Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to D-\(\alpha\)-tocopheryl acid succinate as a source of vitamin E in foods intended for the general population, food supplements and foods for particular nutritional uses

Question number EFSA-Q-2003-074

 Adopted on 26 April 2005

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to advice on the safety and bioavailability of D-\(\alpha\)-tocopheryl acid succinate (TAS) as a source of vitamin E in foods for particular nutritional purposes (PARNUTS), foods intended for the general population and food supplements.

TAS has already been evaluated by the former Scientific Committee on Food (SCF) which evaluated a number of substances, including TAS, intended to be used in the manufacture of foods for particular nutritional purposes (PARNUTS) in May 1999. The SCF considered TAS temporarily acceptable, pending submission of additional information to clarify the extent of hydrolysis of TAS in the gut and, consequently, whether any unhydrolysed TAS is available for absorption. The SCF agreed to extend its temporary acceptance of TAS in September 2000 and in April 2003, respectively. Since then additional data on subchronic toxicity which covered both the bioavailability issue and possible effects of TAS, should any be absorbed intact, have become available.

The Panel evaluated a new 90-day oral toxicity study on TAS in rats. The Panel considered that the NOAEL was 265 mg TAS/kg body weight/day based on increases of alanine aminotransferase and aspartate aminotransferase at a dose of 1123 mg TAS/kg body weight/day. In order to estimate the margin between the NOAEL and a high intake of TAS by humans the following assumptions were made. A rough estimate of the high-level vitamin E intake from supplements by adults consuming more than one supplement per day is 336 mg/day (equivalent to 5.6 mg/kg bw body weight/day). Thus, assuming that all vitamin E supplements taken contain TAS and that the bioavailability in humans is roughly the same as in rats the margin of safety for TAS in adults is 47.3. Since the effects observed at the higher dose level were limited to changes in enzyme markers without accompanying histopathological alterations this margin of safety is considered acceptable. If the intake per kg body weight by children was the same as by adults the margins of safety would be of a similar magnitude.
The use of TAS as a human medicine and studies in humans indicate that tocopherol (vitamin E) is bioavailable from orally ingested TAS. However, the extent of hydrolysis is not clear. Based on the data reviewed the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded TAS, as a source of tocopherol (vitamin E) in foods for particular nutritional purposes (PARNUTS), foods intended for the general population and food supplements is not of concern from the safety point of view.

**KEYWORDS**

D-α-tocopheryl acid succinate (TAS), Vitamin E succinate, D-alpha-Tocopherol hemisuccinate, CAS Registry number: 4345-03-3, other CAS numbers: 120246-47-1, 53532-12-0, 55134-51-5, foods for particular nutritional purposes (PARNUTS)
BACKGROUND

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients. D-α-tocopheryl acid succinate is included as a source of vitamin E in the following European legislative measures:

- Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses\(^1\),

In addition, the Commission proposal for a regulation on the addition of vitamin and minerals and certain other substances to foods lists D-α-tocopheryl acid succinate\(^3\).

On 4\(^{\text{th}}\) April 2003, the SCF made a statement on D-α-tocopheryl acid succinate (TAS) noting that the petitioner had offered to conduct an in vivo study to address both the bioavailability issue and the possible effects of TAS, should any be absorbed intact. The SCF considered that the results of the study should be submitted to the European Commission/EFSA.

TERMS OF REFERENCE

In accordance with Article 29 (1)(a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to provide, based on its consideration of the new evidence submitted by the petitioner, a scientific opinion on the safety and the bioavailability of the substance D-α-tocopheryl acid succinate (TAS) when used as a source of vitamin E in foods intended for the general population, food supplements and foods for particular nutritional uses.

ASSESSMENT

Introduction

D-α-tocopherol, DL-α-tocopherol and their acetates have been accepted by the former Scientific Committee on Food (SCF) as nutrient substances in foods for infants and young children and in Foods for Special Medical Purposes (FSMP) (Commission of the European Communities 1989a, 1991, 1997a, 1997b). The SCF also accepted the use of tocopherols as antioxidants for foods in general (Commission of the European Communities 1989b) and in nutrient preparations for use in infant formulae, follow-on formulae and weaning foods (Commission of the European Communities 1997c), but considered it was not appropriate to establish an Acceptable Daily Intake (ADI). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated DL-α-tocopherol and D-α-tocopherol concentrate a group ADI of 0.15 – 2 mg/kg bw. (WHO, 1987a). The SCF evaluated a number of additives, including the succinate anion, not covered before by Community provisions in May 1990 (Commission of the European Communities, 1991). The SCF noted that the succinate anion occurs in

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\(^1\) OJ No L 52, 22.2.2001, p. 19

\(^2\) OJ L 183, 12.7.2002, p. 51

\(^3\) COM (2003)671 final
nature, plays a role as an intermediate metabolite in the citric acid cycle, and also participates in glucose and fatty acid synthesis. At that time no systematic toxicological studies were available. However, in view of its role as an intermediate metabolite the SCF established a group ADI not specified for succinate and succinic acid. In May 1999, the SCF evaluated a number of substances, including D-α-tocopheryl acid succinate (TAS), intended to be used in the manufacture of foods for particular nutritional purposes (PARNUTS) (SCF 1999). In the case of TAS, the SCF considered it temporarily acceptable, pending submission of additional information within one year of publication of the opinion to clarify the extent of hydrolysis of TAS in the gut and, consequently, whether any unhydrolysed TAS is available for absorption.

Further information, including published papers, was submitted by the petitioner within the deadline but it was not sufficient to clarify the extent of hydrolysis of TAS. At its 122nd Plenary meeting (on 6-7 September 2000), the SCF therefore requested further clarification on this issue from the petitioner. The SCF agreed to extend its temporary acceptance of TAS for a further two years, in the light of the long history of use of TAS as a human medicine and studies in humans showing that tocopherol (vitamin E) is bioavailable when TAS is ingested (though not the extent of bioavailability).

A further submission by the petitioner was received in 2001 informing the SCF that an in vitro hydrolysis study on TAS, using simulated gastric fluids, had been conducted but that the results were inconclusive, in accordance with some earlier published work. Following consideration of this and other published information about possible cellular effects of TAS at the biochemical level by the Additives Working Group of the SCF in 2002, the petitioner offered to conduct an in vivo study to address both the bioavailability issue and the possible effects of TAS, should any be absorbed intact.

In view of this, in a Statement on TAS expressed on 4 April 2003 (SCF 2003b), the SCF recommended extension of the temporary acceptance of TAS for a further 2 years, with the provision that the results of the proposed study are submitted within one year to the European Commission/EFSA for evaluation.

**Chemistry**

<table>
<thead>
<tr>
<th>Name of the substance:</th>
<th>D-α-tocopheryl acid succinate*</th>
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<tbody>
<tr>
<td>CAS number:</td>
<td>4345-03-3</td>
</tr>
<tr>
<td>Other CAS numbers:</td>
<td>120246-47-1, 53532-12-0, 55134-51-5</td>
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<tr>
<td>Synonyms (examples):</td>
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</tr>
<tr>
<td></td>
<td>RRR-alpha-tocopheryl succinate</td>
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<td></td>
<td>D-alpha-Tocopherol acid succinate</td>
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<tr>
<td></td>
<td>D-alpha-Tocopherol hemisuccinate</td>
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<tr>
<td></td>
<td>Vitamin E succinate</td>
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<tr>
<td></td>
<td>Vitamin E hemisuccinate</td>
</tr>
<tr>
<td>Molecular weight:</td>
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</table>

* trivial name used in current legislation
In terms of vitamin E activity, 1 mg of TAS is reported to be equivalent to 1.21 International Units (IU) (FNB, 1981) or 0.81 mg D-α-tocopherol equivalent (TE) (USP, 1995).

Structure:

D-α-tocopheryl acid succinate is the monoester of succinic acid with D-α-tocopherol. It is a solid form of tocopherol and has a technological importance as it does not require addition of a carrier. Other forms of vitamin E are liquid and require a carrier if used in solid foods.

Exposure

TAS is widely used in food supplement tablets, dietetic and fortified food and as a human medicine. Whilst there are data on dietary intake of vitamin E (SCF, 2003a), no data are available specifically on the intake of TAS.

The intake of vitamin E (as well as other vitamins and minerals) resulting from the consumption of food supplements and other food was investigated by Beitz et al. (2004). Information about supplement use in Germany (from supplements and other foods), in particular of persons who use several supplements, was examined. As part of the representative German National Health Interview and Examination Study 1998, in the Nutrition Survey 4030 persons, aged 18 – 79 years, were asked about their dietary habits, including vitamin and mineral supplement use. About 43 % of the population reported using supplements at least once in the observation period of 12 month. Data on vitamin E intake from supplements and other foods are summarised in Table 1 and 2.

<table>
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<th>Mean</th>
<th>Standard Deviation</th>
<th>90th Percentile</th>
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<td>Intake from supplements</td>
<td>86.6</td>
<td>149.8</td>
<td>268.0</td>
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<tr>
<td>Intake from other foods</td>
<td>12.2</td>
<td>5.0</td>
<td>18.9</td>
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<tr>
<td>Total intake</td>
<td>98.8</td>
<td>149.3</td>
<td>277.6</td>
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Table 1: Intake of vitamin E (mg/day) by persons (n = 240) consuming one supplement per day (according to Beitz et al., 2004)
Tocopheryl acid succinate

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>90&lt;sup&gt;th&lt;/sup&gt; Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake from supplements</td>
<td>153.8</td>
<td>111.7</td>
<td>336.0</td>
</tr>
<tr>
<td>Intake from other foods</td>
<td>10.4</td>
<td>3.5</td>
<td>15.0</td>
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<tr>
<td>Total intake</td>
<td>164.3</td>
<td>111.5</td>
<td>346.0</td>
</tr>
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</table>

Table 2: Intake of vitamin E (mg/day) by persons (n = 23) consuming more than one supplement per day (according to Beitz <i>et al.</i>, 2004)

The intake at the 90<sup>th</sup> percentile is in the same range as the currently established tolerable upper intake level of vitamin E for adults (300 mg/day (SCF 2003a)). There is a large uncertainty inherent in the calculation of high percentiles in small population samples. However the 90<sup>th</sup> percentile reported in the 23 persons consuming more than one supplement per day provides a rough estimate of potential intake of vitamin E in high consumers of supplements.

**Toxicological studies**

**Kinetic studies**

The capacity of pancreatic carboxylester hydrolase to hydrolyse TAS <i>in vitro</i> has been shown to be much lower than the capacity to hydrolyse α-tocopheryl acetate. The capacity of porcine pancreatic carboxylester hydrolase to hydrolyse TAS in pancreatic juice <i>in vitro</i> was only 1.5 % of the corresponding capacity to hydrolyse α-tocopheryl acetate (Jensen <i>et al.</i> 1999). This was confirmed by a study of Lauridsen <i>et al.</i> (2001).

Since TAS was shown by Nakamura <i>et al.</i> (1975) to be hydrolysed rather slowly in rat liver homogenate <i>in vitro</i> and only 31 % radioactivity was found in the lymph when tritium-labelled TAS was administered orally to rats while a potential absorption via the portal vein was not investigated, systemic availability of TAS cannot be excluded from this study.

In a feeding study with chickens, the animals received diets with different concentrations of TAS or α-tocopheryl acetate (ranging from 50 to 200 mg TAS or α-tocopheryl acetate per kg feed) for four weeks (Jensen et al. 1999). At all dietary levels, the apparent absorption coefficient (Vitamin E<sub>consumed</sub> - Vitamin E<sub>excreted</sub> / Vitamin E<sub>consumed</sub> x 100) for TAS was significantly lower than that of α-tocopheryl acetate (the mean apparent absorption coefficient for TAS was 58.0 ± 5.4 compared with 70.8 ± 5.6 for α-tocopheryl acetate). This was accompanied by significant differences in α-tocopherol concentrations in plasma, breast muscle, liver and adipose tissue of the chickens. Based on plasma and tissue responses, the succinate ester was utilized 69 – 76 % as efficiently as the corresponding acetate ester. The concentration of TAS in plasma and tissue was not measured.
The oral administration of a sesame oil solution of D-alpha-tocopheryl-5-methyl-\(^{14}\)C-succinate to rabbits led to a rapid elimination of radioactivity in the faeces (Simon et al. 1956). Over 74 % of the administered dose appeared in the faeces within 3 days after administration. More than 93 % of the faecal excretion product was shown by the isotope dilution technique to be either alpha-tocopherol or alpha-tocopheryl succinate. Only traces of radioactivity were found in the urine. Based on a comparison of the rate of faecal excretion, the level of urinary radioactivity, and the nature of the faecal excretion products with corresponding results obtained on intravenous administration, the authors assumed that the amount of alpha-tocopheryl succinate absorbed from the gastrointestinal tract did not exceed 10 % of the oral dose. However, this assumption was not verified by direct measurement.

In a study of Exon et al. (2004) primarily designed to examine the effects of TAS on colon cancer in rats, the levels of \(\alpha\)-tocopherol and TAS in serum, liver, brain, heart, kidney, lung, and colon were measured. Female Sprague-Dawley rats of either 2 or 20 months of age were fed a diet supplemented with or without TAS for 49 days. The TAS content of the feed was adjusted for age-related food consumption differences between the old and the young animals: 1 g TAS/kg diet (for young rats) or 2 g TAS/kg diet (for old rats) (equivalent to 1380 or 2760 IU/kg diet as reported by the authors; or equivalent to 1210 or 2420 IU/kg diet according to FNB, 1981). The control diet contained 49 IU/kg of vitamin E as \(\alpha\)-tocopheryl acetate. The mean daily TAS intake of rats treated with TAS was calculated to be nearly identical in young and old animals (0.08 ± 0.00 and 0.07 ± 0.00 g TAS/kg body weight/day, respectively). The levels of \(\alpha\)-tocopherol and TAS in tissues were measured using HPLC with fluorescence detection (\(\alpha\)-tocopherol) or mass spectrometric detection (TAS). All rats within each age group treated with TAS had significantly greater levels of \(\alpha\)-tocopherol in all tissues than the rats not treated with TAS. Old rats treated with TAS retained significantly higher levels of \(\alpha\)-tocopherol in all tissues compared with the young rats treated with TAS. The greatest \(\alpha\)-tocopherol level (728.6 ± 101.7 nmol/g tissue) was found in the liver of old rats treated with TAS, followed by lung, heart, colon, serum, kidney, and brain (54.5 ± 3.1 nmol/g tissue). For comparison, the \(\alpha\)-tocopherol levels in the liver and brain of old rats not treated with TAS (controls) were 80.8 ± 6.8 and 32.9 ± 1.5 nmol/g tissue, respectively. Levels of TAS were found in all tissues except the brain of old and young rats that were treated with TAS. The greatest TAS level (5.2 ± 0.5 nmol/g tissue) was found in the liver of old rats treated with TAS, followed by colon, lung, serum, heart, and kidney (1.4 ± 0.2 nmol/g kidney) while no TAS was found in tissues of rats not treated with TAS (controls). Thus, this study demonstrates that a certain low but measurable amount of TAS can be absorbed as unhydrolysed ester which is then retained as such in rat tissues.

When TAS was administered parenterally to rats or mice, the unhydrolysed ester was found in several organs and subcellular fractions (Tirmenstein et al. 1997, Trevthick and Mitton 1999, Weber et al. 2002). When 50 microL of 200 mmol/L TAS was injected i.p. in mice for 10 days, TAS was rapidly absorbed and partially hydrolysed to \(\alpha\)-tocopherol (Weber et al. 2002). The plasma TAS level reached 47.2 ± 6.7 micromol/L on day 1 and then declined. Concomitantly, the plasma \(\alpha\)-tocopherol level increased from 4.8 ± 2.1 to 8.3 ± 2.9 micromol/L on day 7. Furthermore, the tissue concentration of TAS in kidney and liver, after administration of TAS for 10 days, was about 2 times higher than that of \(\alpha\)-tocopherol after \(\alpha\)-tocopherol administration.
In a human study of Horwitt et al. (1984) serum concentrations of α-tocopherol were measured at 0, 8, 24 and 48 hours after a single ingestion of 800 IU of one out of five vitamin E preparations including D-α-tocopherol, D-α-tocopheryl acetate, and D-α-tocopheryl acid succinate (800 IU of TAS are equivalent to 660 mg TAS). The greatest percentage increases above basal concentrations of serum α-tocopherol were observed at 24 hours after ingestion of different vitamin E forms. The mean percentage increase in concentration of α-tocopherol in 24 h after ingestion of α-tocopherol, α-tocopheryl acetate, or α-tocopheryl acid succinate was 71.2, 60.9, and 41.2 %, respectively. However, large variations between subjects were observed. It was mentioned by the authors that "Intestinal hydrolysis of tocopherol esters is very efficient and no esters appear in the blood 8h after ingestion". However, since this was pointed out in the section Materials and methods and not detailed in the Results, it is not clear if this is actually a result of measurement or only an assumption. Furthermore, even if no ester was detected in the blood 8h after ingestion, some absorption during the first hours after ingestion cannot be excluded.

In a study of Cheeseman et al. (1995) the bioavailability of D-α-tocopherol from the oral administration of D-α-tocopherol itself and its acetate and succinate esters was determined in human subjects using deuterium-labelled tocopherols. Venous blood samples were withdrawn periodically over a 51-h period following oral administration of a gelatine capsule containing 100 mg of an equimolar mixture of D-α-tocopherol and D-α-tocopheryl acetate or, in a second trial, an equimolar mixture of D-α-tocopheryl acetate and D-α-tocopheryl succinate. The tocopherol forms were differently labelled, respectively. Overall, large inter-individual differences in absorption were noted. D-α-tocopherol was absorbed at similar rates from both the free phenol and acetate ester. In the second trial, there was no significant difference in the extent of absorption of D-α-tocopherol from the acetate ester and the succinate ester, although there was an apparently higher initial rate of absorption from the acetate ester. The authors found it worth stressing that (1) the assessments made are all based on relative bioavailability, comparing one form of tocopherol to another, and (2) the data cannot be used to obtain an accurate measure of absolute bioavailability, which would show what percentage of the dose is absorbed into the circulation. Therefore, from the data presented in this paper the absorption of some unhydrolysed D-α-tocopheryl acid succinate cannot be excluded.

In summary, data from animal studies demonstrate that D-α-tocopherol (vitamin E) is bioavailable in animals from orally administered TAS and that a low but measurable amount of TAS can be absorbed in rats as unhydrolysed ester which is then retained as such in rat tissues. Furthermore, the use of TAS as human medicine and studies in humans indicates that D-α-tocopherol is bioavailable from orally ingested TAS in humans, however, the extent of hydrolysis of TAS in humans is not clear. Consequently, it is not clear whether any unhydrolysed TAS is available for absorption in humans. In terms of bioavailability of D-α-tocopherol from oral administration of TAS, the data from animal and human studies cannot be compared quantitatively since these studies were performed with different protocols.

Biochemical and cellular effects

There is scientific literature concerning biochemical and cellular effects induced by D-α-tocopheryl acid succinate. Several examples of effects in vitro and in vivo including antineoplastic effects provide some indication that D-α-tocopheryl acid succinate may interact
with cell cycling, signalling pathways and cellular functions and that the activity of D-\(\alpha\)-tocopheryl acid succinate is related to the action of the intact molecule (Kline et al., 2001; Neuzil et al., 2001 a-c; Kim et al., 1998; Weber et al., 2002; Kempna et al., 2003; Fukuzawa et al., 2004).

D-\(\alpha\)-Tocopheryl acid succinate is a potent inhibitor of acetylcholinesterase in vitro (\(IC_{50}\) of 1.73 micromol/l) (Chelliah et al., 1994).

In rats treated i.p. with D-\(\alpha\)-tocopheryl hemisuccinate tris salt (0.19 mmol/kg bw) hepatic activity of CYP2E1 and CYP2B1/2 were decreased by about 30 % and 65 % after 18 h, respectively, while heme oxygenase-1 activity was increased about twofold. CYP2E1 was not affected by D-\(\alpha\)-tocopherol (Tirmenstein et al., 1999).

**Acute toxicity**

D-\(\alpha\)-tocopheryl acid succinate was tested for acute oral toxicity in rats (Food and Drug Research Laboratories Inc. 1982). These tests revealed that the acute LD\(_{50}\) was greater than 15 g/kg of body weight.

**Subchronic toxicity**

To address the concerns expressed by the SCF the petitioner conducted a 90-day oral toxicity study on D-\(\alpha\)-tocopheryl acid succinate and D-\(\alpha\)-tocopheryl acetate in rats. The study focussed on the specific concerns on haematological disorders and on cholinergic effects that had been raised by the SCF. Blood disorders were investigated in the haematological part of the study with 13 parameters. Effects on cholinesterase were monitored by ocular assessment and monitoring of clinical symptoms. The activity of acetylcholinesterase was not measured. The petitioner argued that toxic signs based on potential muscarinic or nicotinic effects or central nervous disturbances would have been detected by clinical observations. Symptoms indicating a cholinergic action (e.g. hypothermia, (whole body) tremor/muscle twitching, salivation and diarrhoea) were investigated likewise.

Selection of doses was based on data from subacute, subchronic and chronic rat studies with tocopheryl acetate which induced haematological disorders at doses of 600 mg/kg bw in a 7-day study (Takahashi et al. 1990), 2000 mg/kg bw in a 13-week study (Abdo et al. 1986) and 500 mg/kg bw in a 2-year study (Wheldon et al. 1983). Additionally, in the study of Abdo et al. (1986) tocopheryl acetate caused interstitial inflammation and adenomatous hyperplasia of the lung at all doses tested (125, 500 and 2000 mg/kg bw). Based on these data the petitioner selected the doses of TAS for the 90-day study.

Accordingly, TAS dissolved in corn oil was administered by gavage to groups of 10 male and 10 female CD\(^{\circ}\) rats at dose levels of 0, 265, and 1123 mg/kg body weight/day at 7 days/week for 90 days. Additionally, D-\(\alpha\)-tocopheryl acetate dissolved in corn oil was administered by gavage to groups of 10 male and 10 female CD\(^{\circ}\) rats at a dose level of 1000 mg/kg body weight/day at 7 days/week for 90 days. The study was conducted in compliance with Good Laboratory Practice and examinations were performed according to OECD Guideline No. 408 (1998).

Body weights and food and drinking water consumption showed no treatment-related difference from control groups. Faeces were of normal consistency and form in all dose
groups. None of the rats showed any clinical signs of systemic toxicity. No influence on
behaviour and external appearance were noted. Functional observations and ophthalmological
examination revealed no changes in any of the dosed groups when compared with the control
group. No test item-related changes were noted in any of the haematological parameters
examined including blood clotting parameters. In terms of clinical biochemistry, alanine
aminotransferase was markedly increased (85.3 vs 32.7 U/L plasma) \( (p \leq 0.01) \) in males
treated with 1123 mg TAS/kg bw and slightly increased (45.5 vs 35.0 U/L plasma) \( (p \leq 0.01) \)
in females treated with 1123 mg TAS/kg bw. In addition, an increase of the aspartate
aminotransferase was noted in male animals treated with 1123 mg TAS/kg bw (99.9 vs 63.4
U/L plasma) \( (p \leq 0.01) \). These findings were not accompanied by related histopathological
effects in the liver. A slight decrease in potassium levels was noted for males treated with
1123 mg TAS/kg bw (3.547 vs 3.922 mmol/L plasma) \( (p \leq 0.01) \) and an increase of creatinine
value in males treated with 1000 mg \( \alpha \)-tocopheryl acetate/kg bw (54.0 vs 49.1 \( \mu \)mol/L plasma.
No test item-related histopathological changes were noted. The Panel considered the No
Observed Adverse Effect Level (NOAEL) is 265 mg TAS/kg bw based on increases of alanine
aminotransferase and aspartate aminotransferase at the higher dose.

Reproductive Toxicity
No data are available specifically on TAS.

The results of reproductive toxicity studies with the water-soluble D-\( \alpha \)-tocopherol
polyethylene glycol 1000 succinate in rats indicated that it did not have adverse effects on
reproductive function at doses of up to 2% of the diet (Krasavage and Terhaar, 1977) and D-
\( \alpha \)-tocopherol was not teratogenic in mice (Hook et al., 1974).

Chronic toxicity
No data are available specifically on TAS.

Genotoxicity
TAS did not induce gene mutations in a bacterial mutagenicity assay (Ames Test) (NTP study
ID 582862) nor chromosomal aberrations in an in vitro cytogenetics assay while it induced
sister chromatid exchanges up to 35% over control in the presence of rat liver S9 in vitro

Human studies
Clinical studies have been conducted with TAS primarily designed to assess the efficacy of
TAS as source of vitamin E in the therapy of angina pectoris and muscular dystrophy
(Anderson and Reid 1974, Anderson 1974, Gillian et al. 1977, Berneske et al. 1960). In these
studies TAS was dosed in a range from 400 to 3200 IU/day (equivalent to 331 – 2645 mg
TAS/day which is equivalent to 5.5 – 44 mg TAS/kg body weight/day based on a body weight
of 60 kg). No adverse effects were reported apart from transient diarrhoea and intestinal
cramps, which have also been reported following high doses of other forms of vitamin E
(WHO 1987b). These studies, however, are only of limited value for safety assessment,
because the parameters used for evaluation of possible adverse effects were not reported in
each case and, moreover, studies primarily designed to assess the efficacy differ to some
extent from real tolerance studies.
DISCUSSION

The use of TAS as a human medicine and studies in humans indicates that tocopherol (vitamin E) is bioavailable from orally ingested TAS. While it has been shown that a low but measurable amount of TAS can be absorbed in rats as unhydrolysed ester which is then retained as such in rat tissues, the extent of hydrolysis after oral ingestion in humans is not clear. Accordingly, it is not clear whether any unhydrolysed TAS is available for absorption in humans.

There are only a few data on genotoxicity. The induction of sister chromatid exchanges is only of minor relevance for the evaluation of genotoxicity since functional consequences of sister chromatid exchanges are unknown and other in vitro genotoxicity tests for bacterial mutagenicity and mammalian cytogenetics were negative. Thus, the data available do not give rise to safety concern with respect to genotoxicity nor would such safety concerns be expected based on the structure of TAS.

In the new 90-day toxicity study on TAS in rats, the Panel considered that the NOAEL was 265 mg TAS/kg bw/day based on increases of alanine aminotransferase and aspartate aminotransferase at the high dose of 1123 mg TAS/kg bw/day. The NOAEL of 265 mg TAS/kg bw/day is equivalent to 320.65 IU vitamin E/kg bw/day. This is equivalent to 19239 IU vitamin E/person (based on a body weight of 60 kg).

In order to estimate the margin between the NOAEL and a high intake of TAS by humans the following assumptions were made. A rough estimate of the high-level vitamin E intake from supplements by adults consuming more than one supplement per day is 336 mg/day (equivalent to 5.6 mg/kg bw/day). Thus, assuming that all vitamin E supplements taken contain TAS and that the bioavailability in humans is roughly the same as in rats the margin of safety for TAS in adults is 265 / 5.6 = 47.3. Since the effects observed at the higher dose level were limited to changes in enzyme markers without accompanying histopathological alterations this margin of safety is considered acceptable. If the intake per kg body weight by children was the same as by adults the margins of safety would be of a similar magnitude.

The 90-day toxicity study conducted with TAS in rats covered some concerns that had been raised by the SCF:

- Haematological abnormalities reported in earlier studies were not observed in the new 90-day study and no cholinergic effects were observed at doses up to 1123 mg/kg body weight/day.
- Potential interaction of TAS with cell cycling, signalling pathways and cellular functions were not specifically addressed in this study. However, if there were such effects then they did not result in any adverse effect recognizable in this standard subchronic toxicity study.
- In light of the results from this 90-day study, further data are no any longer considered necessary on hydrolysis and bioavailability initially requested by the SCF are no longer considered necessary for the safety evaluation of TAS.
CONCLUSION

The use of TAS as a human medicine and studies in humans indicate that tocopherol (vitamin E) is bioavailable from orally ingested TAS. However, the extent of hydrolysis is not clear. Based on the data reviewed the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded TAS, as a source of tocopherol (vitamin E) in foods for particular nutritional purposes (PARNUTS), foods intended for the general population and food supplements is not of concern from the safety point of view.
DOCUMENTATION PROVIDED TO EFSA

Unpublished study report on a 90-day subchronic toxicity study by repeated oral administration to CD® rats according to OECD 408 guideline by LPT Laboratory of pharmacology and toxicology KG

REFERENCES


SCIENTIFIC PANEL MEMBERS


ACKNOWLEDGEMENT

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Rainer Gürtler for his contribution to the draft opinion.