

**Opinion on mixed tocopherols, tocotrienol tocopherol and tocotrienols as sources for vitamin E added as a nutritional substance in food supplements<sup>1</sup>**

**Scientific Opinion of the Panel on Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food**

(Question No EFSA Q-2005-146, Q-2005-172, Q-2006-265)

**Adopted on 22 February 2008 by written procedure**

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**SUMMARY**

Following a request from the Commission, the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) has been asked to evaluate the safety and bioavailability of mixed tocopherols, tocotrienol tocopherol and tocotrienols as a source for vitamin E when added for nutritional purposes in food supplements.

The present opinion deals only with the safety and bioavailability of three particular sources of vitamin E, intended for the general population, to be added in food supplements. The safety of vitamin E itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

Vitamin E has been evaluated by the Scientific Committee on Food (SCF) who set a tolerable upper intake level (UL) of vitamin E (as d-alpha-tocopherol) for adults of 300 mg alpha-tocopherol equivalents /day. JECFA has defined an Acceptable Daily Intake (ADI) of 0.15-2 mg/kg bw/day calculated as alpha-tocopherol.

The three preparations, mixed tocopherols, tocotrienol tocopherol and tocotrienols are proposed to be used as sources of vitamin E. These sources contain varying amounts of tocopherols and tocotrienols.

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<sup>1</sup> For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission on mixed tocopherols, tocotrienol tocopherol and tocotrienols as sources for vitamin E. *The EFSA Journal* (2008) 640, 1-34.

Studies indicate that tocopherols and tocotrienols are bioavailable, with tocotrienols having shorter plasma half-lives and probably different tissue distribution than alpha-tocopherol, the major constituent of natural vitamin E.

Since the bioavailability and tissue distribution of tocotrienols appear to be different from that of tocopherols and since the specifications of the two tocotrienol preparations of the present opinion do not match the specifications for E306, the only registered vitamin E additive which has specification including tocotrienols, safety assessment of these tocotrienol-containing preparations cannot be based on upper limits for vitamin E.

From a subchronic toxicity study in rats with tocotrienol rich palm oil extract (70 % tocotrienols) the Panel concluded that a no-observed-adverse-effect level (NOAEL) of 120 mg tocotrienol extract/kg bw/day for male rats and 130 mg tocotrienol extract /kg bw/day for female rats can be derived. The effects observed at this dose level were not considered to be adverse.

The tocotrienol rich fractions were shown in bacterial tests to be not genotoxic and long-term studies gave no indications of neoplastic lesions.

#### *Intake of mixed tocopherols*

Intake of mixed tocopherols from supplement use will be in accordance with the UL for Vitamin E (as d-alpha-tocopherol) of 300 mg /day for adults set by the SCF in 2003 (SCF, 2003).

#### *Intake of tocotrienol tocopherol*

The proposed uses and use levels of tocotrienol tocopherol for supplement intake are based on the recommended daily allowance (RDA) for alpha-tocopherol. In Europe the RDA is set to 10 mg Vitamin E in European Council Directive 90/496/EEC (1990). Since the tocotrienol tocopherol preparation contains 11.5 mg alpha-tocopherol and 15.5 mg tocotrienols per 100 mg these values from the European Directive would amount to a daily intake of 87 mg of the tocotrienol tocopherol preparation containing 13.5 mg tocotrienols amounting to an intake of 0.23 mg tocotrienols/kg bw/day for a 60 kg person. This would be at least 500 times lower than the NOAEL for the tocotrienols in the rat study.

Given the specifications of the tocopherol tocotrienol preparation, a daily intake of 87 mg of tocotrienols plus tocopherols would amount to 10 mg alpha-tocopherol plus 0.44 mg beta-tocopherol (amounting to 0.22 mg alpha-tocopherol equivalents) plus 3.9 mg gamma-tocopherol (0.98 mg alpha-tocopherol equivalents) plus 1.04 mg delta-tocopherol (0.10 mg alpha-tocopherol equivalents). Together this would amount to 11.3 mg alpha-tocopherol equivalents and would be significantly below the UL of 300 mg alpha-tocopherol equivalents established by the SCF in 2003. For a 60 kg person this intake would amount to 0.19 mg alpha-tocopherol equivalents/kg bw/day.

#### *Intake of tocotrienols*

The petitioner indicates that tocotrienols are normally incorporated in softgel capsules providing up to 1000 mg of tocotrienols per daily dose. This would result in a daily intake of 16.7 mg tocotrienols/kg bw/day for a 60 kg person and would be only 7 times below the

NOAEL of the rat study and higher than the 5 mg/kg bw/day frequently demonstrated to be without adverse effects in human studies.

In conclusion, the Panel considers that the use of mixed tocopherols and tocotrienol tocopherol as a source of vitamin E in food supplements for the general population at the proposed levels of use is not of safety concern.

However, the available safety data are insufficient to conclude on the safety of the proposed use and use levels of the tocotrienols (the preparation containing mainly tocotrienols).

**Key words:**

Food supplements, mixed tocopherols, tocotrienol tocopherol and tocotrienols, CAS Registry Numbers 1406-18-4, 59-02-9, 148-03-8, 7616-22-0, 119-13-1, vitamin E.

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## **BACKGROUND AS PROVIDED BY THE COMMISSION**

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of mixed tocopherols, tocotrienol tocopherol concentrate and tocotrienols added for nutritional purposes to food supplements. The relevant Community legislative measure is:

European Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements<sup>2</sup>.

## **TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION**

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of mixed tocopherols, tocotrienol tocopherol concentrate and tocotrienols added for nutritional purposes in food supplements.

## **ACKNOWLEDGEMENTS**

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Fernando Aguilar, Dimitrios Boskou, David Gott, Sandro Grilli, Werner Grunow, Rainer Gürtler, Karin Hulshof, John-Christian Larsen, Jean-Charles Leblanc, Catherine Leclercq, Alicja Mortensen, Dominique Parent-Massin, Iona Pratt, Ivonne Rietjens, Paul Tobback, Gerrit Speijers, Fidel Toldrá.

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<sup>2</sup> OJ L 183, 12.7.2002, p.51.

## ASSESSMENT

### 1. Introduction

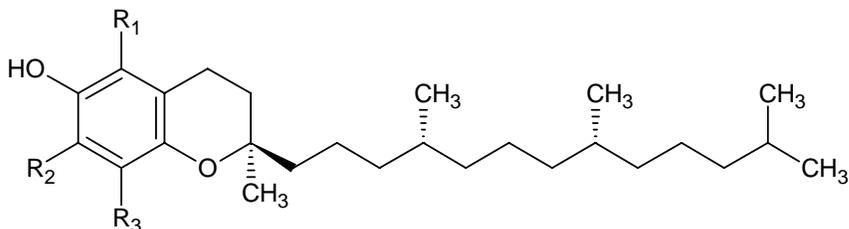
The present opinion deals only with the safety and bioavailability of three particular sources of vitamin E, intended for the general population, to be added in food supplements. The safety of vitamin E itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

### 2. Technical data

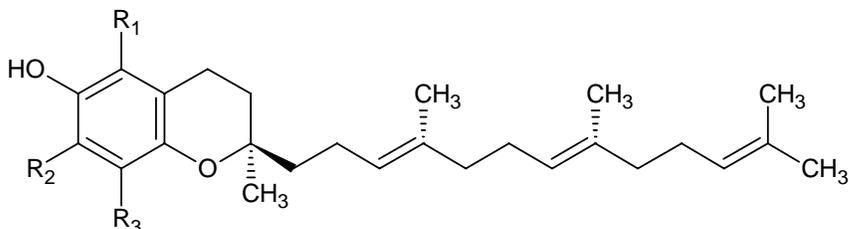
#### 2.1. Chemistry

Vitamin E is the collective term for a family of chemical substances that are structurally related to alpha-tocopherol. Vitamin E occurs naturally in eight different forms: four tocopherols, alpha ( $\alpha$ )-, beta ( $\beta$ )-, gamma ( $\gamma$ )- and delta ( $\delta$ )-tocopherol and four tocotrienols, alpha-, beta-, gamma- and delta-tocotrienol. All of these forms consist of a chromanol ring with a long aliphatic side chain, bound to the chromanol ring at the 2 position. Tocotrienols differ from their corresponding tocopherols in that the saturated phytyl side chain is replaced with an unsaturated isoprenoid side chain. The Greek characters refer to the number and position of the methyl groups at the 5, 7 and 8 positions. Figure 1 gives a schematic presentation of the molecular structures.

Tocopherol



Tocotrienol



Compound	Formulae	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	MW
$\alpha$ -tocopherol	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>		CH <sub>3</sub>	CH <sub>3</sub>	430
$\alpha$ -tocotrienol	C <sub>29</sub> H <sub>44</sub> O <sub>2</sub>				424
$\beta$ -tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>		CH <sub>3</sub>	H	416
$\beta$ -tocotrienol	C <sub>29</sub> H <sub>42</sub> O <sub>2</sub>				410

$\gamma$ -tocopherol	$C_{28}H_{48}O_2$	H	CH <sub>3</sub>	CH <sub>3</sub>	416
$\gamma$ -tocotrienol	$C_{28}H_{42}O_2$				410
$\delta$ -tocopherol	$C_{27}H_{46}O_2$	H	H	CH <sub>3</sub>	402
$\delta$ -tocotrienol	$C_{27}H_{40}O_2$				396

Figure 1. Schematic presentation of the chemical structures of alpha- beta-, gamma- and delta-tocopherol. Tocotrienols have the same basic structure as tocopherols, only with three unsaturated double bonds in the side chain.

The CAS number for mixed tocopherols is 1406-18-4, which is a mixture of the following tocopherols with their own CAS numbers: d-alpha-tocopherol CAS number 59-02-9; d-beta-tocopherol CAS number 148-03-8; d-gamma-tocopherol CAS number 7616-22-0; d-delta-tocopherol CAS Number 119-13-1.

The relative abundance of the vitamin E homologues (tocopherols and tocotrienols) in a specific preparation depends on the species of plant and the extraction procedure used. Palm oil contains significant quantities of tocotrienols. Other sources of tocotrienols are coconut oil, cereal grains including rice, barley, rye and wheat and nuts. Palm, rice bran, oat and barley contain vitamin E mostly as the tocotrienols (Ong, 1993; Sheppard *et al.*, 1993; Souci Fachman Kraut, 2002). The common sources of commercial vitamin E such as soy, corn, cottonseed, canola and sunflower oil distillates contain little or no tocotrienols. Examples of the tocopherol and tocotrienol content of some selected plant-derived oils are presented in Table 1.

Table 1. Tocopherol and tocotrienol content of some edible plant oils in mg/100 g product (Ong, 1993; Sheppard *et al.*, 1993)

Oil	Tocopherols (mg/100 g product)				Tocotrienols (mg/100 g product)			
	alpha	beta	gamma	delta	alpha	beta	gamma	delta
Canola	21.0	0.1	4.2	0.04	0.04	-	-	-
Castor	2.8	2.9	11.1	31.0	-	-	-	-
Coconut	0.5	-	-	0.6	0.5	0.1	1.9	-
Corn	11.2	5.0	60.2	1.8	-	-	-	-
Cottonseed	38.9	-	38.7	-	-	-	-	-
Olive	11.9	-	0.7	-	-	-	-	-
Palm	25.6	-	31.6	7.0	14.6	3.2	28.6	6.9
Palm kernel	6.2	-	-	-	-	-	-	-
Peanut	13.0	-	21.4	2.1	-	-	-	-
Rice bran	32.4	1.8	5.3	-	23.6	-	34.9	-
Safflower	34.2	-	7.1	-	-	-	-	-
Sesame	13.6	-	29.0	-	-	-	-	-
Soybean	7.5	1.5	79.7	26.6	0.2	0.1	-	0.03
Sunflower	48.7	-	5.1	0.8	-	-	-	-
Walnut	56.3	-	59.5	45.0	-	-	-	-
Wheat germ	133.0	71.0	26.0	27.1	2.6	18.1	-	-

*In vivo* vitamin E functions mainly as an antioxidant preventing the oxidation of polyunsaturated fatty acids (Ball, 1988; SCF, 2003). The antioxidant activity of tocopherols and tocotrienols has been well established (Kamal-Eldin and Appelqvist 1996 and references therein). The requirement for vitamin E is determined to a large extent by the intake of

polyunsaturated fatty acids (PUFAs) (SCF, 2003). Vitamin E has also been implicated in a variety of other functions including the preparation for and protection of pregnancy, since resorption of the foetus in pregnant rats can be prevented by vitamin E as a nutrient (Ball, 1988).

The International Unit (IU) of vitamin E is the activity of 1 mg of dl-alpha-tocopheryl acetate. This is the average quantity that, administered orally, prevents resorption-gestation in fifty percent of female rats deprived of vitamin E (Ball, 1988). The accepted biological activities, expressed in IU of different forms of vitamin E are given in Table 2. Table 3 presents the relative biological activities of the various tocopherols and tocotrienols in the rat resorption-gestation test (Ball, 1988; Kamal-Eldin and Appelqvist, 1996).

Table 2. Biological activity of different forms of vitamin E expressed in IU (Bieri and McKenna, 1981).

Form	IU
dl-alpha-tocopheryl acetate	1.00
dl-alpha-tocopherol	1.10
d-alpha-tocopheryl acetate	1.36
d-alpha-tocopherol	1.49

Table 3. Relative biological activities (in %) of vitamin E active compounds in the rat resorption-gestation test (Ball, 1988; Kamal-Eldin and Appelqvist, 1996).

Form	Relative activity in prevention of foet resorption (%)
d-alpha-tocopherol (RRR)	100
d-beta-tocopherol (RRR)	25-50
d-gamma-tocopherol (RRR)	1-11
d-delta-tocopherol	1-3
d-alpha-tocotrienol	29-30
d-beta-tocotrienol	5
d-gamma-tocotrienol	Not known
d-delta-tocotrienol	Not known

**From this it can be concluded that tocopherols and tocotrienols contribute to vitamin E activity, but to a different extent.**

## 2.2. Specifications

For the three tocopherol/tocotrienol preparations to which the present opinion refers specifications provided by the petitioners were as follows:

### *Mixed tocopherols*

The petitioner indicates that natural mixed tocopherols are commercially available in different potencies and physical forms. The product with the highest potency (90% tocopherols) will further be referred to in the dossier, since the other products are made from

it, either by standardising to the specified content of total tocopherols using partially hydrogenated vegetable oil, or by spray-drying onto a suitable carrier.

Mixed tocopherols are a mixture containing all four tocopherols. The proportion of each of the tocopherols in the product reflects the pattern in the vegetable oils used to isolate the tocopherols. The petitioner indicates that the proportion of the individual tocopherols is typically: alpha-tocopherol <20%, beta-tocopherol <10%, gamma-tocopherol 50 – 70% and delta-tocopherol 10 - 30%.

The petitioner indicates that the purity criteria for mixed tocopherols apply to the purity criteria for tocopherol-rich extract (E306) as given in the Annex to the European Directive 96/77/EEC (EC, 1996). They include: sulphated ash not more than 0.1%, arsenic not more than 3 mg/kg, lead not more than 5 mg/kg, mercury not more than 1 mg/kg, heavy metals not more than 10 mg/kg (as lead).

The petitioner indicates that the 90% mixed tocopherol material is also in compliance with the requirements and specification set by the FCC monograph (Tocopherols Concentrate, Mixed, Low- $\alpha$  Type, as published in Food Chemicals Codes IV) (FCC, 1996) and the US NF monograph (Tocopherols Excipient in the 19<sup>th</sup> edition of the U.S. National Formulary) (USP 24-NF 19, 1999).

### ***Tocotrienol tocopherol***

The tocotrienol tocopherol preparation evaluated in the present opinion is a natural blend of tocotrienols and tocopherols extracted and concentrated from rice bran oil distillate.

The petitioner indicates that the preparation contains typically 155 mg/g total tocotrienols (minimum 150 mg/g) and 350 mg/g total tocotrienols plus tocopherols (minimum 325 mg/g). Thus, the tocotrienol tocopherol preparation consists of 35% vitamin E (alpha-, beta-, gamma- and delta-tocopherols and alpha-, beta-, gamma- and delta-tocotrienols).

Possible other components are squalene (5-10%), phytosterols (20%) and 35-40% remaining compounds including mono-, di- and triglycerides of palmitic, stearic and oleic acid as well as free fatty acids.

The level of the individual tocopherols and tocotrienols in the preparation is indicated by the petitioner to amount to typically:

- 115 mg/g alpha-tocopherol (101 mg/g minimum)
- 5 mg/g beta-tocopherol (<1 mg/g minimum)
- 45 mg/g gamma-tocopherol (25 mg/g minimum)
- 12 mg/g delta-tocopherol (3 mg/g minimum)
- 67 mg/g alpha-tocotrienol (30 mg/g minimum)
- <1 mg/g beta-tocotrienol (<1 mg/g minimum)
- 82 mg/g gamma-tocotrienol (45 mg/g minimum)
- 5 mg/g delta-tocotrienol (<1 mg/g minimum)

### ***Tocotrienols***

The tocotrienol preparation is a blend of alpha-, beta-, gamma- and delta- tocotrienol with alpha-tocopherol. The petitioner indicates the following typical composition:

- 180 mg/g d-alpha-tocotrienol

- 10 mg/g d-beta-tocotrienol
- 120 mg/g d-gamma-tocotrienol
- 80 mg/g d-delta-tocotrienol
- 110 mg/g d-alpha-tocopherol

This implies a total amount of tocotrienols of 390 mg/g (39%) and 11% alpha-tocopherol, and a total amount of tocotrienols and alpha tocopherol indicated by the petitioner to be not less than 500 mg/g (50%). The remaining constituents are not specified but the Panel assumes that these are likely to be similar to the residual compounds in the mixed tocopherol tocotrienol preparation, including mono-, di- and triglycerides of palmitic, stearic and oleic acid as well as free fatty acids.

Limits for impurities were specified by the petitioner to be as follows: arsenic < 1 mg/kg, lead < 1 mg/kg, mercury < 1 mg/kg, heavy metals < 5 mg/kg. The peroxide value should be < 1 mEq/kg.

### **2.3. Manufacturing Process**

For the three tocopherol/tocotrienol preparations to which the present opinion refers the manufacturing procedures have been described by the petitioners and can be summarised as follows:

#### ***Mixed tocopherols***

Mixed tocopherols are obtained by vacuum steam distillation of edible vegetable oil products including soya oil. The petitioner indicates that the raw material used for the production of mixed tocopherols is a by-product of vegetable oil refining with the type of vegetable oils used varying with the batch and the supplier. One of the suppliers indicated that the main raw material comes from soybean (approximately 60-100%), that rapeseed may contribute to not more than approximately one third, and that minor sources (below 10%) are sunflower, corn and cottonseed. Manufacturers indicate that they do not use peanut oil.

The 90% mixed tocopherol material is produced from vegetable oil deodorised distillates using a combination of purification and distillation steps. The stereochemistry is preserved so that the mixed tocopherols are identical in all respects to the various forms of tocopherols found in the natural source material. The product is standardised to its final potency and quality by concentration and purification by distillation.

#### ***Tocotrienol tocopherol***

The tocotrienol tocopherol preparation is prepared by distillation of rice bran oil distillate, a product of rice oil refining, followed by extraction of the tocotrienol enriched distillate stream using methanol, crystallization and a subsequent distillation to remove the residual methanol.

### ***Tocotrienols***

The petitioner indicates that the sources for production of tocotrienols are fruits of oil palm (*Elaeis Guineensis*), which may contain the tocopherols and tocotrienols at levels indicated in Table 4.

Table 4. Tocopherol and tocotrienol content for several palm oils (Slover, 1971; Whitte, 1967).

Sources	tocopherols (mg/kg)				tocotrienols (mg/kg)				tocotrienols (mg/kg)	Sum (mg/kg)
	alpha	beta	gamma	delta	alpha	beta	gamma	delta	Total	Total
Palm Oil <sup>1</sup>	256	-	316	70	143	32	286	69	530	1172
Palm Oil <sup>2</sup>	279	-	61	-	274	-	398	69	741	1081

<sup>1</sup> HT Slover

<sup>2</sup> KJ Whittle, *et al.*

The purification process involves extraction and distillation steps and is adequately described by the petitioner.

The petitioner indicates that tocotrienols are obtained from vegetable edible oils extracted from palms that have not been genetically modified and do not contain and/or are not derived from genetically modified organisms.

### **2.4. Methods of analysis in food**

Tocopherols can be analysed by Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC). Tocotrienols can be determined using HPLC with fluorescence detection. Appropriate sample preparation methods are to be employed depending on the various matrices.

### **2.5. Reaction and fate in foods to which the source is added**

Tocopherols and tocotrienols are relatively stable in foods but may suffer oxidation when exposed to air, heat, acid, alkali and metal ions. One of the petitioners indicates that degradation is not expected under normal storage conditions.

### **2.6. Case of need and intended levels of use**

The three tocopherol/tocotrienol preparations to which the present opinion refers are to be used as a source of vitamin E supplied as a nutrient in food supplements.

### ***Mixed tocopherols***

The petitioner indicates that mixed tocopherols are mainly used by food supplement manufacturers as a liquid ingredient in soft capsules and liquid food supplements but may also be used as a spray-dried powder in tablets, caplets, capsules, chewable tablets and effervescent powders that are food supplements.

The petitioner does not describe the intended levels of use but instead refers to the tolerable upper intake level (UL) of vitamin E (as d-alpha-tocopherol) given by the SCF for adults of 300 mg/day (SCF, 2003). The petitioner indicates that the recommended daily amount of

mixed tocopherols to be included in a food supplement depends on the relative proportion of each tocopherol and its conversion factor to d-alpha-tocopherol activity. The petitioner also indicates that the maximum daily recommended dose (as tocopherol equivalents) would be subject to any restrictions set in future EU legislation on maximum permitted levels implementing requirements of the Food Supplements Directive 2002/46/EC.

### ***Tocotrienol tocopherol***

The petitioner indicates that the proposed uses and use levels for tocotrienol tocopherol for supplement intake are based on the recommended daily allowance for alpha-tocopherol. The petitioner indicated that this implies an intake of 15 mg (22.5 IU) alpha-tocopherol/day for adults (IOM, 2000). In Europe the recommended daily allowance (RDA) is set to 10 mg vitamin E in European Council Directive 90/496/EEC (1990). Since the tocotrienol tocopherol preparation contains 11.5 mg alpha-tocopherol and 15.5 mg tocotrienols per 100 mg these values from the IOM or European Directive would amount to daily intakes of 130 mg and 87 mg, respectively of the tocotrienol tocopherol preparation.

### ***Tocotrienols***

The petitioner indicates that tocotrienols are to be used by food supplement manufacturers as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders and liquids that are food supplements. The method of incorporation is determined by the individual manufacturers as appropriate for the particular type of finished products.

The petitioner also indicates that tocotrienols, being an oily liquid, are normally incorporated in softgel capsules providing up to 1000 mg of tocotrienol per daily dose.

## **2.7. Exposure**

For the three tocopherol/tocotrienol preparations to which the present opinion refers exposure can be estimated as follows:

### ***Mixed tocopherols***

The anticipated exposure of the population to mixed tocopherols in food supplements is by self-selection of products containing multivitamins or as more specific combinations providing an alternative source of vitamin E. Given the information provided by the petitioner it is not possible to foresee whether intake of mixed tocopherols from supplement use will be in accordance with tolerable upper intake levels of vitamin E (as d-alpha-tocopherol) given by the SCF for adults of 300 mg/day (SCF, 2003). The petitioner has not provided information on the actual levels to be used in supplements. The maximum daily recommended dose (as tocopherol equivalents) would be subject to any restrictions set in future EU legislation on maximum permitted levels implementing requirements of the Food Supplements Directive 2002/46/EC.

Intake of mixed tocopherols from other food sources has not been provided by the petitioner. Fats and oils are major sources of vitamin E in food products. The relative abundance of the vitamin E homologues depends on the species of plant and the extraction procedures.

Synthetic vitamin E contains only alpha-tocopherol and none of the other tocopherols and tocotrienols.

Intake from tocopherols from food additive intake will add to normal dietary intake. Natural mixed tocopherols are approved as an antioxidant for general use in foods *ad quantum satis* as E306 tocopherol-rich extract. However, intake data from food additive use is scarce. For example, in the recent report on Dietary Food Additive Intake from the European Commission, E306 tocopherol-rich extract was excluded from the monitoring exercise because inclusion of additives was done by prioritising according to potential safety concerns. Mixed tocopherols were categorised into the category with least potential safety concerns, since, 'on the basis of the available scientific data, the total intake was not regarded to represent a hazard to health, so that an ADI 'not specified' was allocated'.

### ***Tocotrienol tocopherol***

The proposed uses and use levels of tocotrienol tocopherol for supplement intake are based on the recommended daily allowance for alpha-tocopherol. The petitioner indicated that this implies an intake of 15 mg (22.5 IU) alpha-tocopherol/day for adults (IOM, 2000). In Europe the recommended daily allowance (RDA) is set to 10 mg vitamin E in the European Council Directive 90/496/EEC (1990). Since the tocotrienol tocopherol preparation contains 11.5 mg alpha-tocopherol and 15.5 mg tocotrienols per 100 mg these values from the IOM or European Directive would amount to a daily intakes of 130 mg and 87 mg, respectively, of the tocotrienol tocopherol preparation containing 20.2 mg and 13.5 mg tocotrienols amounting to an intake of 0.34 mg and 0.23 mg tocotrienols/kg bw/day for a 60 kg person.

Given the specifications of the tocopherol tocotrienol preparation a daily intake of 78 mg of tocotrienols plus tocopherols would amount to 10 mg alpha-tocopherol plus 0.44 mg beta-tocopherol (amounting to 0.22 mg alpha-tocopherol equivalents) plus 3.9 mg gamma-tocopherol (0.98 mg alpha-tocopherol equivalents) plus 1.04 mg delta-tocopherol (0.10 mg alpha-tocopherol equivalents)(for conversion factors see Table 3). Together this would amount to 11.3 mg alpha-tocopherol equivalents.

Intake of tocotrienols from other food sources has not been provided by the petitioner.

Some exposure may come from additive use since E306 might contain tocotrienols, depending on the source of oil used.

### ***Tocotrienols***

Tocotrienols only occur at very low levels in nature, with the highest concentration found in palm oil. Thus it can be foreseen that intake from normal food sources would be limited.

The petitioner indicates that tocotrienols are normally incorporated in softgel capsules providing up to 1000 mg of tocotrienol per daily dose. This would result in a daily intake of 16.7 mg tocotrienols/kg bw/day for a 60 kg person.

Some exposure may come from additive use since E306 might contain tocotrienols depending on the source of oil used.

## **2.8. Information on existing authorisations and evaluations**

In the EU there are several vitamin E containing food additives; E306 tocopherol-rich extract, E307 alpha-tocopherol, E308 gamma-tocopherol and E309 delta-tocopherol, of which only E306 might contain tocotrienols, depending on the source of oil used.

Mixed tocopherols underwent a safety evaluation by the SCF (SCF, 1989) and were approved as antioxidants for foods in general *ad quantum satis*, and as additives in nutrient preparations for use in infant formula, follow-on formulae and weaning foods within the European Community (European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners).

European Commission Directive 96/77/EC of 2 December 1996 laying down specific purity criteria on food additives other than colours and sweeteners describes E306 tocopherol-rich extract as a 'product obtained by the vacuum steam distillation of edible vegetable oil products, comprising concentrated tocopherols and tocotrienols. Contains tocopherols such as d-alpha-, d-beta-, d-gamma- and d-delta -tocopherols'. The content of total tocopherols is specified to be at least 34%'.

At the international level, tocopherols were evaluated for their safety by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The NOAEL was established from short-term studies as 154 mg/kg body weight for alpha-tocopherol. The ADI value was estimated to be 0.15 – 2 mg/kg body weight, calculated as alpha-tocopherol (WHO, 1986). Tocopherols used as a chemical antioxidant in food, as a dietary supplement in food, as a nutrient in food or as an antioxidant for food use (all for human consumption) are GRAS listed (generally recognized as safe) by the US-FDA (21 CFR, Part 182.8890 and 182.3890) when used in accordance with good manufacturing practice (21CFR 182).

The petitioner indicates that in the USA dietary supplements containing the tocotrienol tocopherol preparation have been on the market since 1999. However, in the USA no approval is required for dietary supplements. In the UK a vitamin E food supplement containing the tocotrienol tocopherol preparation has been on the market since at least 2002.

Three international expert groups have recently evaluated the safety of vitamin E: The EU Scientific Committee on Food (SCF, 2003), the UK Expert group on Vitamins and Minerals (EVM, 2003), and the Antioxidant Panel of the Food and Nutrition Board (FNB) at the Institute of Medicine of the US National Academy of Sciences (IOM, 2000). While all three expert groups agreed on the critical adverse event to be used as basis for their evaluation, and draw on the same body of published data, the Upper Safe Levels (UL) set by these groups differ somewhat.

The FNB set the tolerable upper intake level (UL) for adults at 1000 mg (2,325 µmol)/day of any supplemental form of alpha-tocopherol (IOM, 2000).

The SCF, based on a placebo controlled, dose-response supplementation study in 88 healthy humans (Meydani *et al.*, 1998), set the NOAEL at 540 mg alpha-tocopherol equivalents, the highest dose used in the study. Considering an uncertainty factor of 2 to cover for interindividual differences in sensitivity, the SCF established an UL of 270 mg alpha-tocopherol equivalents for adults, which was rounded to 300 mg alpha-tocopherol equivalents (SCF, 2003).

The EVM established a NOAEL of 540-970 mg alpha-tocopherol equivalents, based on three placebo controlled human studies (Gillilan *et al.*, 1977, Meydani *et al.*, 1998, Stephens *et al.*, 1996). An uncertainty factor to account for interindividual differences was not considered necessary since the results of the larger trial by Stephens *et al.* support a NOAEL of 540 mg alpha-tocopherol equivalents, so that the UL (defined by the EVM as a safe upper level for consumption over a lifetime) was established as 540 mg alpha-tocopherol equivalents for supplemental vitamin E (EVM, 2003).

The equivalence of the mixed tocopherols to the standard d- $\alpha$ -Tocopherol Equivalence (TE) is calculated by multiplying each component by its relevant factor (Table 5):

Table 5. Relative TE activity calculations

Homologue	Example Relative conce (%)	Absolute content (mg)	Conversion factor*	Vitamin E Activity (mg $\alpha$ -TE)
$\alpha$ -tocopherol	14	126	1.0	126.0
$\beta$ -tocopherol	2	18	0.5	9.0
$\gamma$ -tocopherol	60	540	0.25	135.0
$\delta$ -tocopherol	24	216	0.1	21.6
Total	100	900		291.6

\* SCF report on the UL for vitamin E (SCF, 2003).

### 3. Biological and toxicological data

#### 3.1. Bioavailability of mixed tocopherols, tocotrienol tocopherol and tocotrienols from their vitamin E source

Reported rates of absorption of vitamin E following intake with food have varied from as high as 51% to 86% to as low as 21% to 29%. All forms of vitamin E, including all of the tocopherol and tocotrienol homologues, are absorbed in a similar manner.

Vitamin E is absorbed from the lumen of the small intestine into the enterocytes by passive diffusion. Prior to its absorption, vitamin E is emulsified together with dietary lipids. Bile acids and salts secreted by the liver aid in the emulsification process. Lipolysis and emulsification of the formed lipid droplets lead to the spontaneous formation of mixed micelles. The micelles containing vitamin E are absorbed at the brush border of the intestinal mucosa in the enterocytes. Vitamin E is secreted by the enterocytes into the lymphatics in the form of chylomicrons. The chylomicrons contain the various forms of vitamin E, including alpha-, beta-, gamma-, and delta-tocopherol, and alpha-, beta-, gamma- and delta-tocotrienol.

The only forms of the vitamin secreted by the liver are the natural RRR-alpha-tocopherol form and the four 2R forms of synthetic tocopherol. Small amounts of the other tocopherol and tocotrienol homologues are secreted.

A major route for elimination of oral vitamin E from the body is by way of biliary recycling and fecal excretion (Machlin, 1991). Faecal excretion of the vitamin includes non-absorbed vitamin E, as well as vitamin E forms not utilized. For example, the forms not secreted by the liver, such as the beta-, gamma- and delta-tocopherol homologues, are excreted via the biliary route.

Recent studies indicate that significant portions of ingested vitamin E homologues are metabolized by cytochrome P450 (CYP)-catalyzed omega-hydroxylation followed by beta-

oxidation to yield hydrosoluble compounds called carboxyethylhydroxychromans (CEHC) that are excreted through the kidney upon conjugation with for example sulphate (Schultz *et al.*, 1995; Brigelius-Flohe and Traber, 1999; Tanabe *et al.*, 2004). CEHCs appear to be the primary metabolites of both tocopherols and tocotrienols and may possess antioxidant activity and pharmacological properties (Yoshida and Niki, 2002; Jiang *et al.*, 2000; Galli *et al.*, 2004; Hensley *et al.*, 2004).

The alpha-tocopherol transport protein (TTP) plays an important role in the secretion and incorporation of vitamin E in the form of alpha-tocopherol into VLDL in the liver and its subsequent transport to the various tissues (Traber and Arai, 1999). However TTP has a low affinity for tocotrienols and does not appear to play a significant role in their metabolism in the body. Very little tocotrienol is secreted by the liver to the circulation in VLDLs (PDR, 2005).

There are some data suggesting that high doses of gamma-tocotrienol are partly converted to alpha-tocopherol (Qureshi *et al.*, 2001).

Most bioavailability studies on vitamin E focus on natural tocopherols as compared to synthetic vitamin E and/or vitamin E acetate, but these are not considered useful in estimating the bioavailability of the mixed tocopherol, the tocotrienol tocopherol or the tocotrienol preparation.

In the next paragraphs only studies are discussed that refer to the bioavailability of the mixed tocopherols, tocotrienol tocopherol and tocotrienols of the present opinion.

### ***Mixed tocopherols***

In a healthy volunteer study, 46 subjects (men and women, age  $52.2 \pm 1.5$ y) were supplemented for 8 weeks with either placebo, or 100 mg *all-rac* alpha-tocopheryl acetate, or mixed tocopherols equivalent to 20 mg alpha-tocopherol, 100 mg gamma-tocopherol and 40 mg delta-tocopherols. Fasting blood samples were drawn before and after the supplementation period. The purpose of the study was to investigate the effects of tocopherols on platelet aggregation and therefore platelet-enriched plasma was prepared and analysed for tocopherols by HPLC, total cholesterol, and triacylglycerol, (Liu *et al.*, 2003). Total cholesterol, and triacylglycerol were within the normal range, remained constant during the supplementation period, and did not differ between the three groups.

Upon supplementation with mixed tocopherols, the concentrations of alpha-, gamma-, and delta-tocopherol increased in platelet-rich plasma and were significantly higher than baseline after 8 weeks of supplementation. In the group supplemented with *all-rac* alpha-tocopheryl acetate only the alpha-tocopherol concentration increased. From these studies it can be concluded that the tocopherols from the mixed tocopherol sample are bioavailable. The data are also in line with the fact that absorption of vitamin E plateaus with increasing oral doses (Machlin, 1991).

### ***Tocotrienol tocopherol and tocotrienols***

Literature data suggest that there is no discrimination between the vitamin E homologues at the level of absorption and chylomicron secretion by the intestines (Kayden and Traber, 1993), although a report based on a rat study suggested that alpha-tocotrienol is preferentially

absorbed as compared to gamma- and delta-tocotrienol when large doses are administered as a mixture (Ikeda *et al.*, 1996).

While tocotrienols and tocopherols are absorbed in a similar manner, they have different mechanisms of transport and tissue uptake that may reflect functional differences (Ikeda *et al.*, 1996; Hayes *et al.*, 1993).

Hayes *et al.* (1993) reported that tocotrienols were transported by chylomicrons and disappear from the plasma during chylomicron clearance. They also reported that in fasting humans, the plasma tocotrienol concentration was not increased significantly after tocotrienol supplementation, whereas the platelet concentration of d-tocotrienol was doubled.

Plasma tocotrienol concentrations have been shown to reach about 1  $\mu\text{mol/l}$  in humans and between 3-20  $\mu\text{mol/l}$  in various animal species following oral administration of tocotrienols (Raederstorff *et al.*, 2002; Schaffer *et al.*, 2005; O'Byrne *et al.*, 2000).

Yap *et al.* (2001) analysed plasma tocotrienol levels of eight subjects following a single oral dose of 300 mg of mixed tocotrienols in both the fed and fasted state and concluded that tocotrienol absorption is enhanced in the presence of food.

Exogenously administered tocotrienols have an extremely short half-life ( $T_{1/2}$ ) and rapid turnover. The apparent  $T_{1/2}$  has been estimated to be 4.4, 4.3 and 2.3 hours for alpha-, gamma-, and delta tocotrienol, respectively whereas the  $T_{1/2}$  for alpha-tocopherol is approximately 73-81 hours (Yap *et al.*, 2001; Schwedhelm *et al.*, 2003).

In another study fasting subjects were given an oral dose of palm oil concentrate (containing 318 mg of alpha tocopherol plus 692 mg of tocotrienols). Plasma samples at various time intervals were analysed for tocopherols and tocotrienols (Fairus *et al.*, 2004). Tocotrienols were barely detectable in plasma, triglyceride plasma, LDL and HDL. Alpha-tocopherol remained the major circulating form of plasma vitamin E regardless of the type of vitamin E ingested. In this study tocotrienols appeared in plasma at 2 hours, peaked between 4 and 6 hours and completely disappeared within 24 hours.

Two self-emulsifying tocotrienol formulations and one non-self-emulsifying formulation were compared in a single dose, cross-over bioavailability study in humans (Yap *et al.*, 2004). Self-emulsifying formulations resulted in higher plasma levels than non-self-emulsifying preparations. The petitioner concludes that this may demonstrate the importance of both droplet size and the rate and the extent of lipolysis in enhancing the bioavailability of tocotrienols.

Tocotrienols, in contrast to alpha-tocopherols are non-detectable or barely detectable in most tissues. Tocotrienols are barely detectable in normal human plasma (Hayes *et al.*, 1993; O'Byrne *et al.*, 2000). With supplementation tocotrienols show a tissue specific uptake in rats, mice and hamsters (Ikeda *et al.*, 2003). In human studies tocotrienol supplementation did not change plasma tocotrienol concentration but did slightly increase the platelet concentration of delta-tocotrienol (Hayes *et al.*, 1993).

A tocotrienol mixture was administered by gavage to Sprague-Dawley rats, and the tocotrienol levels in various tissues were measured 0, 4, 8 and 24 hours after the administration. In blood clots, brain, thymus, testes, vice-testes and muscles, tocotrienol homologues were not detected at all. In epididymal adipose, renal adipose, subcutaneous

adipose and brown adipose tissues and in the heart, the tocotrienol levels were maintained or increased for 24 hours after the administration. In the serum, liver, mesenteric lymph node, spleen and lungs, the tocotrienol levels were highest 8 hours after the tocotrienol administration. These results suggest that the distribution and metabolism of tocotrienol in the rat vary considerably among different tissues (Okabe *et al.*, 2002).

Hamsters supplemented with tocotrienols showed detectable levels of both alpha- and gamma-tocotrienols in adipose tissue but not in other tissues (Hayes *et al.*, 1993). Ikeda *et al.* (2003) found similar results in rats supplemented with tocotrienols with the exception that alpha and gamma-tocotrienols were also detected in the skin. The selective uptake of alpha- and gamma-tocotrienols by the skin has also been shown in rats and mice (Podda *et al.*, 1996; Ikeda *et al.*, 2000).

Lodge *et al.* (2001) have evaluated whether tocotrienols are also metabolised and excreted as urinary CEHC. In healthy volunteers, urine was monitored following tocotrienol supplementation. Complete (24 h) urine collections were obtained for 2 days prior to exposure (baseline), on the day of exposure and 2 days after human subjects (n = 6) ingested tocotrienol supplements. The subjects consumed 125 mg gamma-tocotrienyl acetate the first week, then the next week 500 mg; then 125 mg alpha-tocotrienyl acetate was administered the third week, followed by 500 mg the fourth week. Urinary alpha- and gamma-CEHC were measured by high-performance liquid chromatography with electrochemical detection. Urinary gamma-CEHC levels rose about four- to six-fold in response to the two doses of gamma-tocotrienol and then returned to baseline the following day. Significant ( $P < 0.0001$ ) increases in urinary alpha-CEHC were observed only following ingestion of 500 mg alpha-tocotrienyl acetate. Typically, 1-2% of alpha-tocotrienyl acetates or 4-6% of gamma-tocotrienyl acetates were recovered as their respective urinary CEHC metabolites. A gamma-CEHC excretion time course showed an increase in urinary gamma-CEHC at 6 h and a peak at 9 h following ingestion of 125 mg gamma-tocotrienyl acetate. In summary, tocotrienols, like tocopherols, are metabolised to CEHC; however, the quantities excreted in human urine are small in relation to dose size.

Faecal excretion is the main route of excretion of oral tocotrienols. Faecal excretion products include non-absorbed tocotrienols and tocotrienols that may be excreted by the biliary route (PDR, 2005).

Altogether the bioavailability studies indicate that tocotrienols are bioavailable, with shorter plasma half-lives and probably different tissue distribution than alpha-tocopherol.

### **3.2. Toxicological data**

Several authorities have carried out assessments of the available toxicity data for vitamin E and defined tolerable upper levels (EVM, 2003; SCF, 2003; EVM, 2002; IOM, 2000).

This document will focus on specific safety information in animals and man on mixtures of the natural tocopherol homologues: d-alpha-tocopherol, d-beta-tocopherol, d-gamma-tocopherol and d-delta-tocopherol (mixed tocopherols) and the tocotrienols, alpha-tocotrienol, beta-tocotrienol, gamma-tocotrienol and delta-tocotrienol.

### ***Acute toxicity***

Palm oil extract (containing 80% tocotrienols) administered to young rats and mice at a dose of up to 25000 mg/kg bw in acute toxicity studies showed no appreciable adverse effects in animals with respect to physical manifestations or behavioural changes (Oo *et al.*, 1992).

In an oral acute toxicity study on the tocotrienol-rich fraction from rice bran oil, representing the tocotrienol tocopherol preparation, carried out according to OECD guidelines, exposure of rats up to 5000 mg/kg tocotrienol tocopherol by gavage resulted in no deaths at the highest dosage and no abnormal clinical observations during the 14 day observation period (Shepard, 1996).

### ***Sub-acute and subchronic toxicity***

Palm oil extract (containing 80% tocotrienols) administered to young rats and mice at a dose of up to 25000 mg/kg bw in a subchronic toxicity study (30 days daily dosing) showed no appreciable adverse effects in animals with respect to physical manifestations or behavioural changes (Oo *et al.*, 1992).

A tocotrienol concentrate from palm oil (70% tocotrienols) was evaluated in a 13 week oral toxicity study in Fisher 344 rats of both sexes at dose levels of 0 (control), 1.9, 7.5 and 30 g/kg powdered diet (Nakamura *et al.* 2001). These doses amounted to 0, 119, 474, 2130 mg tocotrienol concentrate /kg bw/day for male rats and 0, 130, 491 and 2047 mg tocotrienol concentrate /kg bw/day for female rats. There was a very small decrease in body weight gain in the male rats fed the highest dose. On haematological examination, significant decrease in mean corpuscular (MCV) was observed in all treated animals. Platelets were significantly reduced in the males of the two highest dose groups. Haemoglobin concentration, MCV, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were significantly decreased in females of the two highest dose groups and haematocrit was reduced in females of the highest dose group.

Serum biochemical examinations revealed an increase in the albumin/globulin ratio and alkaline phosphatase in all treated males. Elevated alanine aminotransferase was observed in both sexes at the highest dose group, and increases in aspartate aminotransferase and gamma-glutamyltransferase in females of the highest dose group.

With regard to relative organ weights, liver weights in both sexes at the highest dose group and adrenal weight in all treated males were increased, and ovary and uterus weights in the highest dose females were reduced. Histopathology revealed slight hepatocellular hypertrophy in males of the two highest dose groups and reduction of cytoplasmic vacuolation in the adrenal critical region in males of the highest dose group.

Other haematological and histopathological examinations in addition to the physical observation and tissue weights were unremarkable, except for the reduction in serum total cholesterol levels that is consistent with the reported inhibition of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase activity by tocotrienols (Qureschi *et al.*, 1986; Parker *et al.*, 1993). A reduction in vacuolation observed histologically in the adrenal cortical region of the male rats was attributed to the hypocholesterolemic effect of the tocotrienols resulting in a reduction in cholesterol and other steroid precursors.

The authors concluded that because of pathological changes in male liver and haematological changes in females, the NOAEL was 1.9 g/kg in the diet amounting to intakes of 120 mg/kg

bw/day for male rats and 130 mg/kg bw/day for female rats. The authors also concluded that a decrease in MCV, an increase in albumin/globulin ratio, elevation of alkaline phosphatase and increase in adrenal weight were not considered adverse.

No additional data on subchronic toxicity testing of the mixed tocopherols, the tocopherol tocotrienol or the tocotrienol preparations were provided by the petitioners.

### ***Reproductive and developmental toxicity***

There have been no standard reproductive toxicology studies carried out with mixed tocopherols, the tocopherol tocotrienol or the tocotrienol preparations considered in the present opinion.

Raghuram and Rukmini (1995) have documented normal reproductive performance and lack of teratogenicity of rice bran oil in albino rats of NIN Wistar strain (15 males and 15 females in a three generation study). A diet containing 10% rice bran oil, as compared to peanut oil containing relatively lower amounts of tocotrienols, did not result in differences in conception, birth weights, litter size, weaning weights and pre-weaning mortality, and had no teratogenic effect. There were no differences in body weight, feed efficiency ratio and nitrogen, phosphorous and calcium balances in the animals over three generations.

### ***Genotoxicity***

There have been no standard toxicology studies carried out to evaluate genotoxicity with the mixed tocopherols, or the tocotrienol preparations considered in the present opinion.

The petitioner provided a study on the mutagenicity of rice bran oil concentrate representing the tocopherol tocotrienol preparation of the present opinion using the Ames test with *Salmonella typhimurium* strain TA98 and TA100 with and without metabolic activation. The tocopherol tocotrienol sample did not cause any increase in mutations compared to vehicle controls at concentrations up to 5000 microgram per plate.

A dimethylsulfoxid (DMSO)-based extract of rice bran oil was reported to reveal no mutagenic activity in an Ames test using *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation (Polasa and Rukmini, 1987).

The mutagenic potential of repeatedly heated refined and crude palm oils has been examined using the Ames test with bacterial strains TA100 and TA98 with and without metabolic activation (Manorama *et al.*, 1989). The unheated and heated samples of crude and refined palm oil did not show mutagenic activity in these tests.

### ***Long term toxicity***

Animals appear to tolerate high levels of vitamin E (*i.e.* at least two orders of magnitude above nutritional requirements, e.g. 1000 – 2000 IU/kg diet) without adverse effects. At very high doses, however, vitamin E can produce signs indicative of antagonism with the function of the other fat-soluble vitamins. Thus, animals with hypervitaminosis E have been found to show impaired bone mineralisation, reduced hepatic storage of vitamin A and coagulopathies. In each case, these signs could be corrected with increased dietary supplements of the appropriate vitamin (*i.e.* vitamins D, A and K, respectively) (EVM, 2002).

The SCF reported two long-term rat studies of up to 16 months and 2 years duration (SCF, 2003; Yang and Desai, 1977, Wheldon *et al.*, 1983).

In one of these studies (Wheldon *et al.*, 1983) the rats received doses of 0, 500, 1000 or 2000 mg dl-alpha-tocopheryl acetate/kg bw/day for 2 years. At all dose levels between 15 and 18 weeks the male animals developed spontaneous haemorrhages in the gut, urinary tract, meninges, orbit and at sites of minor injury. This led to some mortality but in survivors the condition was corrected by administration of 10 mg vitamin K<sub>3</sub>/kg bw. The only other treatment-related effect of significance was the presence of vacuolated lipid staining macrophages in the liver.

The effects of long-term administration of tocotrienol on hepatocarcinogenesis in rats induced by diethylnitrosamine (DEN) and 2-acetylaminofluorene (AAF) were investigated. Twenty-eight male 7- to 8-week-old *Rattus norvegicus* rats, weighing 120-160 g, were used in this study. The rats were divided into four treatment groups: a control group on a basal diet, a group fed a basal diet supplemented with tocotrienol (30 mg/kg food), a group treated with DEN/AAF, and a group treated with DEN/AAF and fed a diet supplemented with tocotrienol (30 mg/kg food). The rats were killed after 9 months, and the livers were examined morphologically. Grayish white nodules (2 per liver) were found in all the DEN/AAF-treated rats (n = 10), of the rats treated with DEN/AAF and supplemented with tocotrienol (n = 6) only one had liver nodules. Tocotrienol supplementation attenuated the impact of the carcinogens in the rats (Rahmat *et al.*, 1993).

### ***Human studies***

The EVM (EVM, 2002) presents a thorough review of case reports and studies reviewing human toxicity of vitamin E. The SCF risk assessment of vitamin E (SCF, 2003) further reviews human and animal safety. Included in these reviews is a major study evaluating natural vitamin E. In this Heart Outcomes Prevention Evaluation (HOPE) study (Yusuf *et al.*, 2000), a total of 9,541 subjects (6,996 men and 2,545 women) aged 55 or over at high risk for cardiovascular events were enrolled in a trial with a 2x2 factorial design. The participants received either 400 IU vitamin E (from natural sources) or placebo and either ramipril or matching placebo for a mean of 4.5 years. The primary endpoint was a combination of myocardial infarction and stroke and death from cardiovascular causes. The secondary outcomes included unstable angina, congestive heart failure, revascularisation or amputation, death from any cause, complications of diabetes and cancer. There were no significant differences in the numbers of deaths from cardiovascular causes (RR 1.05; 95%CI 0.9-1.22) between those receiving vitamin E or placebo or in any of the secondary outcomes.

A number of clinical trials have been conducted with vitamin E and few side effects have been reported. However, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study of Finnish smokers,  $\alpha$ -tocopherol treatment was associated with an increase in deaths from haemorrhagic stroke (ATBC, 1994). In the Cambridge Heart Anti-Oxidant trial (CHAOS), a non-significant excess of cardiovascular deaths was found in the treatment groups (Stephens *et al.*, 1996). The significance and explanation for the latter findings is uncertain. Other large scale epidemiological studies in female nurses and male health professional have not found similar associations (EVM, 2002).

### ***Tocotrienols***

The petitioner for the tocotrienol tocopherol preparation indicates that palm oil is a rich source of tocotrienol that has been a traditional staple food in several Asian countries and also in some African countries, and that rice bran oil, also a rich source of tocotrienols, has a long history of use in Asian culture.

The petitioner also indicates that data from numerous clinical studies are available where chronic administration of pure tocotrienols or tocotrienols derived from tocotrienol-rich fractions of palm oil and rice bran oil were tested to evaluate specific therapeutic effects. Although these studies were not designed to study the adverse effects of tocotrienols they may add to the safety evaluation of tocotrienols in humans.

In a double blind-crossover study, the tocotrienol-rich fraction of palm oil was tested in 25 hypocholesterolemic subjects at a dose of 200 mg per day over a 4-6 week period (Qureshi *et al.*, 1991). Significant reductions in serum total cholesterol, LDL cholesterol, apolipoprotein B, thromboxane B2 and platelet factor were observed. No adverse effects were reported. A follow-up study was conducted with 36 hypercholesterolemic subjects who were maintained on the American Heart Association (AHA) step 1 diet and received either a tocotrienol-rich fraction from palm oil (equivalent to 220 mg/day of tocotrienols) or 200 mg/day of pure gamma tocotrienol over a 4 week period (Komiyama *et al.*, 1989). While significant reductions in serum total cholesterol, apolipoprotein B and *ex vivo* generation of thromboxane B2 were observed with both preparations, no adverse side effects due to tocotrienol administration were reported.

A long-term, randomized, placebo-controlled study is available where 50 subjects with carotid atherosclerosis received 240 mg/day tocotrienols from palm oil or placebo in addition to standard medical therapy over 18 months (Tomeo *et al.*, 1995). Serum measures of oxidative stress (TBARS) showed a significant decrease with no appreciable change in serum lipids or other biochemical markers. Carotid atherosclerotic regression was seen in a significant number of patients receiving the tocotrienols and no adverse effects were reported.

In an additional study 50 patients with hypercholesterolemia and carotid stenosis were randomized to receive 312 mg/day tocotrienols from a tocotrienol-rich rice bran oil (the tocopherol tocotrienol preparation of the present opinion) or a placebo for one year, in addition to conventional medical therapy (Watkins *et al.* 1999). Significant reductions were observed in serum total cholesterol, LDL cholesterol, and measures of oxidative stress. Serum HDL increased in the tocotrienol treated group. No side effects were reported.

Qureshi *et al.* (1995) reported reduction of cholesterol levels but no adverse effects in 39 hypercholesterolemic subjects dosed with 220 mg/day of tocotrienols plus 40 mg alpha-tocopherol for 4 and 8 weeks.

In another study Qureshi *et al.* (1997) performed a randomized double blind placebo-controlled trial in which 41 hypercholesterolemic subjects were first placed on the NCEP step 1 diet for 4 weeks and then given 200 mg/day of tocotrienols from rice bran oil or corn oil as placebo. Significant reductions were seen in serum lipid profile, apolipoprotein B, Lp(a), platelet factor 4 and thromboxane B2 in the tocotrienol group. No adverse effects due to tocotrienol ingestion were observed.

Qureshi *et al.* (2002) also performed a study to investigate the correlation between the dose of tocotrienol and the reduction in serum cholesterol. Eighteen subjects were maintained on AHA step 1 diet and then received a tocotrienol-rich fraction of rice bran oil in a dose ranging from 25 to 2000 mg/day for 35 days. The best serum cholesterol response was obtained at a daily dose of 100 mg of tocotrienols, and no adverse effects due to tocotrienol supplementation were observed.

In another placebo controlled study tocotrienol esters in the form of alpha-, gamma- or delta-tocotrienyl acetates were administered individually to normal subjects at a daily dose of 250 mg for 8 weeks (O'Byrne *et al.* 2000). No adverse effects of tocotrienol ester ingestion were observed in this study.

In recent years, human studies have examined the biological and health effects of tocotrienols for their cholesterol lowering effect (Qureshi *et al.*, 1995; O'Byrne *et al.*, 2000), antioxidant properties (Tomeo *et al.*, 1995) and anti-aggregation of blood platelets (Mensink *et al.*, 1999).

Mensink *et al.* (1999) reported mildly elevated serum lipid concentrations in 20 subjects dosed with 140 mg/day tocotrienols for 6 weeks. No adverse effects were reported.

In a study reported by Yap *et al.* (2001) healthy volunteers were administered a single oral dose of mixed tocotrienols (300 mg) under fed or fasted conditions to study their bioavailability. No adverse effects were reported.

### ***Other studies***

In a study to investigate whether haemorrhage is induced by overdoses of tocopherols and other antioxidants male Jcl:SD rats (six rats/group) were fed d-alpha-, d-beta-, d-gamma- or d-delta-tocopherol at a level of 5 g/kg in the diet for 7 days. The results suggested that the four naturally occurring tocopherols have a tendency to cause haemorrhage in the order of alpha > beta > gamma > delta (Takahashi, 1995).

In recent years, studies in animals have examined the biological and health effects of tocotrienols for their cholesterol lowering effect (Qureshi *et al.*, 1991, 1995), blood pressure lowering effect (Koba *et al.*, 1992), glycaemic control (Nazaimoon and Khalid, 2002), anticancer and tumour suppressive activities (Goh *et al.*, 1994; Nesaretnam *et al.*, 1998) and antioxidant properties (Serbinova *et al.*, 1992).

## **4. Discussion**

For the three tocopherol/tocotrienol preparations to which the present opinion refers specifications were provided by the petitioners. The level of total tocopherols specified in the European Directive 96/77/EC applies to the mixed tocopherols of the present application but not to the tocotrienol tocopherol and the tocotrienol preparations. These preparations contain 15% and 39% of tocotrienols and only 18% and 11% tocopherols, respectively.

Studies indicate that tocopherols and tocotrienols are bioavailable, with tocotrienols having shorter plasma half-lives and probably different tissue distribution than alpha-tocopherol, the major constituent of natural vitamin E.

Since the bioavailability and tissue distribution of tocotrienols appears to be different from that of tocopherols, and since the specifications of the two tocotrienol preparations of the present opinion do not match the specifications for E306, the only registered vitamin E additive which has specification including tocotrienols, safety assessment of these tocotrienol-containing preparations cannot be based on upper limits for vitamin E.

From subchronic toxicity studies in rats with tocotrienol rich palm oil extract (70 % tocotrienols) the Panel concluded that a NOAEL of 120 mg tocotrienol extract/kg bw/day for male rats and 130 mg tocotrienol extract/kg bw/day for female rats can be derived. The effects observed at this dose level were not considered to be adverse.

The available genotoxicity data are limited, however they do not give rise to safety concern. In bacterial tests the tocotrienol rich fractions were not genotoxic. Long term studies on vitamin E, containing tocotrienol, and a limited 9- month study on tocotrienol itself gave no indications of enhanced induction of neoplastic lesions.

Furthermore studies in which human subjects were exposed to dose levels of 200 to 300 mg/day (amounting to 3.3 to 5.0 mg tocotrienols rich extracts/kg bw/day for a 60 kg person) for periods from 2 weeks to up to 18 months did not report adverse effects. Although these studies were not designed to evaluate the safety of tocotrienol-rich extracts they do offer supporting evidence that doses up to 5.0 mg/kg bw/day are not of safety concern.

#### ***Intake of mixed tocopherols***

Intake of mixed tocopherols from supplement use will be in accordance with the tolerable upper intake level of vitamin E (as d-alpha-tocopherol) set by the SCF for adults of 300 mg/day (SCF, 2003).

#### ***Intake of tocotrienol tocopherol***

The proposed uses and use levels of tocotrienol tocopherol for supplement intake are based on the recommended daily allowance for alpha-tocopherol. In Europe the recommended daily allowance (RDA) is set to 10 mg vitamin E in the European Council Directive 90/496/EEC (1990). Since the tocotrienol tocopherol preparation contains 11.5 mg alpha-tocopherol and 15.5 mg tocotrienols per 100 mg these values from the European Directive would amount to a daily intake of 87 mg of the tocotrienol tocopherol preparation containing 13.5 mg tocotrienols amounting to an intake of 0.23 mg tocotrienols/kg bw/day for a 60 kg person. This would be at least 500 times lower than the NOAEL for the tocotrienols from the rat study.

Given the specifications of the tocopherol tocotrienol preparation, a daily intake of 87 mg of tocotrienols plus tocopherols would amount to 10 mg alpha-tocopherol plus 0.43 mg beta-tocopherol (amounting to 0.22 mg alpha-tocopherol equivalents) plus 3.9 mg gamma-tocopherol (0.98 mg alpha-tocopherol equivalents) plus 1.04 mg delta-tocopherol (0.10 mg alpha-tocopherol equivalents). Together this would amount to 11.3 mg alpha-tocopherol equivalents and would be significantly below the UL of 300 mg alpha-tocopherol equivalents established by the SCF (SCF, 2003). For a 60 kg person this intake would amount to 0.19 mg alpha-tocopherol equivalents/kg bw/day

### ***Intake of tocotrienols***

The petitioner indicates that tocotrienols are normally incorporated in softgel capsules providing up to 1000 mg of tocotrienol per daily dose. This would result in a daily intake of 16.7 mg tocotrienols/kg bw/day for a 60 kg person and would be only 7 times below the NOAEL of the rat study and higher than the 5 mg/kg bw/day demonstrated to be without adverse effects in human studies.

### **CONCLUSIONS**

The present opinion deals only with the safety and bioavailability of mixed tocopherols, tocotrienol tocopherol and tocotrienols as three particular sources of vitamin E intended for the general population, to be used in food supplements. The safety of vitamin E itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

Studies indicate that mixed tocopherols and tocotrienols are bioavailable, although tocotrienols have with shorter plasma half-lives and probably different tissue distribution than alpha-tocopherol.

The Panel concludes that the use of mixed tocopherols and tocotrienol tocopherol as sources of vitamin E in food supplements at the proposed levels of use is not of safety concern.

However, the available safety data are insufficient to conclude on the safety of the proposed use and use levels of the tocotrienols (the preparation containing mainly tocotrienols).

### **DOCUMENTATION PROVIDED TO EFSA**

1. Dossier on Mixed Tocopherols Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. May 2005. Submitted by Health Food Manufacturers Association UK.
2. Dossier on the submission to the European commission for the safety evaluation of Tocotrienols by the European Food Safety Authority (EFSA). June 2005. Submitted by Eastman Chemical Company Kingsport Tennessee USA.
3. Dossier on Tocotrienols Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. June, 2005. Submitted by Health Food Manufacturers Association UK.

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**GLOSSARY / ABBREVIATIONS**

ADI	Acceptable Daily Intake
AFC	Scientific Panel on food additives, flavourings, processing aids and materials in contact with food.
bw	Body weight
CAS	Chemical Abstract Service
CoA	Coenzyme A
CEHC	Carboxyethylhydroxychromans
EVM	Expert Group on Vitamins and Minerals
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FNB	Food and Nutrition Board of the Institute of Medicine
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
FCC	Food Chemicals Codex
GRAS	Generally Recognized As Safe
GC	Gas chromatography
HDL	High Density Lipoprotein
HMG	3-hydroxy-3-methyl-glutaryl
HPLC	High Performance Liquid Chromatography
IU	International Units
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDL	Low Density Lipoprotein
NOAEL	No-Observed-Adverse-Effect Level
PUFA	Polyunsaturated fatty acids
SCF	Scientific Committee for Food
TBARS	thiobarbituric acid reactive substances
UL	Upper Levels
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation