

Opinion on Calcium L-threonate for use as a source of calcium in food supplements¹

Scientific Panel on Food Additives and Nutrient Sources added to food (ANS)

(Question number EFSA Q-2005-158)

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PANEL MEMBERS

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SUMMARY

Following a request from the Commission, the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) has been asked to give advice on the safety and bioavailability of the substance calcium L-threonate, when used as a source of calcium in food supplements.

The present opinion deals only with the safety and bioavailability of a particular source of calcium, calcium L-threonate, to be used as a nutritional substance in food supplements. The safety of calcium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

Human and animal studies indicate that calcium from calcium L-threonate is absorbed. In animal studies, the bioavailability of calcium from this source was comparable to or higher than from other sources of calcium.

Threonate is a normal constituent of the body, typically arising from the catabolism of ascorbic acid.

Calcium L-threonate has low oral acute toxicity, with no adverse effects observed at doses as high as 40 g/kg bw in mice or 32 g/kg bw in rats.

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² Editorial changes only: page 3 (table of content) and page 8 – 3.3.2 was updated to 3.1.2, page 4 Terms of Reference – the year of the regulation was changed from 202 to 2002. The changes do not affect the overall conclusion of the opinion. To avoid confusion, the original version has been removed from the website.

In sub-chronic studies with calcium L-threonate, the Panel identified a NOAEL of 4 g/kg bw/day in the rat with regard to effects on blood coagulation time and accretion of the thyroid gland, and of 1 g calcium L-threonate/kg bw/day in the dog with regard to hyperplasia of the thyroid gland. The Panel noted that the effects on blood coagulation time and the thyroid gland were reversible and that a mild accretion in the thyroid gland in rats was limited to males only. The Panel further noted that these effects are likely to be attributed to the high dosage of calcium administered over a long period. A high concentration of calcium ions can result in accelerated blood coagulation. It can influence intestinal absorption of iodine and reduce/suppress the secretion of thyroxin by the thyroid gland. The NOAELs are equivalent to 516 mg calcium/kg bw/day and 3484 mg L-threonate/kg bw/day in the rat, and to 129 mg calcium/kg bw/day and 871 mg L-threonate/kg bw/day in the dog.

Studies using different test systems *in vitro* and *in vivo* indicated that calcium L-threonate was not genotoxic. Although no carcinogenicity studies were available, the Panel considered that such studies were not needed given that L-threonate is an endogenous compound in the body and that calcium L-threonate did not show any genotoxic potential.

Reproductive and developmental toxicity studies in mice indicated that calcium L-threonate in doses up to 6 g/kg bw/day has no adverse effect on the fertility and the developing fetus, and did not cause maternal toxicity.

The Scientific Committee on Food (SCF) has established a tolerable upper intake level of 2500 mg/day for calcium from all sources for adults. The petitioner proposed use levels of 2-4 tablets/person/day of calcium L-threonate with each tablet providing 100 mg calcium and 675 mg threonate. The exposure to calcium through the use and at the use levels of calcium L-threonate proposed by the petitioner, may lead to exposures of 200-400 mg calcium/person/day, which would not represent a safety concern.

Data on dietary intakes of L-threonate are not available. L-Threonate may occur in certain foods. For example, L-threonate is a break-down product of ascorbic acid during food preparation. Exposure to L-threonate at the use and use levels indicated by the petitioner is estimated to amount to 1350 – 2700 mg per person per day corresponding to 22.5 – 45 mg/kg bw/day for a 60 kg person. The margin of safety between the estimated human exposure to L-threonate and the amount of L-threonate equivalent to the NOAELs for calcium L-threonate, as demonstrated in sub-chronic toxicity studies in dogs and rats, is 39 – 19 for the dog and 155-77 for the rat. The Panel considers this margin of safety to be sufficiently large given that threonate is an endogenous compound in the body, and that the NOAELs in the dog and rat studies were identified for effects attributable not to L-threonate but to the calcium dosages.

The Panel concludes that calcium is bioavailable from calcium L-threonate and that the use of calcium L-threonate as a source of calcium in food supplements for the uses and at the use levels proposed by the petitioner is not of safety concern.

Key words:

Calcium L- threonate; CAS Number: 70753-61-6; food supplements.

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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.

The Commission has received a request for the evaluation of calcium L-threonate as a source of calcium in food supplements. The relevant Community legislative measure is:

- 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.³

TERMS OF REFERENCE

In accordance with article 29 (1) (a) of the Regulation (EC) No. 178/2002, 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 calcium L-threonate added for nutritional purposes to food supplements.

ACKNOWLEDGEMENTS

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³ OJ L183,127.2002,p51

ASSESSMENT

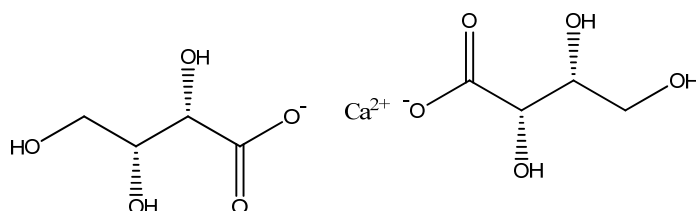
1. Introduction

The present opinion deals only with the safety and bioavailability of a particular source of calcium, calcium L-threonate, to be used as a nutritional substance in food supplements. The safety of calcium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

2. Technical data

2.1 Chemistry

Calcium L-threonate is calcium (2R,3S)-2,3,4-trihydroxybutyric acid with the molecular formula $C_8H_{14}O_{10}Ca$ and structural formula:



The molecular weight of calcium L-threonate is 310.3 g/mol. Its CAS Number is 70753-61-6. The trade name of the compound is calcium L-threonate, and the abbreviation is Ca L-threonate.

2.2 Specifications

Calcium L-threonate is a white granular non-hygroscopic powder with no distinctive odour or taste and slightly soluble in water at ambient temperature.

Specifications for calcium L-threonate as provided by the petitioner are: calcium L-threonate not less than 98.5%, total calcium (as Ca) 12.92% by weight. The loss of weight on drying is 1.5%. The melting point is $>300^{\circ}C$. The pH of a 5% aqueous solution is in the range of 6.0 to 8.0. The total impurities are $<1\%$. The content of arsenic is limited to <0.5 mg/kg, lead <0.2 mg/kg, mercury <0.05 mg/kg, and cadmium <0.3 mg/kg.

2.3 Manufacturing process

The petitioner provided adequate information on the production process of calcium L-threonate. The compound is produced by hydrogen peroxide oxidation of the calcium salt, obtained from ascorbic acid and calcium carbonate.

2.4 Methods of analysis

The petitioner presented a validated capillary electrophoresis method with indirect UV detection for the determination of L-threonate after appropriate extraction and preparation from a calcium L-threonate chewable tablet and dry syrup, published elsewhere (Song *et al.*, 1999).

2.5 Reactions in food, stability

The stability of calcium L-threonate has been investigated under a range of conditions such as high humidity (up to 92.5%), temperature (up to 80 °C) and at intense light (4000 LX), for up to 10 days, at either 40°C or 60°C at 75% relative humidity for up to 6 months. It has also been tested as a component of a tablet upon 1, 2, 3 and 6 months storage at 40°C/75% relative humidity.

The results of the stability tests carried out for tablets containing calcium L-threonate showed no significant differences in the measured physical, chemical and microbiological parameters between the test and control samples, including those stored for six months.

According to the petitioner investigations of samples in the ambient and accelerated stability studies did not indicate the production of any potentially toxic degradation by-products.

2.6 Case of need and proposed uses

The calcium L-threonate food supplement is in tablet form and each tablet weighs 972 mg. The total calcium L-threonate content is 775 mg per tablet, and one tablet provides 100 mg of calcium and 675 mg of L-threonate. According to the petitioner, the proposed use is one to two tablets twice a day (2-4 tablets/person/day).

2.7 Exposure

The daily intake of calcium L-threonate from the proposed uses is estimated by the petitioner to range from 1550 to 3100 mg. These amounts of calcium L-threonate provide from 200 to 400 mg calcium and from 1350 to 2700 mg L-threonate daily.

According to the SCF, the average and 97.5th percentile calcium intakes from food and calcium supplements in European countries vary from 683 to 949 mg/person/day and from 1317 to 1970 mg/person/day, respectively (SCF, 2003). The Panel noted that exposure to calcium through the use of calcium L-threonate as supplements may lead to an additional exposure to calcium of 200-400 mg calcium/person/day. The Panel further noted that the total exposure to calcium for users of calcium L-threonate with the use and use levels proposed by the petitioner will not exceed the tolerable upper level of 2500 mg/day for calcium established for adults by the SCF (SCF, 2003).

Data on dietary intakes of threonate are not available. L-threonate may be ingested as a constituent of certain foods as it is a major breakdown product of ascorbic acid during baking of breads (Thewlis, 1971) and the storage of meats processed using ascorbic acid as a food additive (Thomas and Hughes, 1983). The use of calcium L-threonate supplements may lead to an additional exposure to L-threonate of 1350-2700 mg/person/day, which corresponds to 22.5 – 45 mg/kg bw/day for a 60 kg person.

2.8 Existing authorisations and evaluations

According to the petitioner products containing calcium L-threonate have been officially authorised for sale for human consumption in China, Canada, Singapore, Indonesia and Thailand. Calcium L-threonate is also on the market in the USA.

The calcium cation has previously been evaluated by the Scientific Committee on Food (SCF, 1990). At that time the SCF evaluated several cations and anions and established a group ADIs not specified for the individual ions, although exhaustive systematic toxicological studies have not been carried out. The SCF considered that these ions were constituents of the major electrolytes in all biological material from animal and plant origin and therefore occurred in foodstuffs. Therefore, the SCF concluded that no safety problems were likely to arise, provided that the contribution from food intake did not disturb the homeostatic mechanisms controlling the electrolyte balance of the body.

In 2003, the SCF established a tolerable upper intake level for calcium of 2500 mg/day from all sources for adults, pregnant and lactating women (SCF, 2003).

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) has previously concluded that the use of calcium ascorbate containing up to 2% threonate as a source of vitamin C in food supplements was not of safety concern (EFSA, 2007).

3. Biological and toxicological data

3.1 Bioavailability of calcium from the source

The Panel has been provided with data on human studies on the absorption and pharmacokinetics of calcium after oral intake of calcium L-threonate, and with animal studies investigating the bioavailability of calcium from calcium L-threonate.

3.1.1 Human studies

Ten male healthy volunteers (age: 22 years old) received a daily dose of 1.72 g calcium L-threonate equivalent to 210 mg calcium for seven days, and urinary calcium concentration was measured before and after dosing. Urinary content of calcium was statistically increased after treatment as demonstrated by three indices: total urinary calcium content (before dosing: 27.09 mg \pm 10.81 and after dosing: 73.03 mg \pm 22.72), calcium content per 100 ml urine (before dosing: 20.64 mg \pm 4.02 and after dosing: 36.41 mg \pm 6.68), and calcium content per gram urinary creatine (before dosing: 144.15 mg \pm 53.67 and after dosing 266.62 mg \pm 109.05) (Jiao *et al.* 1996a).

Fourteen healthy subjects (age and sex not given) received 6 tablets of calcium L-threonate daily in two servings of 3 tablets each for at least 6 days. The time interval between the servings was 12 hours. Each tablet contained 675 mg L-threonate and provided 100 mg of calcium. On the fourth, fifth and sixth days of treatment, blood concentrations of calcium were measured before the administration of the tablets and 1.5 hours after the administration. The recorded average calcium concentrations in the blood before calcium L-threonate administration were 4.7 \pm 2.0, 4.6 \pm 2.4 and 6.0 \pm 3.4 μ g/ml on days 4, 5 and 6, respectively.

The recorded average calcium concentrations in the blood 1.5 hours after administration of the test substance were 39.2 ± 14.6 , 40.4 ± 13.7 , 35.8 ± 11.6 $\mu\text{g/ml}$ on days 4, 5 and 6, respectively. The authors concluded that the calcium blood concentration was stable after treatment of healthy subjects with calcium L-threonate, and that all measured pharmacokinetic parameters of plasma calcium (the median time to peak concentration, the peak concentration, the area under the concentration-time curve, and the volume of distribution) were stable (Anonymous a).

The petitioner presented no human studies comparing the bioavailability of calcium from calcium L-threonate and from other sources. No data on the fate of L-threonate in the body were provided.

3.1.2 Animal studies

Studies in rats

The absorption of calcium from the intestine involves both active and passive processes. The active process requires energy (such as ATP) and is of limited duration. The passive process is based on diffusion, is continuous process that does not require energy.

In a rat study the bioavailability of calcium from calcium L-threonate was compared to that from other sources and the relative contributions of the active and passive processes in the absorption of calcium were assessed. The other calcium sources studied were calcium acetate and calcium gluconate. Eight groups of six male Wistar rats (mean body weight 335.0 ± 12.0 g) were anaesthetised and subjected to intestinal injection of calcium acetate, calcium gluconate or calcium L-threonate. After 15, 30, 60 and 90 minutes post injection, blood samples were collected (from the external jugular vein) to determine serum calcium concentrations. The calcium dosages for the three sources were adjusted on the basis that calcium acetate contains 25.3% calcium, calcium gluconate contains 8.9% calcium, and calcium L-threonate contains 13% calcium. Calcium L-threonate was administered in a medium and a high dose, but the dose levels were not stated in the report. For each calcium source there were two groups. One group was administered an ouabain-Tyrode solution in addition to the tested source. The ouabain was used to block the ATPase and thus the active absorption. The other group was able to exhibit both active and passive absorption. The relative passive absorption ratio of the calcium was calculated from the quotient of the absorption value of the two groups. The serum calcium concentration of the untreated five rats was used as baseline to calculate the increase of blood calcium after intestinal administration from the different sources.

At 15, 30, 60 and 90 minutes after dosing, serum concentrations of calcium in groups exhibiting calcium absorption by both active and passive transport were 26 ± 22 , 48 ± 15 , 32 ± 12 , 18 ± 10 $\mu\text{g/ml}$ in the calcium acetate group, 14 ± 2 , 44 ± 15 , 25 ± 3 , 11 ± 8 $\mu\text{g/ml}$ in the calcium gluconate group, 25 ± 9 , 42 ± 7 , 19 ± 9 , 15 ± 9 $\mu\text{g/ml}$ in the calcium L-threonate middle-dose group, and 32 ± 15 , 86 ± 14 , 59 ± 29 , 24 ± 18 $\mu\text{g/ml}$ in the calcium L-threonate high-dose group .

At 15, 30, 60 and 90 minutes after dosing, serum concentrations of calcium in the groups exhibiting calcium absorption only by the passive transport were 10 ± 3 , 16 ± 3 , 13 ± 7 , 9 ± 4 $\mu\text{g/ml}$ respectively in the calcium acetate group, 10 ± 4 , 26 ± 7 , 13 ± 4 , 8 ± 5 $\mu\text{g/ml}$ respectively in the calcium gluconate group, 21 ± 4 , 31 ± 10 , 11 ± 5 , 5 ± 4 $\mu\text{g/ml}$ respectively in the calcium L-threonate middle-dose group, and 30 ± 8 , 70 ± 15 , 33 ± 18 , 12 ± 6 $\mu\text{g/ml}$ respectively in the calcium L-threonate high-dose group.

At 15, 30, 60 and 90 minutes after dosing, the percentages of passive absorption of calcium were 40, 33, 40, and 50 from calcium acetate, 71, 58, 51 and 51 respectively from calcium gluconate, 82, 74, 56 and 36 respectively from the calcium L-threonate middle-dose, and 93, 78, 56, 52 respectively from calcium L-threonate high-dose at 15, 30, 60 and 90 minutes after dosing.

According to the petitioner, the serum calcium value in the calcium L-threonate treated animals was higher than that for either calcium gluconate or calcium acetate at the same calcium dose at any given time point. Furthermore, the proportion of passive absorption of calcium from calcium L-threonate was also greater than for the other two substances (Li *et al.*, 1998).

In another rat study, availability, absorption rate, pure availability and absorption capacity of calcium L-threonate and calcium carbonate were compared. The rats (Sprague-Dawley, 5 males and 5 females per group) were kept individually in metabolic cages, and given a standard feed for the first three days and a calcium depleted feed thereafter. On the seventh day the rats received calcium L-threonate or calcium carbonate adjusted to deliver 40 mg/kg bw calcium, by oral gavage, whereas the control group received an amylum solution. Animals of each group were allowed to drink deionised water *ad libitum*. Urine and faeces were collected 24 hours after the calcium administration and the calcium content was measured in the samples.

Absorption rate was calculated as: (ingested calcium – calcium in faeces)/(ingested calcium) × 100. Availability was calculated as: (ingested calcium – calcium in faeces – calcium in urine) / (ingested calcium – calcium in faeces) × 100. Pure availability was calculated as: (absorption rate × availability)/100. Absorption capacity was calculated as (pure availability × ingested calcium)/ 100. For calcium L-threonate and calcium carbonate, calcium absorption rates were 94% ± 2 and 45% ± 5, respectively; similarly, the availabilities were 94% ± 7 and 90% ± 10 respectively, pure availabilities were 87% ± 6 and 34% ± 6 respectively, and absorption capacities per day were 33 mg ± 3 and 14 mg ± 3 respectively (Liuyi *et al.*, 1996). The Panel concluded that the availability of calcium from both sources was comparable.

Another rat study evaluated calcium bioavailability from calcium L-threonate or tablets containing 600 mg calcium as calcium carbonate, was evaluated using a method of femur calcium storage. Young male Wistar rats (14/group) were kept on a low calcium synthetic diet low in calcium and received, by gavage, daily calcium doses of 100, 200, or 300 mg/kg bw from calcium L-threonate (0.76, 1.54, or 2.31 g/kg bw of the source) or 300 mg/kg bw from the calcium carbonate tablets, as a source, for 40 days. The fifth group received the same volume of deionised water. Additionally one group was killed before the experiment and served as a basic control group for calcium content in the femur. Calcium supplementation from both sources prevented the effects of calcium deficiency on growth (body weight gain: 137, 183, 157 g/6 weeks in the control, calcium L-threonate high dose and calcium carbonate groups, respectively), and on development of the skeleton evaluated as body length (21, 22, 22 cm in the control, calcium L-threonate high dose and calcium carbonate groups, respectively), spinal column length (15, 17, 16 cm in the control, calcium L-threonate high dose and calcium carbonate groups, respectively), tail length (15, 17, 16 cm in the control, calcium L-threonate high dose and calcium carbonate groups, respectively), femur length (30, 32, 31 mm in the control, calcium L-threonate high dose and calcium carbonate groups respectively). Furthermore both calcium sources enhanced the levels of serum calcium (7.7, 9.3, 9.2 mg/dl in the control, calcium L-threonate high dose and calcium carbonate groups, respectively), bone wet weight, dry weight, ash, calcium content, calcium total quantity, calcium storage in femur, bone density, bone mechanical features (force used for tibia

bending, degree of bone calcification, work for bone bending), and decreased serum alkaline phosphatase and bone re-absorption. The authors of the study concluded that calcium L-threonate could effectively prevent the losses of bone salts in young rats and help ensure normal growth and development of the bone (Yuan et al., 1997a; Fan and Li).

A study investigating the rates of calcium absorption and utilisation of calcium L-threonate in a rat calcium deficiency model was presented by the petitioner. In order to attain a consistent calcium state in all rats at the start of the study, the rats (Sprague-Dawley, sex not stated) were fed a calcium-containing feed for three days during a pre-experimental period. From the fourth day i.e. the start of the study the rats were fed a low-calcium diet and given once daily, by oral gavage, either a high dose of calcium L-threonate (N=10) corresponding to 80 mg calcium or a low dose of calcium L-threonate (N=10) corresponding to 40 mg calcium. The control animals (N=10) received a vehicle (an amyllum suspension) once a day. The duration of treatment with calcium L-threonate was 3 days. During the observation period, the animals were given distilled water, whilst all urine and faeces were collected. Blood samples were collected before and after treatment with calcium L-threonate. Absorption rates, availabilities, pure availabilities and absorption capacities were calculated as described elsewhere (Liuyi *et al.* 1996)

Plasma calcium concentrations (mg/dl) before and after dosing with calcium L-threonate were 14.51 ± 4.06 and 14.92 ± 1.11 in the low-dose group, and 13.98 ± 3.72 and 16.13 ± 1.70 in the high-dose group. The absorption rates were $94 \pm 6\%$ and $95 \pm 3\%$, the availabilities were $89 \pm 4\%$ and $77 \pm 8\%$, the pure availabilities were $84 \pm 7\%$ and $74 \pm 6\%$, absorption capacity per day was 34 ± 3 mg and 58 ± 5 mg, and relative calcium absorption was 168 mg/kg bw and 293 mg/kg bw in the low- and high-dose groups, respectively. The daily excretion of calcium from faeces was 4 ± 3 mg/day and 4 ± 2 mg/day in the low- and the high-dose groups. The daily excretion of calcium from the urine was 4 ± 2 mg/day and 18 ± 3 mg/day. The authors of the study concluded that there were no significant differences between the absorption rates at the two dose levels but the availability was higher for the low dose group than the high dose group. The pure availability of the calcium was over 70% in both groups but the low dose group had the higher pure availability (Jiao *et al.*, 1996b; Jiao).

Studies in dogs

Pharmacokinetic parameters of calcium L-threonate and of other sources of calcium were studied in dogs. The kinetic parameters are presented in Table 1.

Table 1. Comparison of pharmacokinetic parameters between calcium L-threonate, calcium gluconate, calcium acetate, and calcium carbonate as determined in dogs.

Parameter	Unit	Calcium L-threonate	Calcium gluconate	Calcium acetate	Calcium carbonate
T_{max}	hours	0.818	0.359	0.540	0.905
C_{max}	µg/ml	27.37	32.64	25.55	22.71
T_{1/2}	hours	4.300	2.975	0.527	0.727
AUC	(µg/ml)·hours	188.26	146.17	29.88	55.15

T_{max}: the time to the peak concentration; **C_{max}**: the observed peak concentration; **T_{1/2}**: elimination half-life, the time taken for the post-peak blood or plasma concentration to halve; **AUC**: the area under the concentration-time curve.

The data demonstrated that the bioavailability of calcium from calcium L-threonate and calcium gluconate was greater than from calcium acetate or calcium carbonate (Anonymous b).

3.1.3 Other studies indicative of bioavailability

Anti-rachitic effect of calcium L-threonate in mouse rachitis model

The petitioner submitted a report concerning the anti-rachitic effect of calcium L-threonate. Weaned Kunming mice were housed in a dark room and maintained on a vitamin D-deficient low-calcium diet and deionised water for 60 days in order to establish a rachitis model. Thereafter the mice (N=18/group) were fed the same diet but received a treatment either with calcium L-threonate in doses equivalent to 300 or 200 mg calcium/kg bw/day, or with calcium gluconate in a dose equivalent to 300 mg calcium, 6 days per week during 51 days. In addition a model control group (mice continuing on the vitamin D-deficient low-calcium diet), and a normal control group (mice maintained on a micro- and macronutrient balanced diet and tap water from weaning) were included. The concentrations of calcium, inorganic phosphorus and alkaline phosphatase in mice treated with calcium L-threonate or calcium gluconate were comparable to those in the normal controls, being statistically significantly higher compared to the values in the model control group. The femoral dry weight, ash content in femur, femoral calcium sedimentation and tibial strength in the calcium L-threonate treated mice were close to the normal values, and significantly higher than in the model control group. These parameters (except for femoral dry weight) were also similar to the normal values for the calcium gluconate treated mice, and significantly higher than in the model control group.

According to the authors of the study, these results indicated that calcium L-threonate could effectively control the loss of bone quantity, prevent the occurrence and development of rachitis and result in higher bone calcium sedimentation than calcium gluconate (Yuan *et al.*, 1997b).

3.2 Metabolic fate of the source and biological distribution

Although the petitioner provided no specific studies on the metabolic fate of the source, the Panel noted that threonate is a normal constituent of the body. For example, it has been identified in human plasma and urine. Threonate typically arises from the catabolism of ascorbic acid (EFSA, 2007).

The tissue distribution and excretion of calcium from the source was investigated in rats using a ⁴⁵Ca tracing technique. Pharmacokinetic parameters were also measured in this study. Three groups of Wistar rats (3 males and 3 females/group) were randomly allocated a dosage of ⁴⁵Ca-labelled calcium L-threonate preparations providing either 100 mg calcium/kg bw (low dose), 200 mg calcium/kg bw (middle dose) or 300 mg calcium/kg bw (high dose). The doses were administered orally after fasting for 16 hours. Blood, urine and faeces were collected at several time points up to 24 hours after administration. The calcium content of heart, liver, spleen, kidney stomach, intestines, brain, femur and sternum was examined at different time points (0.5, 1, 4, and 8 hours) after oral dosing of 200 mg calcium/kg bw. The content of calcium as a measure of calcium deposition in the sternum, femur, jaw and teeth was

measured at 1, 4 and 24 hours after oral administration of the source at the doses providing 100, 200 or 300 mg calcium/kg bw.

The pharmacokinetic parameters for calcium after the low, middle and high dose were: the time to peak concentration (T_{max}): 1.5 h, 1.4 h and 1.2 h; the observed peak concentration (C_{max}): 26, 25, 20 µg/ml; the area under the concentration-time curve (AUC): 359, 317, 217 (µg/ml)·hours.

Twenty-four hours after oral administration of 200 mg/kg bw, approximately 30% of the calcium was excreted in the urine, around 40% was eliminated in the faeces, and about 10 - 20% remained in the blood and bone, and about 10% was stored in organs.

The calcium levels in the heart, liver, spleen, kidney and brain tissues were not increased significantly after oral dosing of 200 mg calcium/kg bw as calcium L-threonate. A significant increase in calcium was found in the sternum (10 and 43 µg/g tissue at 0.5 hours and 8 hours after dosing, respectively) and femur (21 and 38 µg/g tissue at 0.5 hours and 8 hours after dosing, respectively). An increased concentration of calcium was also observed in jaw, incisor and molar teeth (Li *et al.*, 2001; Li and Niu).

3.3 Toxicity data

3.3.1 Acute toxicity

Calcium L-threonate was not toxic when administered orally by gavage at a daily dose of 40 g/kg bw given as two doses of 20 g/kg bw six hours apart to 10 male and 10 female Kunming mice observed for 10 days (Gao *et al.*, 1997a).

Calcium L-threonate was not toxic when administered orally by gavage at a daily dose of 32 g/kg bw given as two doses of 16 g/kg bw six hours apart to 10 male and 10 female Wistar rats observed for 10 days (Gao *et al.*, 1997a).

3.3.2 Subchronic and chronic toxicity

Calcium L-threonate was administered orally by gavage to rats in doses of 0, 2, 4, 6 g/kg bw/day, six days per week, for 24 consecutive weeks. The number of animals was 15/sex in the control and high-dose groups, and 10 animals/sex in the low- and middle-dose groups. Feed and water were supplied *ad libitum*. After 12 weeks of treatment, 5 rats/sex from both the control and high-dose groups were euthanized. For the remaining animals the treatment was terminated after 24 weeks and 7 rats/sex/group were euthanized, while the remaining 3 rats/sex/group were observed for the following 3 weeks (recovery phase). All animals were observed daily for any changes in clinical appearance, weighed once a week during the first 12 weeks of treatment and once every second week thereafter. Blood samples for haematology (red blood cell count, white blood cell count and classification, haemoglobin, platelet count and coagulation time) and clinical chemistry (urea, nitrogen, bilirubin, total protein, albumin, total cholesterol, blood sugar, alkaline phosphatase, glutamic oxalacetic transaminase, glutamate pyruvate transaminase, creatinine) investigations were collected after 12 weeks (the control and high dose groups, only), 24 weeks and at the end of the recovery period.

No mortality was recorded during the study. A decreased spontaneous motor activity and loose stools were recorded on several occasions in some of the animals from the high-dose

group. The body weight of the animals in the low and medium dose groups were comparable to that in the control group. In the high-dose group the terminal body weight was not statistically significantly different compared to the controls, but the body weight of females was statistically significantly lower from week 4 to 22, and for the males from week 4 to 8. The only difference in the haematological parameters was a significantly ($p < 0.01$) shorter coagulation time in the high dose group males (134 sec. \pm 33) and females (164 sec. \pm 42) compared to the controls (males: 222 sec. \pm 33; females: 217 sec. \pm 36). No significant differences were recorded between the treated and control rats of both sexes in the blood chemistry parameters after 12 and 24 weeks and at the end of the recovery period. Gross examination revealed the presence of gas and yellow liquid in the intestines of animals of either sex in the high-dose group. No abnormalities or histopathological changes were found in the low- and medium-dose groups of both sexes. In the high-dose group a mild thyroid gland accretion was observed in males but this change was not recorded after the recovery period. The authors of the study argued that the effects on the coagulation time and the thyroid gland were due to high calcium intake. The Panel concluded that the NOAEL was 4 g/kg bw/day (Gao *et al.*, 1998).

Calcium L-threonate was administered orally to hybrid dogs in doses of 0, 1, 2, or 3 g/kg bw/day, six days per week, for 24 consecutive weeks. The number of animals was 2/sex in the control and high-dose groups, 2 males and 3 females in the low-dose group, and 3 males and 2 females in the middle-dose group. Daily observations included general appearance, psychomotility, appetite, feed and water intake, urine and faeces. Blood samples for haematology (red blood cell count, white blood cell count and classification, haemoglobin, platelet count and coagulation time) and clinical chemistry (alanine aminotransferase, aspartate aminotransferase, total bilirubin, total protein, albumin, blood urea nitrogen, creatinine cholesterol, glucose) were collected prior to treatment, after 2, 4 and 6 months, and two weeks after the last administration (recovery phase). Electrocardiograms were taken before administration and after 3 and 6 months, and two weeks after the last administration (recovery phase). Two to three animals from each group were killed at the end of the treatment period and the remaining animals were killed two weeks after the last administration (recovery phase).

No mortalities were reported, and no differences compared to the controls were recorded for the measured parameters and at necropsy. The only histopathological finding attributable to the treatment was slight hyperplasia of the thyroid gland, which was found in the medium and high dose groups. In these animals there was an apparent decrease or absence of gelatinous substance in follicles and cubical or columnar epithelial cells. Exfoliated cells were also found in some follicles. These effects were found to be reversible.

The authors of the study argued that the effects in thyroid gland at 2 and 3 g/kg bw dose levels were due to the high calcium intake (Zhao *et al.*, 1997). The Panel concluded that the NOAEL was 1 g calcium L-threonate/kg bw/day.

3.3.3 Genotoxicity

The data on potential genotoxicity of calcium L-threonate submitted by the petitioner comprised the reports of an Ames test, a test of *in vitro* mammalian chromosome aberration in Chinese hamster cells and an *in vivo* mouse bone marrow micronucleus assay.

The mutagenic potential of calcium L-threonate was tested in the Ames test with and without metabolic activation (S9 mix) using *S. typhimurium* strains TA 97, TA 98, TA 100, and TA

102. Calcium L-threonate was tested at concentrations of 0, 10, 100, 1000, 2500, and 5000 µg/plate. Calcium L-threonate was found to be not mutagenic under the conditions of the assay (Gao *et al.*, 1997b).

In its opinion on calcium ascorbate with a content of threonate, the AFC Panel has previously evaluated results of two other studies of the mutagenic potential of calcium L-threonate monohydrate in the Ames test. In these studies concentrations up to 10000 µg/plate were used. Calcium L-threonate monohydrate was found to be not mutagenic under condition of the tests (EFSA, 2007).

In an *in vitro* chromosomal aberration assay using Chinese hamster cells, calcium L-threonate at concentrations up to 2.5 mg/ml, both in the absence and in the presence of an exogenous source of metabolic activation, demonstrated no genotoxic activity (Gao *et al.*, 1997c).

In an *in vivo* mouse bone marrow micronucleus assay, calcium L-threonate in doses of 5, 10 or 20 g/kg bw administered by oral gavage to groups of 6 male mice did not induce any changes in the frequency of micronucleated polychromatic erythrocytes (Gao *et al.*, 1997d).

3.3.4 Carcinogenicity

No studies investigating carcinogenicity of calcium L-threonate have been provided by the petitioner.

3.3.5 Reproductive and developmental toxicity

Calcium L-threonate was administered by gavage in doses of 0, 2, 4 or 6 g/kg bw/day to groups of 20 Kunming male mice for 60 days, and to groups of 20 female Kunming mice for 14 days prior to mating one-to-one. The treatment continued for the pregnant females through the period of organogenesis. The dams were sacrificed on day 21 of gestation. Pregnancy rate, number of living fetuses, number of dead fetuses, weight of the living fetuses, implantation numbers and the number of resorptive fetuses were recorded, and viable fetuses were examined for external abnormalities. Thereafter, half of the fetuses were sectioned for examination of visceral alterations and the remaining were processed and examined for skeletal abnormalities.

Calcium L-threonate in doses up to 6 g/kg bw/day had no effect on body weight and on reproductive performance of the F₀ generation. The authors of the study reported that no external, visceral and bone malformations were seen in the fetuses but these results were not presented in the report (Wu *et al.*, 1997a).

Calcium L-threonate was administrated orally by gavage to groups of 20 pregnant Kunming mice at doses of 0, 2, 4, and 6 g/kg bw/day on days 6-15 of gestation. Body weights of dams were recorded on days 0, 3, 7, 10, 13, 16, and 20. The dams were sacrificed on day 21 of gestation. The number of implantations, resorptions, dead and viable fetuses, the weight of viable fetuses, and external abnormalities were recorded. Half of the fetuses were sectioned for examination of visceral alterations. The remaining fetuses were examined for skeletal abnormalities.

Treatment with calcium L-threonate at doses up to 6 g/kg bw/day did not affect the clinical appearance or body weight of the dams. The number of implantations, resorptions, and dead and viable fetuses, as well as body weights of viable fetuses in the test groups was comparable to those in the control group. The authors of the study reported that there were no

external abnormalities, no visceral alterations or skeletal abnormalities attributable to the treatment, but the results were not presented in the report. The authors of the study concluded that calcium L-threonate was not teratogenic under conditions of this assay (Wu *et al.*, 1997b).

Calcium L-threonate was administered orally by gavage to groups of 20 pregnant Kunming mice at doses of 0, 2, 4, and 6 g/kg bw/day from day 15 of gestation to the end of weaning (day 21 post delivery). Body weights of the dams were recorded on days 0, 3, 7, 10, 13, 16, and 20 of gestation. Other observations included duration of gestation, the number of live and dead pups, external abnormalities, and indices of physiological and behavioural development. Sixty days old offspring (F₁ generation) were mated one-to-one within the same treatment group to assess their reproductive performance.

Calcium L-threonate up to 6 g/kg bw/day had no effect on the length of gestation, litter size, number of live or dead fetuses, survival rate, developmental parameters, behaviour or reproductive performance of the F₁ generation. Behavioural studies in the form of a net-climbing test showed no significant difference between each of the test groups and the control group. A coordination test using the rotating rod method also indicated that there was no significant difference between the groups. Furthermore, calcium L-threonate had no effect on mebunal-induced sleeping time in the animals of the F₁ generation (Wu *et al.*, 1997c).

The results from these studies indicated that calcium L-threonate had no adverse effect on fertility and the developing fetus.

3.3.6 Special studies

The possible pharmacological effects of calcium L-threonate were investigated in two studies, one on the nervous system of Kunming mice and the other on the cardiovascular and respiratory systems of conscious or anaesthetised hybrid dogs (Tang *et al.*, 1998). Calcium L-threonate at dose levels of 0.77-6.16 g/kg bw (equivalent to 100-800 mg calcium/kg bw), had no effect on voluntary behaviour and Nembutal sleeping period of the mice. In addition calcium L-threonate at dose levels of 0.385-1.54 g/kg bw (equivalent to 50-200 mg calcium/kg bw) had no effect on blood pressure, heart rate, electrocardiogram, respiration frequency and depth in anaesthetised dogs, and on heart rate and blood pressure in conscious dogs.

4. Discussion

Human and animal studies indicate that calcium is absorbed from orally ingested calcium L-threonate. In animal studies, the bioavailability of calcium from calcium L-threonate was comparable to or higher than that from other sources.

Although, no data were provided on the metabolic fate of threonate, the Panel noted that threonate is a normal constituent of the body. For example, it has been identified in human plasma and urine. Threonate typically arises from the catabolism of ascorbic acid.

Acute oral toxicity studies revealed that calcium L-threonate is of low toxicity, with no adverse effects observed at doses as high as 40 g/kg bw in mice or 32 g/kg bw in rats.

In sub-chronic studies with calcium L-threonate, the Panel identified a NOAEL of 4 g/kg bw/day in the rat with regard to its effect on blood coagulation time and accretion of the thyroid gland, and of 1 g calcium L-threonate/kg bw/day in the dog with regard to hyperplasia

of the thyroid gland. The Panel noticed that the effects on blood coagulation time and the thyroid gland were reversible and that a mild accretion in the thyroid gland in rats was limited to males only. The Panel further noticed that these effects were likely to be attributed to the high dosage of calcium administered over a long period. A high concentration of calcium ions can result in accelerated blood coagulation. It can influence intestinal absorption of iodine and reduce/suppress the secretion of thyroxin by the thyroid gland. The NOAELs are equivalent to 516 mg calcium/kg bw/day and 3484 mg L-threonate/kg bw/day in the rat and to 129 mg calcium/kg bw/day and 871 mg L-threonate/kg bw/day in the dog.

Studies using different test systems *in vitro* and *in vivo* indicated that calcium L-threonate was not genotoxic. Although no carcinogenicity studies were available, the Panel considered that such studies were not needed given that L-threonate is an endogenous compound in the body and that calcium L-threonate did not show any genotoxic potential.

Reproductive and developmental toxicity studies in mice indicated that calcium L-threonate in doses up to 6 g/kg bw/day had no adverse effect on the fertility and on the developing foetus, nor did it cause maternal toxicity.

The SCF has established a tolerable upper intake level of 2500 mg/day for calcium from all sources for adults. The petitioner proposed use levels of 2 - 4 tablets/person/day of calcium L-threonate with each tablet providing 100 mg calcium and 675 mg threonate. The exposure to calcium through the use and at the use levels of calcium L-threonate proposed by the petitioner, may lead to exposures of 200-400 mg calcium/person/day, which would not represent a safety concern.

Data on dietary intakes of L-threonate were not available. L-threonate may occur in certain foods. For instance, L-threonate is a breakdown product of ascorbic acid during food preparation. Exposure to L-threonate at the uses and use levels indicated by the petitioner is estimated to amount to 1350 – 2700 mg L-threonate per person per day corresponding to 22.5 – 45 mg/kg bw/day for a 60 kg person. The margin of safety between the estimated human exposure to L-threonate and the amount of L-threonate equivalent to the NOAELs for calcium L-threonate, as demonstrated in sub-chronic toxicity studies in dogs and rats, is 39 – 19 for the dog and 155-77 for the rat. The Panel considers this margin of safety to be sufficiently large given that threonate is an endogenous compound in the body, and that the NOAELs in the dog and rat studies were identified for effects attributable not to L-threonate but to the calcium dosages.

CONCLUSIONS

The present opinion deals only with the safety and bioavailability of a particular source of calcium, calcium L-threonate, to be used as a nutritional substance in food supplements. The safety of calcium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

The Panel noted that the foreseen supplementation with calcium L-threonate will not exceed the tolerable upper level for calcium established in Europe for adults.

The Panel concludes that calcium is bioavailable from calcium L-threonate and that the use of calcium L-threonate, as a source of calcium, in food supplements for the uses and at the use levels proposed by the petitioner is not of safety concern.

DOCUMENTATION PROVIDED TO EFSA

Dossier on calcium L-threonate proposed for addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council relating to food substances submitted by Biocalth UK Limited, May 2005. United Kingdom.

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GLOSSARY / ABBREVIATIONS

ADI	Acceptable Daily Intake
ADI not specified	It is a term used when, on the basis of the available toxicological, biochemical and clinical data, the total daily intake of the substance, arising from its natural occurrence and/or its present use in food at the levels necessary to achieve the desired technological effect, will not represent a hazard to health
AFC	Scientific Panel on food additives, flavourings, processing aids and materials in contact with food
ATP	Adenosine triphosphate
AUC	The area under the concentration-time curve
CAS	Chemical Abstract Service
C _{max}	The observed peak concentration
EC	European Commission
EFSA	European Food Safety Authority
NOAEL	No-Observed-Adverse-Effect Levels
RH	Relative Humidity
SCF	Scientific Committee on Food
T _{max}	The time to the peak concentration