in body weight gain and food consumption was also recorded in animals treated at 500 mg/kg bw/day.

There was no histopathological correlation with necropsy findings. When considering histopathological changes, there were minimal eosinophilic droplets in the kidneys of animals treated at 500 mg /kg bw/day. This finding may be of equivocal toxicological significance, however, the authors indicated that under the conditions of the study, a NOAEL in both sexes was considered to be 100 mg/kg bw/day. The Panel noted that effects on AST and ALT activities were also observed in the new 13 week study described below but appeared to be reversible upon a 4 week recovery phase

included in this new study. Thus the Panel concluded that the NOAEL of this 28 week toxicity study was 100 mg/kg bw/day.

The new studies that became available following a public call for data also included a 13 week toxicity study on β-apo-8'-carotenal 10% WS/N in rats incorporating a neurotoxicity screen with administration by the diet with a 4 week recovery period (Edwards et al. 2007). The study design was in compliance with OECD guideline 408 and the study was performed in accordance with GLP. Five groups of 10 male and 10 female Sprague-Dawley rats were dosed continuously by diet for 13 consecutive weeks at levels of 0, 0 (placebo), 10, 30 and 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. In addition, a further 3 groups of 5 male and 5 female rats were assigned to the recovery study and dosed at 0, 0 (placebo), 100 mg β-apo-8'-carotenal active ingredient/kg bw/day for 13 weeks followed by a 4 week recovery period. The achieved levels of intake were very close to nominal. The rats were regularly monitored for any signs of ill health or reaction to treatment. Detailed functional observations were performed weekly, with additional functional investigations performed prior to dosing and week 12 of treatment. Body weights and food consumption were recorded at regular intervals until the end of the treatment period. Blood and urine samples were collected for laboratory investigations at the end of the treatment and recovery periods. Blood samples were also collected from all animals for analysis of the test item concentration in plasma. On completion of 13 weeks of treatment (main study) and a further 4 week treatment free period (recovery study), all rats were subjected to necropsy with pre-specified organs being weighed and/or placed in fixative. During necropsy, plasma and liver samples were obtained from each rat for analyses. Histopathological evaluation was performed on all animals receiving 0 and 100 mg β-apo-8'-carotenal active ingredient/kg bw/day; liver and kidneys were also examined from all animals receiving 10 and 30 mg β-apo-8'-carotenal active ingredient/kg bw/day.

Dietary administration with β-apo-8'-carotenal 10% WS/N for 13 weeks resulted in the expected orange/red colored faeces in all treated animals and orange colored skin in animals treated at 30 mg β-apo-8'-carotenal active ingredient/kg bw/day and above. In males at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day the discoloured skin lasted for 2 weeks after the completion of treatment. There were no signs of neurotoxicity, body weight or food consumption effects that could be attributed to administration of β-apo-8'-carotenal 10% WS/N.

Statistically significant increases in white blood cells and some of the associated parameters were seen in males receiving 30 and 100 mg β-apo-8'-carotenal active ingredients/kg bw/day. Minor increases were seen in AST and ALT levels in females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. These differences were not evident after the recovery phase.

Discolouration of body tissues was seen at necropsy at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day, which persisted to the end of the recovery period. There was an increase in liver weight in females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. This was not evident at the end of the recovery phase.

In groups receiving β-apo-8'-carotenal 10% WS/N, plasma and liver levels of β-apo-8'-carotenal showed a dose related increase. Also, mean plasma values at the different sampling points during the treatment period were similar at the respective dosages. Plasma and liver levels for three metabolites (β-apo-8'-carotenol, β-apo-8'-carotenoic acid and a polar metabolite) also increased with dose. Values for metabolites were generally up to 3 fold higher for females than for males. In the recovery study there was evidence at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day of complete plasma clearance for β-apo-8'-carotenal and the metabolite β-apo-8'-carotenol. For liver samples there was a significant clearance of β-apo-8'-carotenal and metabolites, although clearance was not totally complete over this 4 week time period.

Histopathologically, administration of β-apo-8'-carotenal 10% WS/N in the diet was associated with:

- eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above,

- multinucleate hepatocytes in the liver of females at 30 mg β-apo-8'-carotenal active ingredient/kg bw/day and above,

- increased numbers of inflammatory cell foci in the liver of females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day.

The finding of eosinophilic droplets at 10 and 30 mg β-apo-8'-carotenal active ingredient/kg bw/day was minimal in degree in all affected animals and, in the absence of other changes in the kidney, was considered by the authors not to be an adverse effect. The Panel does not agree with this conclusion.

Overall, the authors concluded in first instance that the NOAEL was considered to be 10 mg β-apo-8'-carotenal active ingredient/kg bw/day.

An amendment to this fist description of the study was also submitted (Perry and Shearer 2008). In this report it was stated that it was realized that the originally defined NOAEL in the 13-week rat study for β-apo-8'-carotenal (Edwards et al., 2007) may be open to question. Specifically the histopathological change of multinucleated hepatocytes (MNHs) was reported as an adverse change whereas there are literature references to this change being adaptive and not adverse (Williams and Iatropoulos, 2002). The Panel was also informed that a limited peer review was organized with a pathologist experienced with compounds from the same chemical category as β-apo-8'-carotenal. The liver slides from all females were reviewed by the pathologist. In addition, the input of an expert in the field of hepatotoxicity was sought.

The work carried out in these additional reviews was not part of the original study.

The Panel was informed that based on the peer review, expert opinion and also taking into account published information on the long half life of multinucleated hepatocytes (MNHs), the study pathologist and the peer review pathologist came to the mutually agreed-upon conclusion that the change of MNHs as observed in this study is not adverse. Accordingly the overall interpretation of the NOAEL in this study was reviewed. The Panel agreed that the change of MNHs as observed in this study is reversible, although, given the long half life of MNHs a study with a longer recovery period to demonstrate full reversibility would have been better in order to fully prove this assumption. The Panel however also noted that the finding of eosinophilic droplets in the kidney of all exposed groups point at the kidney as the target tissue.

The amendment indicates that histopathologically, administration of β-apo-8'-carotenal 10% WS/N in the diet was associated with eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above, for which there was no NOAEL. The finding of eosinophilic droplets at 10 and 30 mg β-apo-8'-carotenal active ingredient/kg bw/day was minimal in degree in all affected animals and, in the absence of other changes in the kidney, was considered by the study pathologist and the peer review pathologist not to be an adverse effect.

Administration of β-apo-8'-carotenal 10% WS/N was also associated with increased numbers of inflammatory cell foci in the liver of females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day, for which the NOAEL was 30 mg β-apo-8'-carotenal active ingredient/kg bw/day.

The amendment report concluded that overall, for males the NOAEL was 100 mg β-apo-8'-carotenal active ingredient/kg bw/day and for females it was 30 mg β-apo-8'-carotenal active ingredient/kg bw/day (based on increased hepatic inflammatory cell foci).

The Panel closely evaluated the data on the eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above. Table 5 presents the data which were not suitable for a BMD analysis. From these data the Panel concluded that 10 mg/kg bw/day is a lowest observed adverse effect level (LOAEL) for this effect.

Table 5: Dose-dependent incidence of eosinophilic droplets in the kidneys of rats exposed for 13 weeks to β-apo-8'-carotenal 10% WS/N, as reported by Edwards et al. (2007).

Gender	Dose level (mg/kg bw/day)	No of animals	No of animals with eosinophilic droplets in the kidney (minimal)	No of animals with eosinophilic droplets in the kidney (mild)	No of animals with eosinophilic droplets in the kidney (total incidence)
male	0	10	0	0	0
	0 (placebo)	10	0	0	0
	10	10	2	0	2
	30	10	1	0	1
	100	10	3	0	3
females	0	10	0	0	0
	0 (placebo)	10	0	0	0
	10	10	10	0	10
	30	10	10	0	10
	100	10	5	5	10

In rats given 100 mg β-apo-8'-carotenal active ingredient/kg bw/day followed by a recovery period, eosinophilic droplets were found in the kidney of 1/5 males and 4/5 females. The severity in males was minimal, and in females was minimal or mild.

3.2.2.2. Dogs

In addition the results of 14 week toxicity and metabolism studies of β-apo-8'-carotenal in dogs (strain and body weight not given) were provided (Bagdon 1961). The study report states that β-apo-8'-carotenal was well tolerated, that toxic manifestations were not detected in these animals despite prolonged treatment with large doses ranging to doses as high as 1000 mg per day. Three groups of dogs (6 animals/group) were given dose levels of 0 (control), 100 and 1000 mg per day β-apo-8'-carotenal given as a single administration orally in gelatin capsules each day for 14 weeks. The dogs were observed closely each day for signs of toxicity and body weights were recorded at weekly intervals. Blood counts, consisting of measurements of the haematocrit, haemoglobin, erythrocyte sedimentation rate, total and differential leukocytes were taken during the 0th, 6th and 13th experimental weeks. Also before treatment and after 6 and 13 weeks administration of β-apo-8'-carotenal a series of liver function tests was performed (including AST and ALT). At autopsy, the organs were weighed and examined for gross pathological changes. Sections of the tissues were examined microscopically. The results obtained did not show abnormalities. β-Apo-8'-carotenal was well tolerated. General health and growth was unimpaired. The occurrence of death in 1 control and 1 treated dog was unrelated to treatment. Haematopoietic tissues were unaffected. Also liver and kidney function tests did not deviate from normal. Gross and microscopic findings were not remarkable except for some deposition of pigment in the tissues of dogs given the highest dose. The Panel identified a NOAEL of 1000 mg/ dog/day which, assuming a body weight of 10-15 kg would amount to 67-100 mg/kg bw/day.

3.2.3. Genotoxicity

TemaNord mentions two in vitro genotoxicity studies with β-apo-8'-carotenal, an Ames test with *Salmonella typhimurium*, and a test with cultured hamster lymphocytes exposed for 48 hours (no further details). In both tests, β-apo-8'-carotenal was found to be non-genotoxic (BIBRA, 1996).

A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell lines was reported by Ishidate et al. (1988). On the basis of the evaluation of the results abstracted from the literature, they concluded that 'apocarotenal', assumed to be β-apo-8'-carotenal (250 and 1000 μg/ml), is not clastogenic in mammalian cell cultures.

In a study on the antimutagenic and anticarcinogenic effects of carotenoids, Azuine et al. (1992) also tested the mutagenicity of β-apo-8'-carotenal with and without metabolic activation in *Salmonella typhimurium* strains TA 98 and TA 100. β-Apo-8'-carotenal was not mutagenic in this test system.

Rauscher et al. (1998) examined β-apo-8'-carotenal at concentrations up to 100 µg β-apo-8'-carotenal per plate for mutagenicity in the histidine-deficient strains of *Salmonella typhimurium* TA98, TA 98NR and/or TA100. They found negative results.

Several new studies on genotoxicity have been published since the previous evaluations.

Three publications by Alija et al. (2004, 2005, 2006) are available, which describe experiments investigating the genotoxic potential of β-carotene and its oxidation products, among which β-apo-8'-carotenal.

Alija et al. (2004; 2005) investigated the genotoxic potential of β-apo-8'-carotenal, β-carotene, and a β-carotene cleavage product (CP) mixture in primary rat hepatocytes at concentrations of 0, 0.01, 0.1, 1, 5 or 10 µM. The endpoints tested were: the mitotic indices, percentage of necrotic and apoptotic cells, cells with micronuclei (MN), chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs). A statistically significant increase of micronuclei and chromosomal aberrations was observed when cells were treated with CP or β-apo-8'-carotenal at concentrations from 0.1 to 10 µM. In the same experimental conditions, a dose related increase of sister chromatid exchanges, which attained statistical significance at top dose, was also observed. In the same study, β-carotene (0.01 – 10 µM) induced neither significant cytotoxic nor genotoxic effects.

The Panel noted that the CP mixture is produced by degradation using NaClO. The similarity of the degradation products obtained by this method and those produced under normal conditions of use is not discussed. The authors determined the composition of the CP mixture but only the main products are quantified: "The CP mixture obtained from a 0.5 mM β-carotene stock solution contained β-carotene (0.16 mM), apo-15'-carotenal (0.08 mM), apo-12'-carotenal (0.12 mM) and apo-8'-carotenal (0.006 mM) and a number of products which could not be identified by HPLC". Thus 13.4% was not identified. Using GC-MS, the authors determine semi quantitatively some other components : "Related to all peaks detected during GC-MS analysis was a peak area of ~4.8% accounting for β-cyclocitral, 0.1% for ionene, 9.9% for β-ionone, 1.9% for β-ionone-5,6-epoxide and 4.5% for dihydroactinidiolide. Furthermore, 4-oxo-β-ionone was detected in trace amounts." But some components of the CP are non-identified. The use of NaClO may produce chlorinated derivatives that are generally more mutagenic than the same non-chlorinated products. Concerning the cell type used in this assay system, the Panel noted that the incidence of spontaneous aberrations observed with the control cultures was unusually high: 7.90±0.90% for the frequency of micronuclei and 1.20±0.03 aberrations per metaphase. This shows genomic instability of this cell system.

The Panel identified more pitfalls in this study (see Discussion), which is, therefore, considered of limited validity for the evaluation of the genotoxicity of β-carotene and its cleavage products.

In a follow up study, Alija et al. (2006) investigated the genotoxic action of 0.01 - 10 µM of a β-carotene cleavage product mixture in a rat primary hepatocyte assay in the presence or absence of DMNQ (2,3-dimethoxy-1,4-naphtoquinone) or hypoxia/reoxygenation (Hy/re) induced oxidative stress. The mitotic indices, the percentage of necrotic and apoptotic cells, the percentages of micronucleated cells, chromosomal aberrations and sister chromatid exchanges were measured.

In combination with DMNQ (40 µM), the cleavage product mixture (CP) induced a significant increase in micronuclei (at 0.01 and 1 µM), increased chromosomal aberrations (at 1 µM) and induced SCE (at 1 µM). Cytotoxic effects were observed at 40 µM DMNQ in combination with 10 µM of the cleavage product mixture. In combination with hypoxia/reoxygenation, the cleavage product mixture induced a significant increase in micronuclei (at 0.01, 1 and 10 µM), increased CAs (at 0.01, 1 and 10 µM), and induced SCEs (at 1 and 10 µM). No cytotoxicity was observed. The Panel noted that this study, which concerns the enhancement by CP of the genotoxic effects of the free radical-generator DMNQ or hypoxia/reoxygenation, has limited relevance for the assessment of the genotoxic potential of CP *per se*.

Kalariya et al. (2009), studied the genotoxic effects of the β-carotene breakdown product β-apo-8'-carotenal in Human Retinal Pigment Epithelial Cells (ARPE-19) using the Comet assay. They concluded that their results suggest that β-apo-8'-carotenal, when applied at partially toxic doses, is genotoxic inducing DNA strand breaks prevented by high levels of glutathione (GSH). The authors stated that the mechanism of genotoxicity of β-apo-8'-carotenal.

However, for the Kalariya study, the same limitations hold as for the Alija et al. (2004; 2005) studies about the representativeness of the degradation products because the same method was used to produce them. The same group showed (Kalariya et al., 2008) that these degradation products induced cell toxicity and apoptosis in the same range of concentrations that was tested in the study with ARPE-19 cells. To which extent ghost cells are taken into account is not described. Therefore the Panel noted that the comets could be, at least partly, a consequence of the effects of cytotoxicity and/or apoptosis.

Overall the Panel concluded that the genotoxicity studies of the β-carotene breakdown products in primary rat hepatocytes and in ARPE-19 cells provide very limited evidence of genotoxicity.

In another in vitro study by Marques et al. (2004), calf thymus DNA was allowed to react with β-apo-8'-carotenal, β-carotene and retinal at 37°C for 72 hours in the presence and absence of 50 mM H_2O_2 in neutral (pH 7.4) and basic (pH 9.4) conditions. 1,N^2-etheno-2'-deoxyguanosine formation was measured. This adduct was also formed in all control incubations (DNA only, in the absence and presence of H_2O_2 in neutral and basic conditions). 1,N^2-etheno-2'-deoxyguanosine formation was significantly increased at both pHs in the presence of β-apo-8'-carotenal, β-carotene and retinal, compared with control incubations. Also, in the presence of H_2O_2, adduct formation was increased by all test compounds. In addition, significant increases in 8-oxo-7,8-dihydro-2'-deoxyguanosine were observed when all three compounds were incubated in the presence of H_2O_2. 1,N^2-etheno-2'-deoxyguanosine has been proven to be mutagenic in *E. coli* uvrA$^-$ (Langouët et al., 1998).

In an in vitro study by Yeh and Wu (2006), it was found that β-apo-8'-carotenal induced DNA strand breaks, lipid peroxidation and expression of CYP1A2 in A549 cells (human alveolar epithelial cells). Furthermore, both β-apo-8'-carotenal and β-carotene significantly enhanced DNA strand breaks and CYP1A2 expression, induced by benzo[a]pyrene at 20 µM. However, β-carotene at 2 µM significantly suppressed BaP-induced strand breaks. DNA damage was found to be associated with expression of CYP since the effects were diminished in the presence of 1-aminobenzotriazole, a CYP inhibitor.

Additional studies on genotoxicity have been provided upon a public call for data, which are described in more detail hereafter.

A *Salmonella typhimurium/Escherichia coli* reverse mutation assay (standard plate test) with β-apo-8'-carotenal was conducted according OECD guidelines and under GLP (BASF, 1998a). The strains used were TA 100, TA 1537, TA 98 and *E. coli* WP2 uvrA, concentrations tested were 20 µg- 5,000 µg/plate (all tester strains) and 20 µg - 6,000 µg/plate (TA 100), and tests were performed in the absence and presence of S9. Precipitation of the test substance was found from about 100 µg/plate onward. A weakly bacteriotoxic effect was occasionally observed depending on the strain and test conditions from about 2,500 µg/plate onward.

No increase in the number of revertants was observed for all strains except for TA 100. For TA100 without S9 mix a slight increase in the number of his+ revertants was observed depending on the test conditions from about 500 µg - 2,500 µg/ plate onward up to 4,000 µg - 6,000 µg/plate (factor 1.9 - 4.1). The authors concluded that β-apo-8'-carotenal is weakly mutagenic in the *Salmonella typhimurium* coli reverse mutation assay under the experimental conditions chosen.

β-Apo-8'-carotenal 10% WS/N was assayed for mutagenicity in five histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) in the absence and in the presence of metabolic activation (S9) (Loget and Johnson, 2006). β-Apo-8'-carotenal 10% WS/N consists of free flowing particles (beadlets), containing β-apo-8'-carotenal dispersed in a cornstarch-coated matrix of gelatin, sucrose and corn oil. An initial toxicity range finding experiment was carried out in strain TA100 only in the absence of S9, using final concentrations of β-apo-8'-carotenal 10% WS/N at 0.0889, 0.4446, 2.223, 11.12, 55.58 and 277.9 µg/plate. The highest concentration was

limited by the maximum achievable solubility of the test article. No evidence of toxicity was observed. No reproducible increases in revertant numbers were observed in any of the strains in the absence or presence of S9. It was concluded that β-apo-8'-carotenal 10% WS/N did not induce mutation in five histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) at concentrations up to 277.9 µg/plate. The Panel noted that the concentrations of β-apo-8'-carotenal active ingredient tested in this study were relatively low.

A mixture of β-apo-8'-carotenal (95.5%) plus crocetindial (0.47%) was tested in an in vitro genotoxicity assay using Chinese hamster ovary (CHO) cells (Loget and Whitwell, 2006) in two experiments. Treatment covering a broad range of doses up to 5000 µg/ml, both in the absence and presence of metabolic activation (S9). The test article was formulated as a suspension in 0.5% methyl cellulose. In experiment 1, treatment in the absence and presence of S9 was for 3 hours followed by a 17-hour recovery period prior to harvest (3+17). The test article concentrations for chromosome analysis were selected by evaluating the effect of β-apo-8'-carotenal on population doubling. Chromosome aberrations were analysed at three or four concentrations, the highest concentrations chosen for analysis being 200.0 µg/ml in the absence of S9 and 800.0 µg/ml in the presence of S9, inducing approximately 69% and 74% reduction in population doubling respectively. In experiment 2, treatment in the absence of S9 was continuous for 20 hours (20+0). Treatment in the presence of S9 was for 3 hours followed by a 17-hour recovery period prior to harvest (3+17). Chromosome aberrations were analysed at three dose levels the highest concentrations chosen for analysis being 50.00 µg/ml (20+0 –S9), and 125.0 µg/ml (3+17 +S9), inducing approximately 49% and 46% reduction in population doubling, respectively.

Treatment of cultures with β-apo-8'-carotenal plus crocetindial in the presence of S9 (experiments 1 and 2) resulted in frequencies of cells with structural chromosome aberrations that were similar to those seen in concurrent vehicle controls for the majority of concentrations analysed. However, increased frequencies of cells with aberrations were observed and associated with cultures inducing high cytotoxicity (greater than 50%) but not at concentrations inducing moderate to low cytotoxicity. The increases observed were therefore considered by the authors to be of questionable biological relevance.

Treatment of cultures with β-apo-8'-carotenal plus crocetindial in the absence of S9 (20+0 hour and 3+17 hour treatments), resulted in frequencies of structural chromosome aberrations that were similar to those seen in concurrent vehicle controls for all but a single concentration analysed. With the exception of one replicate culture at the highest concentration analysed following 3+17 hour treatment (200 µg/ml, a concentration inducing 69% cytotoxicity), all treated cultures exhibited aberrant cell frequencies that fell within historical vehicle control (normal) values. The increase observed was therefore considered by the authors to be of questionable biological relevance.

With the exception of sporadic increases in endoreduplicated and polyploid cells (single cultures at 400.0 and 600.0 µg/ml respectively following 3+17 hour +S9 treatment in experiment 1), no increases in numerical aberrations, (exceeding historical vehicle control values), were observed in cultures treated with β-apo-8'-carotenal plus crocetindial. The authors concluded that β-apo-8'-carotenal plus crocetindial showed evidence of inducing structural chromosome aberrations in cultured Chinese hamster ovary (CHO) cells when tested in excess of its limit of cytotoxicity (50%) in both the absence and presence of metabolic activation (S9). These increases were sporadic and in all instances associated with high cytotoxicity (greater than 50% as assessed by population doubling data). No such increases were observed in cultures inducing less than 50% cytotoxicity, or at any concentration analysed following continuous 20+0 hour –S9 treatment. Therefore, the increases observed were considered of questionable biological relevance as they were observed only at cytotoxic (or highly cytotoxic) concentrations. The Panel noted that this study has limited relevance for evaluating the genotoxicity of β-apo-8'-carotenal given the combined exposure with crocetindial.

The new studies submitted to EFSA also included an in vivo rat bone marrow micronucleus test (Lodget and Beevers, 2006). β-Apo-8'-carotenal 10% WS/N was assayed in vivo in a rat bone marrow micronucleus test at three dose levels. β-Apo-8'-carotenal 10% WS/N consists of free flowing particles (beadlets), containing β-apo-8'-carotenal dispersed in a cornstarch-coated matrix of gelatin,

sucrose and corn oil. The choice of dose levels was based on an initial toxicity range-finding study in which β-apo-8'-carotenal 10% WS/N, formulated in purified water (water for injection) was administered to rats via oral gavage. The test article was administered once daily on two consecutive days to a group of three male and three female rats at a dose of 800 mg β-apo-8'-carotenal active ingredient/kg bw/day. This dose was the maximum practicable dose based on the formulation used and a dose volume of 20 ml/kg bw. Observations were made over a 2 day period following the second administration and signs of toxicity recorded. No clinical signs of toxicity were observed, thus confirming that 800 mg β-apo-8'-carotenal active ingredient/kg bw/day was well tolerated and therefore a suitable maximum dose for the micronucleus test. Two further doses of 200 and 400 mg/kg/day were chosen. As there were no gender-specific differences in toxicity, male animals only were used in the micronucleus test.

In the micronucleus test, β-apo-8'-carotenal 10% WS/N, formulated as described above, was administered at 200, 400 and 800 mg β-apo-8'-carotenal active ingredient/kg bw/day to groups of six male rats. Doses were administered once daily for two consecutive days and rats killed 24 hours after the second administration. No clinical signs of toxicity were observed in any animal receiving β-apo-8'-carotenal 10% WS/N. The negative (vehicle) control in the study was water for injection. In addition, a placebo group was dosed with empty beadlets at a dose equivalent to the maximum test article dose (800 mg/kg/day). Groups of six male rats were treated with vehicle or placebo once daily on two consecutive days via oral gavage. The animals were killed and sampled 24 hours after the second administration. Cyclophosphamide (CPA), the positive control, was dissolved in saline and administered via oral gavage as a single dose of 30 mg/kg bw to a group of six male rats which were killed after 24 hours. Negative (vehicle) control rats exhibited an acceptable group mean frequency of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE; ratio expressed as %PCE) and the frequencies of micronucleated PCE fell within the historical negative control (normal) data. Positive control animals exhibited increased numbers of micronucleated PCE such that the frequency in the positive control group was significantly greater than in concurrent controls. Placebo treated rats resulted in %PCE and micronucleated PCE (MN PCE) frequencies that were consistent with data from the concurrent vehicle control group, thus confirming that the beadlets used in preparation of the test article had no adverse effect on the assay system and did not induce micronuclei under the conditions tested.

Groups of rats treated with β-apo-8'-carotenal 10% WS/N exhibited %PCE and MN PCE values that were similar to vehicle controls and consistent with the laboratory's historical control range. There were no statistically significant increases in MN PCE frequency in any of the test article treated groups. Analysis of plasma confirmed that rats dosed with β-apo-8'-carotenal 10% WS/N were systemically exposed to β-apo-8'-carotenal and three metabolites.

It was concluded by the authors that β-apo-8'-carotenal 10% WS/N did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of rats treated up to 800 mg/kg/day (the maximum practicable dose for this study). The Panel agreed with this conclusion.

Overall the Panel concluded that the available in vitro genotoxicity studies and the in vivo micronucleus study with β-apo-8'-carotenal do not give reason for concern with respect to genotoxicity. A few in vitro studies with β-carotene cleavage products provide limited evidence of DNA damaging activity and covalent binding to DNA. In this respect the Panel noted that such results may reflect a pro-oxidant effect, common to other antioxidants, which is elicited under specific in vitro conditions which may not occur in vivo.

3.2.4. Chronic toxicity and carcinogenicity

JECFA describes a single long-term study conducted with the ethyl ester of β-apo-8'-carotenoic acid. In this study 15 male rats were fed β-apo-8'-carotenoic acid ethyl ester at 1% of their diet (equivalent to 500 mg/kg bw/day) for 2 years. Two control groups received either the basic diet or 7500 International Units (IU) of vitamin A/animal/day. No effects were noted compared to animals

receiving the basic diet regarding general health, mortality, weight gain, or fertility. In the vitamin A control group some of these parameters were affected (no further details given) (Anonymous, 1966).

Upon a public call for data results from chronic studies performed in the 1960's became available for evaluation (Schärer, 1960; Schärer, 1961; Schärer and Studer, 1961a; Schärer and Studer, 1961b; Schärer and Studer, 1961c; Schärer and Studer, 1961d; Schärer and Studer, 1961e; Schärer and Studer, 1962a; Schärer and Studer, 1962b; Schärer and Studer, 1962c; Schärer, 1963; Schärer, 1965).

A summary of these chronic toxicity studies with β-apo-8'-carotenal was also provided (Schärer and Studer 1961b). β-Apo-8'-carotenal was administered at 0.1% in the diet to the first generation of Wistar rats and their offspring (second generation) for a period of 2 years and to a third generation for a period of 1 year. The average intake of β-apo-8'-carotenal was about 40 mg/kg bw/day. The number of animals per group amounted to 20 males and females in the control and treatment groups of the first generation and the rats in this treatment group received 0.1% β-apo-8'-carotenal in the diet for 104 weeks. From the offspring of this first generation 14 respectively 15 males and 15 respectively 15 females were included in the control and treatment groups of the second generation of which the treatment group also received 0.1% β-apo-8'-carotenal in the diet for 104 weeks. From the offspring of this second generation 5 males and 12 females were included in the control group of the third generation and 12 males and 11 females were included in the treatment group of the third generation which received 0.1% β-apo-8'-carotenal in the diet for 52 weeks

The results were as follows:

1) The number of spontaneous deaths was greater among controls (25 out of 122 animals) than among the treated animals (18 out of 147 animals).

2) The average weight of the animals treated with 0.1% β-apo-8'-carotenal in both the 1 year and the 2 year experiments were slightly below those of the respective controls but still within the permissible range.

3) Results of the haematology test were not influenced by addition of 0.1% β-apo-8'-carotenal to the diet.

4) The treated animals exhibited orange-yellow discolouration of the body fat and yellow to ochre discolouration of the liver.

5) The absolute weights of the liver, kidneys and heart did not reveal any important differences between treated and untreated rats. Some of the testes of the treated animals were lighter but their weights varied greatly; thus it was concluded by the authors that the weight of the gonads of rats given β-apo-8'-carotenal did not differ significantly from the controls.

6) Histopathological examination of sections stained for fat revealed golden-yellow to yellow-brown, iron-free pigment granules in the liver and kidneys of treated animals which were absent in the controls. The interstitial changes in the kidneys as well as the tubular distensions and calcium incrustations at the medulla cortex margin were less pronounced in the treated animals than in the controls. The other organs (lungs, heart, spleen, bone marrow, stomach, duodenum, jejumen, pancreas, ovaries, skeletal musculature, thyroid and skin) did not reveal any differences when examined microscopically. Degenerative changes in the epithelium of the seminiferous tubules were slightly, but not statistically significantly more frequent ($p=0.17$) in the animals treated with β-apo-8'-carotenal than in the controls.

7) The spontaneous tumours were distributed evenly over both groups (11/147 treated rats and 10/122 untreated rats) and consequently the authors concluded that their occurrence was not connected with the administration of β-apo-8'-carotenal.

8) The reproductive power and the average number of animals per litter were approximately the same in the treated and untreated rats.

Based on these results the Panel considered that there were no adverse effects in this chronic toxicity study at the single dose level tested amounting to about 40 mg/kg bw/day.

3.2.5. Reproductive and developmental toxicity

JECFA describes two multi-generation studies with β-apo-8'-carotenal.

Three generations of rats received diets containing 0, 1000, 2000 or 5000 mg/kg diet β-apo-8'-carotenal (equivalent to 0, 50, 100 and 250 mg/kg bw/day, respectively) for 2 years.

No adverse effects were observed in any generation (no further details) (Anonymous, 1966).

The second study examined four generations of rats (20-40/group/sex) fed diets containing 0, 0.1, 0.2, 0.5 or 1% β-apo-8'-carotenoic acid methyl ester (equivalent to 0, 50, 100, 250 and 500 mg/kg bw/day, respectively) for 52 - 104 weeks.

No compound-related differences were observed with regard to food consumption, general health, or mortality (no further details) (Anonymous, 1966).

Additional studies on developmental toxicity have been provided upon a public call for data which are described in more detail hereafter.

In a range-finding study (Loget and Marsden, 2006) information was obtained for the selection of appropriate dose levels for a subsequent embryo toxicity study with β-apo-8'-carotenal 10% WS/N following administration from implantation to the day of caesarean section. The study was performed according to OECD guidelines. Three groups each consisting of 6 mated female SD rats were given β-apo-8'-carotenal 10% WS/N beadlets by admixture in powdered diet from days 6 to 20 of gestation inclusive at the concentrations of 2064, 10344 and 51720 mg/kg diet (groups 3, 4 and 5 respectively) corresponding to β-apo-8'-carotenal active ingredient target doses of 20, 100 and 500 mg β-apo-8'-carotenal/kg bw/day. A fourth group of 6 mated rats received placebo WS/N beadlets over the same period and served as a control (group 2). A second control (group 1) of 6 mated rats received basic powdered diet only over the same period. The total beadlet concentration (β-apo-8'-carotenal 10% WS/N and/or placebo WS/N) was equalised in groups 2 to 5.

The authors concluded that within the context of this study, dietary concentrations of β-apo-8'-carotenal 10% WS/N up to 51720 mg/kg diet (equivalent to a target dose of 500 mg/kg bw/day of the β-apo-8'-carotenal active ingredient β-apo-8'-carotenal) are considered to be NOAELs for both maternal and embryo-fetal toxicity.

Consistent with the nature of the test item (orange-red colouring agent), the only findings related to treatment were orange coloured integuments, faeces, tissues and organs in all β-apo-8'-carotenal 10% WS/N treated groups. All doses could be employed in a subsequent embryo-fetal development study.

In this subsequent study the effect of β-apo-8'-carotenal 10% WS/N on embryonic and fetal development of the rat was investigated when administered by the oral route (dietary admixture) from implantation to the day of caesarean section on day 20 of gestation (Loget et al. 2006). The study was performed according to OECD guidelines and under GLP. Three groups each consisting of 25 mated female SD rats were given β-apo-8'-carotenal 10% WS/N beadlets by admixture in powdered diet from days 6 to 20 of gestation inclusive at dose levels of 2064, 10344 and 51720 mg/kg diet (groups 3, 4 and 5 respectively) corresponding to β-apo-8'-carotenal active ingredient target doses of 20, 100 and 500 mg β-apo-8'-carotenal/kg bw/day. A fourth group of 25 mated rats received placebo WS/N beadlets over the same period and served as a control (group 2). A second control (group 1) of 25 mated rats received basic powdered diet only over the same period. The total beadlet concentration (β-apo-8'-carotenal 10% WS/N and/or placebo WS/N) was equalised in groups 2 to 5. Clinical condition, body weights and food consumption were monitored throughout the study. Surviving females were killed on day 20 of gestation for examination of their uterine contents, including examination of the placentae. At necropsy, the females were examined macroscopically and fetuses were weighed, sexed and examined for external abnormalities. Half of the fetuses were examined internally prior to processing for skeletal examination. There was no skeletal malformation noted in any group. The

remaining fetuses were preserved for fixed-visceral examination by the modified Wilson-Barrow technique. Blood sampling was performed on day 20 of gestation whilst the animals still had access to the diets. The liver of the first 5 surviving animals per group was sampled and frozen at necropsy. Plasma and liver samples were collected for bioanalysis.

There were two unscheduled deaths in the 500 mg/kg bw/day group, neither of which was clearly attributable to treatment. Consistent with the nature of test item, orange stained fur and/or orange coloured faeces and/or orange coloured integuments were noted amongst the β-apo-8'-carotenal treated animals. The placebo group was not affected. There was a slight reduction in mean body weight gain and food consumption in the 100 and 500 mg/kg bw/day groups compared with the vehicle control group. The other groups were not affected.

Overall mean intake of the β-apo-8'-carotenal active ingredient, β-apo-8'-carotenal, was consistent with the target dose (within 1%) in each group during the treatment period (days 6 to 20 of gestation).

There were no treatment-related macroscopic abnormalities noted in any group at the terminal necropsy examinations. However, consistent with the nature of the test item, essentially all β-apo-8'-carotenal 10% WS/N treated animals had orange coloured organs and tissues.

Consistent with an effect on mean fetal weight (see below), gravid uterus weight was slightly lower (6%) in all beadlet treated groups (including the placebo group) than in the vehicle control group with the exception of the 500 mg/kg bw/day group. The absence of a similar finding in the high dose group was due to an incidentally slightly greater mean litter size compared with the other groups.

There were 24, 24, 22, 25 and 19 pregnant females at the terminal caesarean sections in groups 1 to 5 respectively, all of which had viable fetuses. There was no adverse effect of treatment on embryo-fetal survival in any group. Mean live litter size in all beadlet treated groups (with or without the test item) was comparable with, or slightly superior to (particularly in group 5), that in the vehicle control group.

Mean fetal weight tended to be slightly lower than in the vehicle control group in each of the beadlet treated groups (with or without the test item). This finding suggested an association with the reduced nutritional content of the test diets due to the beadlet content rather than an effect of the test item.

There was one malformed fetus in each of groups 1, 2, 3 and 5, and two in group 4. Neither the incidence, nor the types of malformation detected, suggested any association with β-apo-8'-carotenal 10% WS/N.

There was no β-apo-8'-carotenal or any of the three metabolites detected in the plasma or liver samples in either of the control groups. All three treated groups were exposed to β-apo-8'-carotenal and the three metabolites in the plasma and liver. There was some evidence of a dose proportional increase in exposure (at least for β-apo-8'-carotenal levels) between the low and mid doses. The increase was then under proportional between the mid and high dose groups suggesting that levels were becoming saturated. Liver levels of β-apo-8'-carotenal and the three metabolites were greater than the equivalent plasma values in each group, particularly for β-apo-8'-carotenal and the β-apo-8'-carotenol and β-apo-8'-carotenoic metabolites.

The authors concluded that within the context of this study, dietary concentrations of 2064, 10344 and 51720 mg/kg diet of β-apo-8'-carotenal 10% WS/N (equivalent to target doses of 20, 100 and 500 mg/kg bw/day of the β-apo-8'-carotenal active ingredient β-apo-8'-carotenal) were associated with slight reductions in mean body weight gain and food consumption at the two higher doses. Other treatment-related findings in all β-apo-8'-carotenal 10% WS/N treated groups, such as orange coloured integuments, faeces, tissues and organs, were consistent with the nature of the test item (orange-red colouring agent).

The NOAEL for maternal toxicity was therefore considered by the authors to be the dietary concentration of 2064 mg/kg diet (20 mg β-apo-8'-carotenal/kg bw/day).

The Panel noted that the reduction in mean body weight gain was observed during the first 9 days of treatment (days 6 to 15 of gestation) in the 100 and 500 mg/kg bw/day groups compared with the vehicle control group and that this effect attained statistical significance only between days 6 and 11

of gestation. As a consequence, net mean body weight gain during the treatment period (days 6 to 20 of gestation) was slightly but not statistically significantly lower in these groups. The Panel therefore considered the slight reduction in mean body weight and food intake not as adverse and concluded that the NOAEL of this study was 500 mg/kg bw/day.

Evidence of an embryo-fetal effect was restricted to slightly lower mean fetal weight in all beadlet treated groups (with or without the test item) compared with the vehicle control. This finding suggested an association with the reduced nutritional content of the test diets due to the beadlet content rather than an effect of the test item. The NOAEL for embryo-fetal toxicity was therefore considered to be the dietary concentration of 51720 mg/kg diet (500 mg β-apo-8'-carotenal /kg bw/day). Overall the Panel concluded that there was no evidence of developmental toxicity of β-apo-8'-carotenal 10% WS/N.

3.2.6. Allergenicity, hypersensitivity and intolerance

In a study with a group of 135 persons diagnosed with urticaria or atopic dermatitis, oral exposure to 100 mg β-apo-8'-carotenal and 100 mg β-carotene induced one positive and one equivocal reaction. One positive reaction was also noted after exposure to a placebo. In a group of 123 persons with contact dermatitis, no response was observed (BIBRA, 1996).

As no cases of intolerance/allergenicity have been reported after oral exposure to the compound, it appears that at the current levels of exposure the incidence is very low, if any.

4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

β-Apo-8'-carotenal (E 160e) is allowed as a food additive in the EU and has been previously evaluated by JECFA in 1974 and the SCF in 1975. The SCF and JECFA both established an ADI of 0-5 mg/kg bw/day. JECFA defined the ADI for the sum of the carotenoids β-apo-8'-carotenal, β-carotene, β-carotenoic acid methyl ester and β-carotenoic acid ethyl ester, while the SCF used the same definition but omitted β-carotenoic acid methyl ester. However, more recently, the SCF withdrew the entire group ADI based on a recent evaluation of β-carotene (SCF, 2000). The SCF decided that there was insufficient scientific basis, either from human or experimental studies, on which to set a new ADI for β-carotene and related carotenoids, but was nonetheless of the opinion that currently permitted food additive uses of β-carotene and related carotenoids are temporarily acceptable from a health point of view at the estimated levels of intake (SCF, 2000). Although it is not mentioned explicitly in the SCF opinion, the Panel considered it reasonable to assume that the term related to carotenoids referred to β-apo-8'-carotenal and β-apo-8'-carotenoic acid ethyl ester, since these were the compounds included in the group ADI defined by the SCF in 1975.

At present only β-apo-8'-carotenal and β-apo-8'-carotenoic acid ethyl ester are specifically defined in Commission Directive 2008/128/EC and JECFA. According to specifications, β-apo-8'-carotenal has to comprise at least 96% of the final product in commercial β-apo-8'-carotenal products. The sum of all subsidiary colouring matters is less than 3% and these subsidiary colouring matters are related carotenoids, mainly β-carotene. The Panel noted that the specifications should be updated to more clearly define the purity of the material. The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg. The Panel also noted that specifications should be extended to include maximum residue limits for residual solvents.

The Panel concluded that the available data indicate that after oral administration absorption of β-apo-8'-carotenal and/or its metabolites is at least 15% (JECFA 1974a,b; Thommen, 1962; Brubacher et al., 1960; Wiss and Thommen, 1963; Bagdon et al., 1960; Rümbeli et al. 2007). The absorbed β-apo-8'-carotenal is metabolically converted to β-apo-8'-carotenoic acid as well as to its ethyl and methyl

esters in rats (Wiss and Thommen, 1963) and β-apo-8'-carotenol (Rümbeli et al. 2007; Edwards et al. 2007; Loget et al. 2006). In rats and monkeys, β-apo-8'-carotenal accumulates in the liver together with β-apo-8'-carotenoic acid and vitamin A (rats) and an unidentified carotenoic acid (monkeys) (Thommen, 1962; Brubacher et al., 1960; Tiews 1963). In monkeys, accumulation of orange material has also been found in the body fat (Tiews, 1963). In dogs, β-apo-8'-carotenal accumulated in serum, and vitamin A levels were increased in the kidney and pigmentation was observed in adipose tissue, kidney and adrenal cortex (Bagdon et al., 1962). In rats, conversion of β-apo-8'-carotenal to vitamin A in the gut is low (4%) compared to that for β-carotene (Wiss and Thommen, 1963; Glover, 1960). β-Apo-8'-carotenal is excreted in the urine in dogs (Bagdon et al., 1960) predominantly as an (undefined) ester of β-apo-8'-carotenoic acid. In human infants the ester of β-apo-8'-carotenal was rapidly eliminated from the blood (Kűbler, 1963).

The Panel noted that the SCF (2000) has questioned the suitability of rodents as a test species for evaluating the bioavailability and effects of β-carotene in human, since rodents were considered to convert β-carotene to vitamin A much more efficiently than humans. Most laboratory animals were reported to degrade β-carotene in their intestines and absorb almost no β-carotene intact, due to high dioxygenase activity converting β-carotene to retinal, which, according to SCF, is in contrast to humans where β-carotene is mainly (20-75%) absorbed intact. Therefore rodent studies were considered to lack relevance for human risk assessment, and the Panel considered that such limitations may also apply to β-apo-8'-carotenal evaluated in the present opinion. Therefore, the Panel carefully evaluated the ADME characteristics for β-apo-8'-carotenal in both rodent and human focusing on possible differences.

An overview of the major results from an ADME study with β-apo-8'-carotenal in rats (Rümbeli et al., 2007) compared to those from a human study (Zeng et al., 1992) was made. According to the metabolite pattern in plasma the uptake and metabolism of β-apo-8'-carotenal seems qualitatively similar in rats and humans. Comparison of the plasma kinetic data from the rat with the data from the human study indicates also quantitative similarities between the two species.

Based on the metabolite pattern in blood plasma and based on the plasma kinetics the Panel concluded that for β-apo-8'-carotenal rats are a suitable model for humans concerning uptake and systemic exposure to β-apo-8'-carotenal and metabolites.

A direct comparison of the formation of vitamin A from β-apo-8'-carotenal in rats and humans is more difficult, since the experimental setup of the two studies was not identical. Results obtained reveal that in rats as well as in humans vitamin A is formed from β-apo-8'-carotenal. From the existing data a quantitative comparison can not be made.

No acute oral toxicity of β-apo-8'-carotenal was seen at relatively high doses (JECFA, 1975a, b; Bächthold, 1972; Bächthold, 1975; Bächthold, 1976; Bächthold, 1973; Bächthold, 1977; Bächthold, 1980; BASF 1972; Bächthold, 1977; Loget O and Arcelin G, 2006a; Loget and Arcelin, 2006b) and acute toxicity is considered to be of little relevance for the safety evaluation of these compounds as food colours.

Several in vitro genotoxicity studies (Alija et al., 2004; 2005; 2006; Marques et al., 2004; Kalariya et al., 2009; Yeh and Wu, 2006) were performed to further investigate the genotoxicity of β-carotene cleavage products.

A β-carotene cleavage products mixture and β-apo-8'-carotenal were reported to induce increases in MN, CAs and SCEs (Alija et al., 2004; 2005; 2006) in primary rat hepatocytes. In this respect, however, the Panel noted that there is limited experience with cytogenetic assays in primary rat hepatocytes, which show a very high spontaneous incidence of both MN and CAs. Moreover, the increase in the frequency of MN and CAs observed in presence of β-carotene cleavage products, and of micronuclei in presence of apo-8'-carotenal, were not clearly dose-related over a 104-fold concentration range. The statistically significant increases in the frequency of MN were only 20 and 11% (at 0.1 and 1 μM respectively) over control incidence, which is within the range of experimental variation for the end-points studied, and thus has limited or no biological significance. The frequencies

of CAs in presence of apo-8'-carotenal show an apparent treatment-related increase, but data are unreliable since they are based on only 20 metaphases/culture. The increase in SCEs observed in presence of both β-carotene cleavage products and apo-8'-carotenal is more credible, but the biological significance of this indicative assay in relation to genotoxicity is indirect.

Kalariya et al. (2009), studied the genotoxic effects of the β-carotene breakdown product β-apo-8'-carotenal in Human Retinal Pigment Epithelial Cells (ARPE-19) using the Comet assay. They concluded that their results suggest that breakdown products of dietary carotenoids could be genotoxic in ARPE-19 cells. The authors also stated that the mechanism of genotoxicity of β-apo-8'-carotenalwas not elucidated..

The Panel concluded that the genotoxicity studies with β-apo-8'-carotenal, β-carotene and a β-carotene cleavage product in primary rat hepatocytes reported by Alija et al. (2004; 2005; 2006) and in ARPE-19 cells by Kalariya et al. (2009), provide very limited evidence of genotoxicity.

In in vitro studies it was found that β-apo8'-carotenal induced DNA strand breaks in A549 cells (Yeh and Wu, 2006), strand breakage in supercoiled DNA (Zhang and Omaye, 2001), and etheno-adduct to calf thymus DNA (Marques et al., 2004).

The Panel also noted that a few in vitro studies with β-carotene cleavage products provide limited evidence of DNA damaging activity and covalent binding to DNA, and that such results may reflect a pro-oxidant effect, common to other antioxidants, which is elicited under specific in vitro conditions which may not occur in vivo.

Additional studies on genotoxicity provided upon a public call for data revealed generally negative results in Salmonella typhimurium/Escherichia coli reverse mutation assays (BASF, 1998; Loget and Johnson, 2006), an in vitro genotoxicity assay using Chinese hamster ovary (CHO) cells (Loget and Whitwell, 2006) and an in vivo rat bone marrow micronucleus test (Lodget and Beevers, 2006).

Overall the Panel concluded that the available in vitro genotoxicity studies and the in vivo micronucleus study with β-apo-8'-carotenal do not give reason for concern with respect to genotoxicity.

Upon a public call for data two subchronic toxicity studies in rats performed according to OECD guidelines and under GLP became available for evaluation.

These included a study in which four groups of 5 male and 5 female Sprague Dawley rats were dosed continuously by diet for at least 4 consecutive weeks with β-apo-8'-carotenal 10% WS/N, at levels of 0, 20, 100 and 500 mg β-apo-8'-carotenal active ingredient/kg bw/day (Loget and Morgan 2006). Increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were noted in all treated groups along with increases in creatinine and total bilirubin levels in treated female animals. Increases in liver weight were also noted in all treated female groups. There were no other in-life findings that could be attributed to administration with β-apo-8'-carotenal 10% WS/N. There was no histopathological correlation with necropsy findings. When considering histopathological changes, there were minimal eosinophilic droplets in the kidneys of animals treated at 500 mg /kg bw/day. This finding may be of equivocal toxicological significance. The authors indicated that under the conditions of the study, a NOAEL in both sexes was considered to be 100 mg/kg bw/day. The Panel noted that effects on AST and ALT activities were also observed in the new 13 week study described below but appeared to be reversible upon a 4 weeks recovery phase included in this new study. Thus the Panel concluded that the NOAEL of this 28 week toxicity study was 100 mg/kg bw/day.

The new studies that became available following a public call for data also included a 13 week toxicity study on β-apo-8'-carotenal 10% WS/N in rats incorporating a neurotoxicity screen with administration by the diet with a 4 week recovery period (Edwards et al., 2007). The study design was in compliance with OECD guideline 408 and the study was performed in accordance with GLP. Five groups of 10 male and 10 female Sprague-Dawley rats were dosed continuously by diet for 13 consecutive weeks at levels of 0, 0 (placebo), 10, 30 and 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. Dietary administration with β-apo-8'-carotenal 10% WS/N for 13 weeks resulted in the expected orange/red coloured faeces in all treated animals and orange coloured skin in

animals treated at 30 mg β-apo-8'-carotenal active ingredient/kg bw/day and above. In males at 100 mg β-apo-8'-carotenal active ingredient /kg bw/day the discoloured skin lasted for 2 weeks after the completion of treatment. There were no signs of neurotoxicity, body weight or food consumption effects that could be attributed to administration of β-apo-8'-carotenal 10% WS/N. Statistically significant increases in white blood cells and some of the associated parameters were seen in males receiving 30 and 100 mg β-apo-8'-carotenal active ingredients/kg bw/day. Minor increases were seen in AST and ALT levels in females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day. These differences were not evident after the recovery phase.

Histopathologically, administration of β-apo-8'-carotenal 10% WS/N in the diet was associated with:

- eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above,

- multinucleate hepatocytes in the liver of females at 30 mg β-apo-8'-carotenal active ingredient/kg bw/day and above,

- increased numbers of inflammatory cell foci in the liver of females at 100 mg β-apo-8'-carotenal active ingredient/kg bw/day.

The Panel closely evaluated the data on the eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and above. From these data the Panel concluded that 10 mg/kg bw/day is a lowest observed adverse effect level (LOAEL) for this effect.

In rats, testicular weights were significantly decreased after being fed 500 mg/kg bw/day β-apo-8'-carotenal or β-apo-8'-carotenoic acid *methyl* ester (Anonymous, 1962; 1966) (or possible *ethyl* ester, discrepancy between JECFA and TemaNord evaluations).

Following administration of β-apo-8'-carotenal in rats granular pigment deposition in the liver and kidneys was noted (Brubacher et al., 1960; Schärer et al. 1961; Loget and Morgan 2006; Edwards et al. 2007) and in dogs pigmentation was found in adipose tissue, kidney and adrenal cortex (Bagdon 1961; Bagdon et al., 1962). Besides pigment deposition, a marked increase in the vitamin A level in the kidney was noted in dogs. As none of the latter findings appear to have been accompanied by any signs of pathology, these effects are considered to be of no toxicological concern.

Two studies on reproductive and developmental toxicity have been conducted well before the introduction of (OECD) GLP guidelines, both in rats (Anonymous, 1966). Although on the basis of these two studies, there appears to be no direct reason for concern, it should be considered that the available studies were conducted well before the introduction of (OECD) GLP guidelines, and that in addition, the studies have been described in very little detail in an evaluation conducted over three decades ago.

Upon a public call for data two additional studies on reproductive and developmental toxicity became available for evaluation; a range-finding study (Loget and Marsden, 2006) and a subsequent study investigating the effects of β-apo-8'-carotenal 10% WS/N on embryonic and fetal development of the rat when administered by the oral route (dietary admixture) (Loget et al., 2006). These studies were performed according to OECD guidelines and under GLP. Within the context of this study, dietary concentrations of 2064, 10344 and 51720 mg/kg diet of β-apo-8'-carotenal 10% WS/N (equivalent to target doses of 20, 100 and 500 mg β-apo-8'-carotenal/kg bw/day) were associated with slight reductions in mean body weight gain and food consumption at the two higher doses. Other treatment-related findings in all β-apo-8'-carotenal 10% WS/N treated groups, such as orange coloured integuments, faeces, tissues and organs, were consistent with the nature of the test item (orange-red colouring agent).

Based on reduction in mean body weight gain the NOAEL for maternal toxicity was considered by the authors to be the dietary concentration of 2064 mg/kg diet (20 mg β-apo-8'-carotenal/kg bw/day).

The Panel noted that the reduction in mean body weight gain was observed during the first 9 days of treatment (days 6 to 15 of gestation) in the 100 and 500 mg/kg bw/day groups compared with the

vehicle control group and that this effect attained statistical significance only between days 6 and 11 of gestation. As a consequence, net mean body weight gain during the treatment period (days 6 to 20 of gestation) was slightly but not statistically significantly lower in these groups. The Panel therefore considered the slight reduction in mean body weight and food intake not as adverse and concluded that the NOAEL of this study was 500 mg/kg bw/day.

Long term carcinogenicity studies on β-apo-8'-carotenal were made available to the Panel upon a public call for data and were performed well before the introduction of (OECD) GLP guidelines. A summary of these chronic toxicity studies with β-apo-8'-carotenal was also provided (Schärer and Studer 1961b). β-Apo-8'-carotenal was administered at 0.1% in the diet to the first generation of Wistar rats and their offspring (second generation) for a period of 2 years. The average intake of β-apo-8'-carotenal was about 40 mg/kg bw/day.

Histopathological examination of sections stained for fat revealed golden-yellow to yellow-brown, iron-free pigment granules in the liver and kidneys of treated animals which were absent in the controls. The interstitial changes in the kidneys as well as the tubular distensions and calcium incrustations at the medulla cortex margin were less marked in the treated animals than in the controls. The other organs (lungs, heart, spleen, bone marrow, stomach, duodenum, jejumen, pancreas, ovaries, skeletal musculature, thyroid and skin) did not reveal any differences when examined microscopically. Degenerative changes in the epithelium of the seminiferous tubules were slightly, but not statistically significantly more frequent (p=0.17) in the animals treated with β-apo-8'-carotenal than in the controls.

The spontaneous tumours were distributed evenly over both groups (11/147 treated rats and 10/122 untreated rats) and consequently the authors concluded that their occurrence was not connected with the application of β-apo-8'-carotenal.

The reproductive performance and the average number of animals per litter were approximately the same in the treated and untreated rats.

Based on these results the Panel considered that there were no adverse effects in this chronic toxicity study at the single dose level tested amounting to about 40 mg/kg bw/day. Overall, the Panel concluded that the present database on β-apo-8'-carotenal provides a basis to revise the ADI of 5 mg/kg bw/day.

The Panel concluded that based on the LOAEL of 10 mg/kg bw/day from the 13 week study in rats showing an increased incidence of eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and an uncertainty factor of 200, an ADI for β-apo-8'-carotenal of 0.05 mg/kg bw/day can be established. The Panel considered an uncertainty factor of 200 to derive the ADI from the LOAEL sufficient, given the fact that the increase in the eosinophilic droplets in the kidneys at the LOAEL was minimal.

The Panel considered whether this carotenoid could be included in a group ADI with β-carotene.

However, since the re-evaluation of β-carotene concluded that no ADI for β-carotene could be established the Panel concluded that a group ADI including β-apo-8'-carotenal and β-carotene cannot be established.

Exposure estimates based on Tier 2 using maximum permitted levels would result in exposures to β-apo-8´-carotenal of 0.9 mg/kg bw/day for adults on average and of 3.3 mg/kg bw/day at the 95th percentile. For children, Tier 2 estimates would result in exposures to the colour in the range of 0.5-3.4 mg/kg bw/day on average and in the range of 1.2-7.2 mg/kg bw/day at the 95th/97.5th percentile.

It was indicated by food industry however that the colour is relatively rarely used due to its comparability to β-carotene. Based on this information and the maximum reported use levels provided, Tier 3 estimates lead to exposure to β-apo-8´-carotenal of adults of 0.05 mg/kg bw/day on average and of 0.19 mg/kg bw/day at the 97.5th percentile with non-alcoholic flavoured drinks being the main contributor (92%). Exposure estimates at Tier 3 for children were calculated across European countries in the range of 0.02-0.22 mg/kg bw/day on average and in the range of 0.09-0.71 at the

95th/97.5th percentile. Main contributors for children´s exposure to β-apo-8´-carotenal were non-alcoholic flavoured drinks (50-91%) and fine bakery wares (11-50%).

Exposure estimates at Tier 3 even at the reported maximum use levels in only few food categories would lead to exposure of adults on average at the level of the ADI of 0.05 mg/kg bw and to exceedance of the ADI by adults at the 95th percentile and by children both on average and at the 95th/97-5th percentile for all European countries.

CONCLUSIONS

β-Apo-8'-carotenal (E 160e) is authorised as a food additive in the EU and has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 and the EU Scientific Committee for Food (SCF) in 1975 and 2000. Both committees have established an ADI of 0-5 mg/kg bw/day, which was withdrawn by SCF in 2000.

The Panel concluded that based on the LOAEL of 10 mg/kg bw/day from the 13 week study in rats showing an increased incidence of eosinophilic droplets in the kidneys of both sexes at 10 mg β-apo-8'-carotenal active ingredient/kg bw/day and an uncertainty factor of 200, an ADI for β-apo-8'-carotenal of 0.05 mg/kg bw/day can be established. The Panel considered an uncertainty factor of 200 to derive the ADI from the LOAEL sufficient, given the fact that the increase in the eosinophilic droplets in the kidneys at the LOAEL was minimal.

The Panel concluded that a group ADI including β-apo-8'-carotenal and β-carotene cannot be established.

Exposure estimates for β-apo-8'-carotenal at Tier 2 using maximum permitted levels in all food categories where the use of the additive is authorised would lead to exposures of both adults and children exceeding the ADI of 0.05 mg/kg bw at average level and at the 95th/97.5th percentile.

Exposure estimates at Tier 3 even at the reported maximum use levels in only few food categories would lead to exposure of adults on average at the level of the ADI of 0.05 mg/kg bw and to exceedance of the ADI of adults at the 95th percentile and of children both on average and at the 95th/97-5th percentile for all European countries.

The Panel noted that the specifications should be updated to more clearly define the purity of the material. The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 10 mg/kg.

The Panel noted that specifications should be extended to include maximum residue limits for residual solvents.

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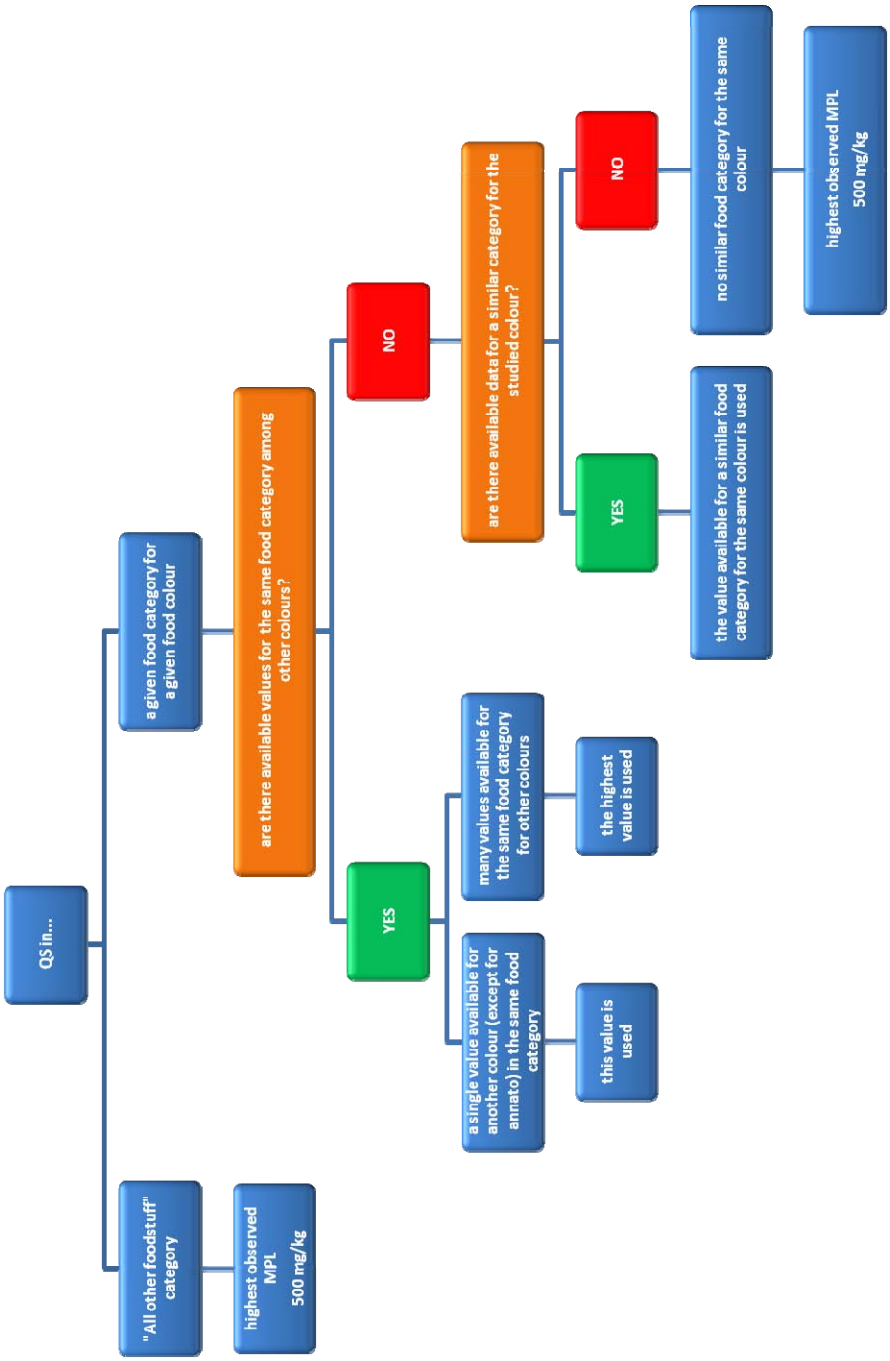
ANNEX A

RULES DEFINED BY THE PANEL TO DEAL WITH QUANTUM SATIS (QS) AUTHORISATION, USAGE DATA OR OBSERVED ANALYTICAL DATA FOR ALL REGULATED FOOD ADDITIVES TO BE RE-EVALUATED

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

Rules defined by the Panel to deal with *quantum satis* (QS) authorisation.



GLOSSARY / ABBREVIATIONS

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
ALT	Alanine Transaminas
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
AST	Aspartate Transaminase
BMD	Benchmark Dose
CA	Chromosomal aberration
CAS	Chemical Abstracts Service
CEN	Committee for Standardization
CHO	Chinese Hamster Ovary
CP	Cleavage Product
CPA	Cyclophosphamide
CRL	European Union Reference Laboratory for GM Food and Feed formerly known as Community Reference Laboratory
DMNQ	2,3-dimethoxy-1,4-naphtoquinone
DNA	Deoxyribonucleic acid
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
EINECS	Existing Commercial chemical Substances
EU	European Union
EXPOCHI	Individual food consumption data and exposure assessment studies for children
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practise
GSH	Glutathione
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
LOAEL	Lowest-Observed-Adverse-Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
MN	Micronuclei

MNH	Multinucleated Hepatocytes
MPL	Maximum Permitted Level
NCE	Normochromatic Erythrocytes
NDNS	UK National Dietary and Nutrition Survey
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic Erythrocytes
SCE	Chromatid Exchange
SCF	Scientific Committee on Food
SCOOP	A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety
TLC	Thin Layer Chromatography
US EPA	US Environmental Protection Agency
UV	Ultraviolet
WHO/FAO	World Health Organization/Food and Agriculture Organization