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
Calcium ascorbate with a content of threonate
for use as a source of vitamin C in food supplements

Question number EFSA-Q-2005-044

Adopted on 17 April 2007

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 the substance calcium ascorbate with a content of threonate, when used as a source of vitamin C in food supplements.

The present opinion deals only with the safety and bioavailability of the particular source of vitamin C, calcium ascorbate with a content of threonate, to be used as a nutritional substance in food supplements. The safety of vitamin C itself, in terms of the amounts that may be consumed, is outside the remit of this Panel and has previously been evaluated by EFSA’s Scientific Panel on Dietetic Products, Nutrition and Allergies and by the Scientific Committee for Food (SCF).

Calcium ascorbate with a content of threonate is a product consisting of the calcium salt of L-ascorbic acid (vitamin C) and less than 2% of L-threonic acid along with calcium carbonate and water.

The bioavailability of vitamin C from calcium ascorbate with a content of threonate is comparable to that of ascorbic acid.

The toxicity studies and mutagenicity tests with calcium ascorbate with a content of threonate, with calcium threonate or L-threonic acid, hemicalcium salt indicate that the compounds are of low toxicity and are not mutagenic. Data on carcinogenicity, long-term studies, reproductive and developmental toxicity of calcium ascorbate with a content of threonate were not presented. Such toxicity studies are not needed in the light of the dissociation of calcium ascorbate with a content of threonate to substances which are physiologically present in the body (ascorbate, threonate, calcium) and considering that the safety of ascorbic acid (and its calcium and sodium salts), and calcium was previously evaluated.

The intended conditions of use of calcium ascorbate with a content of threonate correspond to those of other approved sources of vitamin C. The additional exposure to calcium and threonate through
use of supplements with calcium ascorbate with a content of threonate does not represent a cause of safety concern.

The Panel noted that threonate is a normal metabolite in the body and concluded that the use of calcium ascorbate containing up to 2% threonate as a source of vitamin C in food supplements is not of safety concern.

**KEYWORDS**
Calcium ascorbate with a content of threonate; Calcium ascorbate; calcium threonate; calcium ascorbate/threonate, vitamin C, L-ascorbic acid, L-threonic acid, CAS Registry Number. 5743-28-2

**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.

**TERMS OF REFERENCE**
In accordance with article 29 (1) (a) of the Regulation (EC) No. 178/202, the European Commission asks the European Food Safety Authority (EFSA) to provide a scientific opinion, based on its considerations of the safety and bioavailability of the substance calcium ascorbate/threonate (hereinafter referred to as calcium ascorbate with a content of threonate) when used as a source of vitamin C in food supplements.

**ASSESSMENT**

**INTRODUCTION**
The present opinion deals only with the safety and bioavailability of a particular source of vitamin C to be used as a source of vitamin C in food supplements. The safety of vitamin C itself, in terms of the amounts that may be consumed, is outside the remit of this Panel and has previously been evaluated by EFSA’s Scientific Panel on Dietetic Products, Nutrition and Allergies and by the Scientific Committee for Food (SCF, 1989, 1990, 2003; EFSA, 2004).

The Panel noted that calcium ascorbate is already authorised as a source of vitamin C in Annex II of Directive 2002/46/EC and as a food additive (E 302) in Directive 95/2/EC2 and that specification for the substance is laid out in Directive 96/77/EC3. This opinion will therefore address the question of the threonate content in calcium ascorbate.

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2 OJ L 61, 18.3.1995, p.1
CHEMISTRY

According to the petitioner calcium ascorbate with a content of threonate consists of (in average) 95.5% calcium ascorbate dihydrate, 1.2% calcium threonate, 1.1% calcium carbonate and 1.1% free water.

The constituents:

Calcium L-ascorbate dihydrate
Chemical name: Ca-di-1-3-oxo-L-gulofuranolactone (enol form); Ca-di-1-L-3-ketothreohexuronic acid; Ca-di-4-L-ascorbate dihydrate, Calcium salt of 2,3-didehydro-L-threo-hexono-1,4-lactone dihydrate, CAS Number: 5743-28-2
Synonyms: Calcium ascorbate, calcium ascorbate dihydrate
Molecular formula: C_{12}H_{14}CaO_{12} 2H_{2}O

![Structural formula of Calcium L-ascorbate dihydrate]

Molecular weigh: 426.35

Calcium L-threonate
Chemical name: Ca-di-L-threonate; CAS Number: 70753-61-6
Molecular formula: C_{8}H_{14}O_{10}Ca
Structural formula:

![Structural formula of Calcium L-threonate]

Molecular weight: 310.27

SPECIFICATIONS

Specifications for calcium ascorbate with a content of threonate as indicated by the petitioner are: L-ascorbic acid between 77.37 and 80.47%, calcium between 8.54 and 9.94% and L-threonic acid not less than 0.9%. The loss on drying is between 0.2 and 1.8%. The pH of 0.1% aqueous solution is in the range from 5.9 to 7.1. The petitioner has submitted analytical data showing that the substance complies with the limits on fluoride and heavy metals in the specification of calcium ascorbate (E 302). Based on the analytical data presented by the petitioner, the Panel assumed that the content of L-threonic acid would not exceed 2%.
The Panel noted that the specification for calcium ascorbate (E 302) (Directive 96/77/EC) gives no limits for content of threonate.

**MANUFACTURING PROCESS**

The method of manufacture is described in the United States Patent No. 4, 822,816, granted April 18, 1989. In this process ascorbic acid is dissolved in water then neutralized by the addition of calcium carbonate with precise temperature control. The threonate is formed from the ascorbate during the manufacturing process.

**METHODS OF ANALYSIS IN FOODS**

According to the petitioner, the following analytical methods can be used to confirm the identity and presence in foods of calcium, ascorbic acid and L-threonic acid. The level of L-ascorbic acid can be determined by iodometric titration. The level of calcium can be tested by ICP-MS, and the level of L-threonic acid (calcium L-threonate) can be tested by HPLC. These methods are standard protocols as applied generally to ascorbic acid and the salts thereof, except in case of the test protocol for L-threonic acid, according to United States Pharmacopoeia guidelines for validation of non-compendial methods.

**REACTIONS IN FOOD AND STABILITY**

The stability of vitamin C in calcium ascorbate with a content of threonate was determined in several various formulations under conditions of ambient and elevated temperature of storage. The samples were analyzed for ascorbate content. The results presented by the petitioner indicated that the content of ascorbic acid in the source as a raw material in powder and tablet forms was stable. No information on the reactions in foods was provided.

**CASE OF NEED AND PROPOSED USES**

According to the petitioner, calcium ascorbate with a content of threonate is an alternative source of vitamin C.

Calcium ascorbate with a content of threonate is proposed as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders, and beverages that are food supplements.

According to the petitioner supplements containing calcium ascorbate with a content of threonate typically provide between 646 mg and 1292 mg of the source per day. This is equivalent to between 500 mg to 1000 mg of ascorbic acid, 60 to 120 mg calcium and 6-12 mg threonate per day.

**EXISTING AUTHORISATIONS AND EVALUATIONS**

According to the petitioner calcium ascorbate with a content of threonate is on the market in over 40 countries around the world.

Calcium L-ascorbate dihydrate, has been evaluated by SCF in 1987. At that time the Committee found ascorbic acid and its calcium and sodium salts acceptable for food additive use (SCF, 1989).

L-ascorbic acid, its calcium, potassium and sodium salts were included in the EFSA opinion on the upper safe levels of vitamin C (EFSA, 2004).
The Joint FAO/WHO Expert Committee on Food Additives (JECFA) on its 25th meeting included calcium ascorbate in the ADI not specified for ascorbic acid and its sodium and potassium salts (JECFA, 1981).

Calcium carbonate has been accepted as safe for use as a food additive by the SCF (SCF, 1990).

**BIOLOGICAL AND TOXICOLOGICAL DATA**

**BIOAVAILABILITY OF VITAMIN C FROM THE SOURCE**

Human and animal studies have been provided by the petitioner showing that the bioavailability of vitamin C from calcium ascorbate with a content of threonate is comparable to that of ascorbic acid (Bernal *et al.*, unpublished; Hunt and Rice, 1995; Bush *et al.*, 1987). No studies have been submitted comparing the bioavailability of vitamin C from this source with calcium ascorbate not containing threonate.

**METABOLIC FATE OF THE SOURCE AND BIOLOGICAL DISTRIBUTION**

According to the petitioner, the metabolic fate and biological distribution of calcium ascorbate with a content of threonate is identical to that from already approved calcium ascorbate. Upon ingestion, ascorbate salts dissociate in the stomach. Ascorbate in single dosages up to about 100 mg is absorbed and no ascorbate is excreted in the urine. At dosages above 100 mg, some unchanged ascorbate and its metabolites (e.g. threonate) are excreted in the urine.

The petitioner provided no details on metabolic fate of threonate.

L-threonic acid, or its dissociated salt form, L-threonate, has been identified in both plant and animals. In mammals it typically arises from the catabolism of ascorbic acid in the liver. Two mechanisms for threonate appearance in the body have been proposed. The first involves the spontaneous decomposition of the oxidation product, dehydroascorbic acid, which is relatively unstable in aqueous environment, and subsequent formulation of precursors of threonate. The second mechanism is thought to be the liver’s glyoxalase system, composed of two enzymes - glyoxylase I and II – together with a glutathione cofactor. This system catalyzes the conversion of alpha-oxoaldehydes to their corresponding aldonic acids, including threonate (Thornalley, 1998).

Threonate is a normal constituent of the body. It has been identified in human plasma (Deutsch *et al.*, 1999, Harding *et al.*, 1999) and in urine (Thompson *et al.*, 1975, Chalmers *et al.*, 1976, Lawson *et al.*, 1976). Furthermore, threonic acid has been identified in the aqueous humour of the eye (Harding *et al.*, 1999).

**TOXICITY DATA ON CALCIUM ASCORBATE WITH A CONTENT OF THREONATE**

*Acute toxicity of calcium ascorbate with a content of threonate, calcium threonate and L-threonic acid, hemicalcium salt*

Three unpublished reports concerning the acute toxicity of calcium ascorbate with a content of threonate, one unpublished report on the acute oral toxicity of calcium threonate and one unpublished report on the acute oral toxicity of L-threonic acid, hemicalcium salt in rats were submitted by the petitioner.
Calcium ascorbate with a content of threonate did not induce signs of toxicity when administered orally by gavage at a single dose of 7500 mg/kg bw in a water solution or in corn oil to rats observed for 14 days (Unilab Research, 1982; Northview Pacific Laboratories, 1983, 1986).

Calcium threonate did not induce signs of toxicity when administered orally by gavage at a single dose of 5000 mg/kg bw in a water solution to rats observed for 14 days (Northview Pacific Laboratories, 1989).

L-threonic acid, hemicalcium salt did not induce signs of toxicity when administered orally by gavage in a single dose of 5000 mg/kg bw in a water solution to rats observed for 14 days (Northview Pacific Laboratories, 1995a).

**Subchronic and chronic toxicity of calcium threonate**

The subchronic toxicity of calcium threonate and effects on survival were studied in rats and mice, respectively.

Young male rats received a casein starch based synthetic diet (control, N=10) or this diet containing 1% calcium threonate equivalent to a dose of 1g/kg bw (N=10) for 120 days. The diets were adjusted for isocaloricity and calcium content. Feed intake was measured at the intervals and body weights were recorded daily. At termination, blood samples were collected for measurements of haemoglobin, packed cell volume, plasma cholesterol. Cytochrome P-450 activity and weights of liver, spleen, adrenals and brain were recorded. No differences were found between the two groups in feed intakes, growth rates, haematological and clinical chemistry parameters and absolute and relative organ weights, except for a significantly lower relative liver weight in calcium threonate group (Thomas and Hughes, 1985).

Five-week old mice (65 females and 65 males per group) were given either a casein-starch synthetic diet (control), or this diet supplemented with 0.05% or 0.20% calcium threonate equivalent to doses of 75 and 300 mg/kg bw/day until death occurred. The survival was the only parameter on which the data were presented. The mean life span in weeks was 63, 60, 61 for males, and 68, 66 and 71 for females, in the control, low, and high dose groups, respectively. Calcium threonate in doses of 75 and 300 mg/kg bw/day had no significant influence on the mean life span of the mice (Thomas and Hughes, 1985).

**Mutagenicity**

Whilst the Panel noted that the submission only contained bacterial mutation tests, the Panel was aware of genotoxicity tests on calcium threonate in mammalian systems.

The mutagenic potential of calcium L-threonate monohydrate was tested in Ames test with and without metabolic activation (S9 mix) using *S. typhimurium* strains TA1535, TA 1537, TA 1538, TA98, and TA100. Calcium L-threonate monohydrate was tested at concentrations of 167, 500, 1670, 5000, 7500, and 10000 µg/plate. Calcium L-threonate monohydrate was found to be not mutagenic under the conditions of the test (Pharmacon Research International, Inc., 1989).

In another study, the mutagenic potential of calcium L-threonate monohydrate was tested in Ames test with and without metabolic activation (S9 mix) using *S. typhimurium* strains TA97A, TA98, TA100, TA102, and TA1535. The compound was tested at concentrations ranging from 10 to
10000 µg/plate. The compound was found to be not mutagenic under the conditions of the test (Northview Pacific Laboratories, Inc., 1995b).

The mutagenic potential of calcium ascorbate with a content of threonate was tested in the Ames test with and without metabolic activation (S9 mix) using \textit{S. typhimurium} strains TA98 and TA100. Calcium ascorbate with a content of threonate was tested at concentrations ranging from 5.0 to 5000 µg/plate. The compound was found to be not mutagenic under the conditions of the test (IIT Research Institute, 1990).

The mutagenic potential of calcium ascorbate with a content of threonate was investigated in the Ames test with and without metabolic activation (S9 mix) using \textit{S. typhimurium} strains TA98, TA100, TA1535, TA1537 and TA1538. Calcium ascorbate with a content of threonate was tested in concentrations of 5-5000 µg/plate. The compound was found to be not mutagenic under the conditions of the test (SITEK Research Laboratories, 1994).

\textit{Other studies}

The petitioner submitted two studies investigating the effect of calcium L-threonate on ascorbic acid uptake in cell cultures. The Panel considered these publications, not directly applicable for the evaluation of the bioavailability and the safety of calcium ascorbate with a content of threonate as a source of vitamin C for humans. Therefore the studies are not further discussed.

\textbf{DISCUSSION}

Human and animal studies have been provided by the petitioner showing that the bioavailability of vitamin C from calcium ascorbate with a content of threonate is comparable to that of ascorbic acid.

The toxicity data on calcium ascorbate with a content of threonate are limited to three studies of acute oral toxicity in rats, and to two Ames tests. Acute toxicity studies in rats reveal calcium ascorbate with a content of threonate is of low toxicity, with no adverse effects observed at the dose of 7500 mg/kg bw. Studies using Ames tests indicate no mutagenic activity.

The toxicity data on threonate are limited to studies of acute oral toxicity in rats with calcium threonate or L-threonic acid hemicalcium salt, one 120-day feeding study in rats with calcium threonate, a survival study in mice with calcium threonate, and two Ames tests with calcium L-threonate monohydrate.

Acute oral toxicity studies in rats reveal that threonate is of low toxicity, with no adverse effects observed at 5000 mg/kg bw. A 120-day feeding study in male rats with doses of 0 (control) or 1g/kg bw/day calcium threonate did not reveal any adverse effects. Calcium threonate in doses of 75 and 300 mg/kg bw/day had no significant effect on the mean life span of the mice. Studies using Ames tests indicate no mutagenic activity of calcium L-threonate monohydrate. Whilst the Panel noted that the submission only contained bacterial mutation tests, the Panel was aware of genotoxicity tests on calcium threonate in mammalian systems.

Data on carcinogenicity, long-term toxicity, reproductive and developmental toxicity of calcium ascorbate containing calcium threonate and of threonate were not presented. The Panel concluded that such toxicity studies are not needed in the light of the dissociation of calcium ascorbate with a content of threonate to substances which are physiologically present in the body (ascorbate,
threonate, calcium) and considering that the safety of ascorbic acid (and its calcium and sodium salts), and calcium was previously evaluated (SCF, 1989, 1990, 2003; EFSA, 2004).

The SCF established a tolerable upper level of 2500 mg/day for calcium from all sources for adults. Additional exposure to calcium through use of supplements of calcium ascorbate with a content of threonate is between 60-120 mg calcium/person/day, which is not of safety concern.

Data on dietary intakes of L-threonate are not available. However, L-threonate may occur in certain foods. For instance, L-threonate can occur as a break down product of ascorbic acid. Additional exposure to L-threonate from the use of supplements of calcium ascorbate containing around 1.2% threonate may lead to an intake of up 12 mg/person/day when consuming the supplement in the recommended dose equivalent to 1 g ascorbic acid. This additional intake of L-threonate is of no safety concern.

**CONCLUSIONS**

The Panel noted that the bioavailability of vitamin C from calcium ascorbate with a content of threonate is comparable to that of ascorbic acid.

The Panel noted that threonate is a normal metabolite in the body and concluded that the use of calcium ascorbate containing up to 2% threonate as a source of vitamin C in food supplements is not of safety concern.

**DOCUMENTATION PROVIDED TO EFSA**


**REFERENCES**

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