European Food Safety Authority

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SCIENTIFIC OPINION

Choline-stabilised orthosilicic acid added for nutritional purposes to food supplements [1]

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food

(Question No EFSA-Q-2006-189)

Adopted on 28 January 2009

PANEL MEMBERS

F. Aguilar, U.R. Charrondiere, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Guertler, G.E.N. Kass, J. Koenig, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R.A. Woutersen.

SUMMARY

Following a request from the Commission to the European Food Safety Authority, the Scientific Panel on Food Additives and Nutrient Sources added to Food was asked to provide a scientific opinion on the safety of choline-stabilised orthosilicic acid (ch-OSA) added for nutritional purposes as a source of silicon in food supplements and on the bioavailability of silicon from this source.

Choline-stabilised orthosilicic acid is a mixture of orthosilicic acid and choline chloride.

The present opinion deals only with the safety of ch-OSA as source of silicon and with the bioavailability of silicon from this source. The safety of silicon itself, in term of amounts that may be consumed and the consideration of silicon as a nutrient are outside the remit of this Panel.

Silicon occurs naturally in foods as silicon dioxide (SiO_2) and silicates. High levels of silicon are found in foods derived from plants, particularly cereals. Silicon levels are lower in foods from animal sources.

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on choline-stabilised orthosilicic acid added for nutritional purposes to food supplements following a request from the European Commission. *The EFSA Journal* (2009) 948, 1-23.

Orthosilicic acid [$Si(OH)_4$] is the major silicon species present in drinking water and other liquids, including beer, and is the most readily available source of silicon to man.

The essentiality of silicon for man has not been established and a functional role for silicon in man has not yet been identified.

The bioavailability of silicon under the form of orthosilicic acid has been proven for choline-stabilised orthosilicic acid.

Acute oral toxicity studies in male and female rats have been performed. The calculated mean LD_{50} value for ch-OSA exceeds (for both animal species) 5000 mg/kg bw.

Concerning the acute oral toxicology of choline, an LD_{50} value of 6640 mg/kg bw as choline chloride was found.

Subchronic toxicity studies on ch-OSA were conducted both in animals (rodents and mammals) and humans (supplementation studies). No adverse effects were observed.

The proposed dosage of ch-OSA in food supplements is 5 to 10 mg silicon/day (equivalent to 0.083-0.17 mg silicon/kg bw/day for a 60 kg person). The equivalent intake of choline amounts to 101-203 mg choline/day for the ch-OSA liquid, and to 117-234 mg choline/day for the ch-OSA pellets. This results in an intake of 135-272 mg choline chloride/day for ch-OSA liquid and of 157-314 mg choline chloride/day for ch-OSA pellets.

The European Food Safety Authority estimated the typical dietary intake of silicon to be 20-50 mg/day, corresponding to 0.3-0.8 mg silicon/kg bw/day for a 60 kg person, and concluded that these intakes are unlikely to cause adverse effects.

The Panel concludes that silicon is bioavailable from choline-stabilised orthosilicic acid and that its use in supplements, at the proposed use levels of the source, is of no safety concern provided that the upper level for choline is not exceeded.

Key words:

Choline-stabilised orthosilicic acid, silicon CAS No. 7440-21-3, orthosilicic acid CAS No. 10193-36-9, choline chloride CAS No. 67-48-1.

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BACKGROUND AS PROVIDED BY THE COMMISSION

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

The Commission has received a request for the evaluation of choline-stabilised orthosilicic acid added for nutritional purposes to 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[2].

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of choline-stabilised orthosilicic acid added for nutritional purposes to food supplements.

ACKNOWLEDGEMENTS

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[2] OJ L 183, 12.7.2002, p.51.

ASSESSMENT

1. Introduction

The present opinion deals only with the safety of choline-stabilised orthosilicic acid (ch-OSA) as a source of silicon and with the bioavailability of silicon from this source. The safety of silicon itself, in terms of amounts that may be consumed, and the consideration of silicon as a nutrient are outside the remit of this Panel.

2. Chemical data

2.1. Chemistry

Choline-stabilised orthosilicic acid (ch-OSA) is a mixture of orthosilicic acid, (CAS Registry number 10193-36-9) and choline chloride (CAS Registry number 67-48-1).

No CAS Registry number is known for 'choline-stabilised orthosilicic acid'.

The molecular formulae of the components are: orthosilicic acid $Si(OH)_4$, molecular weight 96.11 g/mol; choline chloride $[HOCH_2CH_2N^+ (CH_3)_3]Cl^-$, molecular weight 139.63 g/mol.

2.2. Specifications

There are two physical forms of ch-OSA, a liquid form and a pellets form.

The percentage composition of both forms is given in Table 1.

Table 1. **Percentage composition of the ch-OSA formulations as provided by the petitioner.**

Nature of component	ch-OSA liquid	ch-OSA pellets
Microcrystalline cellulose (E460i) (excipients)	none	60 - 65% (w/w)
Choline chloride (stabiliser)	44 - 48% (w/w)	20 - 28% (w/w)
Glycerol (excipient)	33% (v/v)	none
Water (excipient)	13 - 18% (w/w)	4 - 10% (w/w)
Silicon (mainly as orthosilicic acid)	1.7 - 2.3% (w/v)	0.6 - 1.0% (w/w)

The petitioner states that the microcrystalline cellulose complies with the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP).

The Panel notes that the purity criteria for microcrystalline cellulose (E460i), as laid down in Commission Directive 2008/84/EC, are a follows: arsenic, not more than 3 mg/kg; lead, not more than 5 mg/kg; mercury, not more than 1 mg/kg; cadmium, not more than 1 mg/kg; heavy metals (as Pb), not more than 10 mg/kg; particle size: not less than 5 µm (not more than 10 % of particles of less than 5 µm); carboxyl groups not more than 1% (EC, 2008b).

- ***ch-OSA liquid*** is transparent with a light-yellow colour. Its specific gravity is 1130-1180 kg/m^3 and pH 1.0-1.5. ch-OSA liquid is completely soluble in water and there are no concentration limits when water is used as solvent.

  Impurities: lead < 20 µg/L, mercury < 20 µg/L, arsenic < 1 mg/L, cadmium < 20 µg/L.

- ***ch-OSA pellets*** have the following size distribution: 55 % has a particle size between 800 µm and 500 µm; 0.05 % has a particle size larger than 1180 µm; 0.06 % has a particle size below 200 µm. The pellets are of white colour and very hygroscopic. The excipient, microcrystalline cellulose, is not soluble in water but ch-OSA is released when the pellets are mixed with water.

  Impurities: lead < 20 µg/kg, mercury < 20 µg/kg, arsenic < 1 mg/kg, cadmium < 20 µg/kg.

The Panel notes that according to Commission Regulation (EC) No 629/2008 the maximum levels of lead, mercury and cadmium in food supplements as sold should be 3.0 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively (EC, 2008a).

The petitioner provided data of spectroscopic studies on the commercial products. From these studies it can be concluded that Si-C or Si-O-C covalent bonds are not found in ch-OSA. Post ingestion simulation in an artificial gastric fluid indicates the formation of substantial concentrations of orthosilicic acid. NMR analysis, after dilution of a 2 % silicon containing [^{29}Si]-ch-OSA sample in water, demonstrates that the majority of silicon is present as orthosilicic acid. On dilution of ch-OSA in water, the dilute primarily contains orthosilicic acid stabilised with choline chloride. Orthosilicic acid is a soluble form of silica present in mineral water.

The petitioner also provided microbiological specifications.

For *ch-OSA liquid*, the total aerobic bacterial count is < 1 CFU/mL and the fungi < 1 CFU/mL. For *ch-OSA pellets,* the total aerobic bacterial count is < 10 CFU/g and the fungi < 10 CFU/g.

In both commercial products, specified microorganisms (*Escherichia coli*, *Salmonella* sp., Coliforms and /or Enterobacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) are absent (expressed either as absent/mL or absent/g).

2.3. Manufacturing process

ch-OSA liquid

The petitioner describes that choline chloride is treated with dry hydrochloric acid and silicon tetrachloride. Water is added and the ch-OSA containing solution is neutralized by the addition of sodium hydroxide. The solution is concentrated by distillation under vacuum. To this concentrated ch-OSA solution, glycerol is added. The final product is analysed for its silicon, choline chloride, heavy metal (As, Cd, Hg, and Pb) concentration and for its microbiological load.

ch-OSA pellets

As for ch-OSA liquid, choline chloride is treated with dry hydrochloric acid and silicon tetrachloride. Water is added and the ch-OSA containing solution is neutralized by adding sodium hydroxide. The solution is concentrated by distillation under vacuum.

To this concentrated solution, microcrystalline cellulose is added under continuous mixing. Water is also added to obtain the desired granulate properties. The wet mass is extruded and the extrudate is spheronised[3]. The resulting pellets are dried. The final product is also analyzed for its silicon, choline chloride, heavy metal (As, Cd, Hg, and Pb) concentration and for its microbiological load.

2.4. Methods of analysis in food

Silicon can be determined by Electrothermal Atomic Absorption Spectrometry (ETAAS) with inverse longitudinal Zeeman background correction. Choline chloride is analysed by Liquid Chromatography - Mass Spectrometry (LC-MS) (Grumbach *et al.*, 2004).

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

The petitioner indicates that ch-OSA liquid is marketed in a drop dispensing bottle and is diluted in beverages (water, juice) prior to use. The ch-OSA pellets are pre-dosed in hard gelatine or a hypromellose EP/USP (hydroxypropyl-methylcellulose, CAS No. 9004-65-3) capsule or as tablets.

The petitioner provided data on the stability of ch-OSA. Jellification, silicon concentration and choline chloride concentration were evaluated during incubation at 60 % relative humidity (RH) and 25 °C. The supplements remained stable within the certified range for up to 3 years.

2.6. Case of need and proposed uses

The petitioner states that the recommended daily dose of ch-OSA, to be used as a source of silicon in food supplements, is 5-10 mg silicon/day. Details are given in Table 2.

Table 2. **Data on dosage of ch-OSA and intakes as provided by the petitioner**

Silicon daily dose (mg/day)	ch-OSA liquid		
	Volume (mL/day)	Choline intake (mg/day)	Choline chloride intake (mg/day)
5	0.25	101	135
10	0.50	203	272

[3] Spheronisation is a process by which an extrusion (paste) product is mechanically broken up into small segments which are then loaded onto a rotating frictional plate where the paste segments are rounded into spherical pellets. To achieve product dose uniformity and reproducibility well-formed spheres of narrow size distribution (0.7 mm to 3.0 mm) should be generated.

Silicon daily dose (mg/day)	ch-OSA pellets		
	Quantity (g/day)	Choline intake (mg/day)	Choline chloride intake (mg/day)
5	0.56	117	157
10	1.12	234	314

2.7. Information on existing authorisations and evaluations

Silicon

The essentiality of silicon for man has not been established and a functional role for silicon in man has not yet been identified (EFSA, 2004). In addition, a recommended intake for silicon has not been set (SCF, 1993; IOM, 2000).

In 2004, EFSA concluded there were no suitable dose-response data to establish a Tolerable Upper Intake Level (UL) for silicon, and also IOM reported that due to lack of data indicating adverse effects of silicon it was not possible to establish a UL (IOM, 2000). EFSA estimated the typical dietary intake to be 20-50 mg silicon/day, corresponding to 0.3-0.8 mg silicon/kg bw/day for a 60 kg person, which is unlikely to cause adverse effects (EFSA, 2004).

The Expert group on Vitamins and Minerals (EVM) carried out a risk assessment and set a safe upper level (SUL) for supplemental daily exposure to silicon at 700 mg silicon/day for adults over a lifetime. In terms of elemental silicon, this is equivalent to a safe upper level of 12 mg silicon/kg bw/day for a 60 kg adult for supplemental silicon (EVM, 2003).

Silicon in the form of silica (E551), silicates and dimethylpolysiloxane is added as an excipient to foods, i.e. anti-caking and anti-foaming agent.

Choline

There is little information about requirements for choline for most age and gender groups. The US Food and Nutrition Board has set an Adequate Intake (AI) level of 550 and 425 mg choline/day for men and women, respectively (IOM, 1998)

The UL of choline for adults is 3.5 g/day (IOM, 1998).

The petitioner stated that choline chloride is considered by the US Food and Drug Administration (FDA) as Generally Recognised As Safe (GRAS.

2.8. Exposure

Silicon

High levels of silicon are found in foods derived from plants, particularly cereals such as oats (3910 -4310 mg/kg dry weight), barley (2610 - 2720 mg/kg dry weight), white wheat flour (81 - 103 mg/kg dry weight) or polished rice (55 - 57 mg/kg dry weight). Silicon levels are lower in foods from animal sources like meat or dairy products (milk, 25 – 27 mg/kg dry

weight) (Bowen *et al.,* 1984). Beer is also a rich source of silicon (9 - 39 mg silicon/L) (Sripanyakorn *et al.*, 2004). In drinking and mineral water, silicon is found as orthosilicic acid in the range of 2 to 5 mg silicon/L (Barnett *et al.,* 1969; EVM, 2003).

There is little information on the intake of dietary silicon. In Finland, a mean intake of 29 mg silicon/day was reported (Varo *et al*., 1980). The intake from the British diet has been estimated to be 20-50 mg silicon/day (Bellia *et al*., 1994; Bowen *et al*., 1984, Pennington, 1991), corresponding to 0.3-0.8 mg/silicon/kg bw/day for a 60 kg person. These data are in the same range as mean estimated silicon intakes in the USA (30 and 33 mg silicon/day in men, and 24 and 25 mg silicon/day in women in two cohorts, respectively) (Jugdaohsingh *et al*., 2002).

Choline

Choline is widely distributed in foods, mainly in the form of phosphatidylcholine in membranes. Foods that are especially rich in choline compounds include milk, liver, eggs, and peanuts (IOM, 1998).

Data on choline intake are scarce. Estimated average choline dietary intake in adults consuming a typical US or Canadian diet (as free choline and the choline in phosphatidylcholine and other choline esters) is approximately 730-1040 mg/day (7 to 10 mmol/day) (LSRO/FASEB, 1981; Zeisel, 1981). There are no recent national representative estimates of choline from foods or food supplements (IOM, 1998).

As indicated by the petitioner, the dosage of ch-OSA to be used in food supplements is 5 to 10 mg silicon/day (equivalent to 0.083-0.17 mg silicon/kg bw/day for a 60 kg person). The equivalent intake of choline amounts to 101-203 and to 117-234 mg choline/day for the ch-OSA liquid and the ch-OSA pellets, respectively. On that basis the intake of choline chloride amounts to 135-272 mg choline chloride/day (ch-OSA liquid) and 157-314 mg choline chloride/day (ch-OSA pellets).

The Panel notes that according to the EVM (2003), the highest reported silicon content in nutrient supplements on the market is 500 mg. If all the silicon in food supplements would be provided entirely by choline-stabilised orthosilicic acid (liquid form or pellets form) the equivalent intake of choline would be between 10.2-11.7 g choline/day (equivalent to 169-195 mg choline/kg bw/day). The equivalent intake of choline chloride would then be 13.6-15.7 g/day. In that case, the UL for choline (3.5 g/day) would be exceeded.

3. Biological and toxicological data

3.1. Bioavailability

Introduction

Silicon occurs in nature as silica (SiO_2) or the corresponding silicic acids formed by hydration of silicon dioxide. Orthosilicic acid [$Si(OH)_4$] is the simplest acid and the main silicon species soluble in water. Supersaturation causes it to dehydrate and polymerise into less soluble forms.

The bioavailability of silicon depends on the solubility or speciation of the compound concerned (Carlisle, 1997).

Popplewell *et al.* (1998) used ^{32}Si labelled ammonium silicate to determine silicon in urine in one healthy male. Thirty-six percent of the ingested dose was absorbed and nearly completely excreted in the urine within 48 h. Two first-order phases of elimination with half-lives of 2.7 and 11.3 h were found.

Reffitt *et al*. (1999) studied silicon kinetics following intake of orthosilicic acid in water (27-55 mg silicon/L) in healthy individuals (6 men, 2 women). Based on urinary excretion, the uptake of orthosilicic acid was about 50 % (range of uptake was between 21 and 74%). The authors reported that after food consumption, there is a 4 h delay before peak silicon absorption appears in the serum, compared to 1 h after orthosilicic acid intake.

Jugdaohsingh *et al*. (2000) compared the bioavailability of orthosilicic acid and polymeric silicic acid. Following administration of orthosilicic acid, 53 % was excreted in the urine, whereas the ingestion of polymeric silicic acid only caused a marginal increase of silicon in the urine.

Sripanyakorn *et al*. (2004) studied the orthosilicic acid content of beer as well as its bioavailability from beer. The orthosilicic content of beer was found to be 80 % of the total silicon content. The absorption of silicon from beer was 55 %, which was comparable with that of a solution of orthosilicic acid, suggesting that silicon in beer is present mainly as orthosilicic acid and is readily bioavailable. This study is consistent with the finding by Bellia *et al*. (1994) who reported that 42-72 % of silicon in beer is excreted in urine.

These studies illustrate that orthosilicic acid is readily absorbed from the gastrointestinal tract in humans and then readily excreted in urine. Thus the quantity of orthosilicic acid liberated from a specific source of silicon could form the basis for the evaluation of the bioavailability of silicon from that source.

Bioavailability of silicon from choline-stabilised orthosilicic acid

Human studies.

The petitioner provided data to prove that incubated dilutions of ch-OSA in water contain primarily silicon in the orthosilicic acid form (Vanden Berghe, 2000).

The absorption of silicon from ch-OSA was studied in a cross-over protocol with 14 healthy subjects (8 females and 6 males, aged 22-34 years). None of them had taken silicon supplements for 3 months before the start of the study. Each fasting subject received orally successively 20 mg silicon in the form of ch-OSA, 20 mg stabilised monomeric silicic acid, herbal silica (533 mg of a dry *Equisetum arvense* extract containing 8% w/w of silicon dioxide), colloidal silicic acid (2 mL of a solution containing 28 g of H_2SiO_3/L) or a placebo (10 mL mineral water) with 1 week wash-out between each supplement or placebo.

A significant increase in serum silicon concentration from the baseline value was observed for ch-OSA and the peak silicon concentration was earlier compared to the mineral water placebo (1 and 4 h respectively). The mean area under the time curve to 8 h (AUC_{0-8}) was significantly higher after ch-OSA supplementation ($p<0.005$), compared to the placebo.

A significant correlation was found between the individual under the time curves (AUC) and the individual urinary silicon concentration. Urinary silicon excretion was significantly higher after ch-OSA supplementation compared to placebo.

A significant correlation was found between the individual AUC and the individual urinary silicon concentration ($r = 0.28$, $p<0.05$, $n=56$). The urinary silicon excretion was significantly higher after ch-OSA supplementation ($p<0.005$) but was not significantly different for respectively herbal silica and colloidal silica compared to the placebo (Calomme *et al.,* 1998).

The petitioner concluded that the studies illustrated that the bioavailability of silicon was to a great extent dependent on the chemical form of the silicon compound. The absorption was faster, higher and much less subject-dependent in the case of ch-OSA, compared to phytolytic silica[4] and colloidal silicic acid which contain polymerized forms of orthosilicic acid. No ch-OSA related side effects were observed.

In a study by Vanden Berghe (2001), the bioequivalence of ch-OSA liquid as compared to that of ch-OSA pellets was studied. Twelve healthy volunteers received orally 10 mg silicon in the form of ch-OSA liquid or ch-OSA pellets. The increase in silicon concentration in the serum of the volunteers was comparable after intake of ch-OSA pellets or ch-OSA liquid. The relative bioavailability calculated as AUC was not different for ch-OSA pellets compared to ch-OSA liquid (765 ±407 µg h/L versus 748 ± 314 µg h/L, respectively).

In another comparative cross-over study (Van Dyck *et al*., 1999) the bioavailability of ch-OSA was compared to that of the herbal extract of *Equisetum arvense* and a silicon-rich diet. The study tested the three different silicon sources in one healthy female test subject. The silicon content of blood and serum samples was determined. For each silicon source, the experiment started with a 4-6 days period during which the test subject was given a normal diet (silicon intake: ± 14 mg silicon/day). During the first experiment this blank period was followed by 31 days of a silicon rich diet (silicon intake: ± 45 mg silicon/day), followed by a wash out period of 5 days (normal diet administration). In the second experiment silicon, in the form of phytolytic silica, was administered (silicon intake: 23 mg silicon/day; 7days), while in the third experiment silicon in the form of ch-OSA was administered (silicon intake: 10 mg silicon/day; 7days).

The authors indicate that strongly diverging results were obtained for the three different sources. No increase in the urinary silicon content was observed when administering the silicon rich diet. Urinary silicon excretion did rise significantly ($p<0.05$) during supplementation with tablets containing dry extract of horsetail. Intake of a solution of ch-OSA resulted in a significantly ($p<0.05$) increased urinary silicon excretion and serum silicon content, showing a higher bioavailability of ch-OSA compared to dietary silicates. The study did also demonstrate that the speciation (chemical form, matrix) strongly influences the bioavailability of silicon.

In addition, in order to prove the bioavailability of ch-OSA the petitioner also referred to a double-blind, placebo-controlled study in which 50 women (mean age ± SD = 50.5 ± 5.5) with photo-damaged facial skin were administered daily 10 mg silicon in the form of ch-OSA pellets (n=25, BMI: 26.3 ± 5.7) or placebo pellets (n=25, BMI: 24.1 ± 4.4, microcrystalline cellulose) for 20 weeks. The serum silicon concentration increased significantly after 20

[4] Supersaturation of orthosilicic acid causes it to dehydrate and to polymerise into less soluble polymeric forms. In plant materials the polymeric forms are called 'phytolytic silicon'.

weeks supplementation in the ch-OSA group but did not change in the placebo group (Barel, 2004).

Supplementation studies with animals.

The bioavailability of ch-OSA was investigated in a supplementation study with calves. Calves (1-week old) were supplemented for 23 weeks with ch-OSA (n=30) or a placebo (n=30). The mean administered ch-OSA dose in this study was 0.3 mg silicon/kg bw/day. A placebo group was supplemented with a solution of choline chloride in ultra pure water. After the supplementation period with ch-OSA the total dietary silicon intake was increased by 4.9 % resulting in a 1.7-fold higher serum silicon concentration compared to non-supplemented controls ($p<0.0001$). Serum concentrations of calcium, phosphorus, magnesium and the calcium/phosphorus ratio were not statistically different for supplemented versus control (placebo) calves (Calomme and Vanden Berghe, 1997).

Vanden Berghe (2003) investigated the bioavailability of ch-OSA in a supplementation study with pigs. A group of 21 sows were randomized in two groups. Both groups were similar regarding the number of previous litters. One group was supplemented with ch-OSA (0.3 mg silicon/kg bw) during both gestation (16 weeks) and lactation (4 weeks), resulting in a total supplementation period of 20 weeks. The supplementation started 3 days prior to artificial insemination. Serum was collected from randomly chosen piglets and analysed for total calcium, phosphorus, magnesium and silicon. Only the serum silicon concentration was found to be significantly higher (+ 150 %) for piglets from supplemented sows compared to piglets from control sows whereas the levels of calcium, phosphorus and magnesium were not significantly different. The authors concluded that the significant higher silicon concentration in the serum of weanling piglets from supplemented sows compared to controls indicates bioavailability of silicon from ch-OSA and the maternal transfer of the absorbed silicon between sows and their offspring during lactation. The high bioavailability of ch-OSA did not interfere with the calcium, phosphorus and magnesium levels in the serum.

3.2. Subsequent metabolic fate of the source and biological distribution

General information on the metabolic fate of silicon

Silicon is not protein-bound in plasma. In plasma it is believed to exist almost entirely as un-dissociated monomeric silicic acid (Berlyne *et al*., 1986). Silicon concentration in blood remains relatively constant, implying rapid distribution to tissues or excretion into the urine. While early analyses showed that serum contains 50-60 µg silicon/dL (Dobbie and Smith, 1982; Carlisle, 1984), more recent analyses indicate that human serum contains 11-25 µg silicon/dL (Van Dyck *et al.,* 2000; Calomme *et al*., 1998). Exceptions included pregnant women who had very low serum silicon concentrations (3.3-4.3 µg/dL) and infants who had higher concentrations (34-69 µg/dL).

Jugdaohsingh *et al*. (2000) and Reffitt *et al*. (1999) reported that fasting concentrations of silicon in plasma are 5.6-28 µg/dL. After meals, plasma concentrations of silicon increase to 56-84 µg/dL.

The human body contains approximately 1 g of silicon, which is present in various tissues and body fluids (Schmidt, 1998). Connective tissues, including aorta, bone, skin (and its

appendages), tendon and trachea, contain much of the silicon that is retained in the body (Adler *et al*, 1986; Carlisle, 1997). This silicon is thought to be present as a silanolate, an ether- or ester-like derivative of silicic acid cross-linking the structural carbohydrates of connective tissue (Schwarz, 1973; 1978). The silicon content, however, declines with age in aorta, other arterial vessels and skin (Carlisle, 1984).

Loeper *et al*. (1978) reported that the concentration of silicon in the arterial wall of humans decreases with the development of atherosclerosis. Leslie *et al*. (1962) found that although silicon levels decreased in skin, the silicon concentrations of most organs (e.g., kidney, brain, liver, spleen, and lung) increased with ageing.

Absorbed silicon is mainly eliminated via the urine, where it probably exists as orthosilicic acid and/or magnesium orthosilicate (Berlyne *et al.*, 1986; Carlisle, 1997). Goldwater (1936) detected about 9 mg of silicon in urine daily. Kelsay *et al.* (1979) found that men excreted between 12 to 16 mg silicon/day. Berlyne *et al*. (1986) found slightly higher urinary silicon excretion in 23 normal individuals (about 33 mg of silicon/day). Jugdaohsingh *et al*. (2002) reported urinary excretion of silicon around 20 mg/day in young adults.

The upper limits of urinary excretion apparently are set by the rate and extent of silicon absorption, and not by the excretory ability of the kidney, because peritoneal injection of silicon can elevate urinary excretion above the upper limit achieved by dietary intake (Sauer *et al*., 1959). This suggests that silicon homeostasis is controlled more by absorption mechanisms than by excretory mechanisms. The view is supported by the finding that in rats, guinea pigs, cattle and sheep, the urinary excretion of silicon initially increases with an increasing intake of siliceous substances, reaching a maximum that is not exceeded by increasing the intake (Bailey, 1981). In sheep, the amount of silicon increased in the urine when dietary silica increased from 0.10 to 0.94%. A further increase of dietary silica up to 2.84% did not markedly affect the amount of silicon excreted in the urine (Jones and Handreck, 1965). The importance of renal elimination of silicon is highlighted by the finding of higher serum or plasma concentrations of silicon in patients with chronic renal failure compared to healthy controls (Dobbie and Smith, 1986; Gitelman *et al.,* 1992).

As already described above (Popplewell *et al*., 1998), following ingestion of a tracer dose of ^{32}Si labelled ammonium silicate in a healthy male volunteer, uptake was complete within 2 hours. Thirty-six percent of the ingested dose was absorbed and nearly completely excreted in the urine within 48 h. Elimination occurred by two simultaneous first-order processes with half-lives of 2.7 and 11.3 h, representing about 90% and 10%, respectively, of the total output. Popplewell *et al.* (1998) suggested that the rapidly eliminated silicon was probably retained in the extracellular fluid volume, while the slower component may have represented intracellular uptake and release. In a study of silicon kinetics by Reffitt *et al.* (1999), silicon peaked in blood after about one hour following an intake of 27-55 mg silicon as orthosilicic acid in water in eight healthy subjects. Renal clearance was 82-90 mL/min, which is similar to that found by Berlyne *et al.* (1986), suggesting high renal filtration. A significant correlation was found between creatinine clearance and silicon in urine or serum. Overall, absorbed silicon is excreted by the kidneys without accumulation in the body.

Specific information on the fate of silicon from choline-stabilised orthosilicic acid

The petitioner provided data of a speciation study using ^{29}Si-NMR. A ^{29}Si labelled ch-OSA was prepared with [^{29}Si]-SiO_2 resulting in a ch-OSA solution containing ^{29}Si. The ^{29}Si-NMR spectrum of this ch-OSA solution showed that the majority of the silicon was in the othosilicate form. In the study, 40 mg ^{29}Si- ch-OSA were administered to one healthy volunteer (male, age: 41 year, BMI: 22.8, bw: 79 kg). The volunteer fasted overnight and during the entire experiment (6 h after intake). The increase in silicon serum concentration was the highest 2 h after administration of ch-OSA. ^{29}Si-NMR analysis of serum, collected 2 h after administration of ch-OSA, demonstrated that only orthosilicic acid was present. ^{29}Si-NMR analysis of urine also indicated the presence of orthosilicic acid in a sample collected during the first 3 h after intake of ch-OSA.

No other silicon compounds were detected in serum or urine. In a 6-h time frame after administration of ch-OSA, 43.6 % of the administered silicon was excreted in urine. It is concluded that after administration of ch-OSA only orthosilicic acid is found in serum which is excreted as such (Kinrade, 2004).

3.3. Toxicological data

The petitioner states that no complete toxicological evaluation was carried out for ch-OSA since it contains choline and a stabilised form of a natural occurring silicon compound (orthosilicic acid). Instead, the petitioner provided the results of a large number of animal and human supplementation studies.

3.3.1. Animal data

Acute toxicity

Pels Rijcken, (1996) found the LD_{50} value for ch-OSA to exceed 5000 mg/kg bw, for both female and male rats (Wistar Cr1) as well as mice (Crl:NMRI BR).

The oral LD_{50} for choline was found to be 6640 mg/kg bw, expressed as choline chloride (Merck Index, 1989). Consequently, the LD_{50} for ch-OSA is comparable with the LD_{50} of choline.

Subchronic and chronic studies

The petitioner provided data on the effects of ch-OSA supplementation investigated in a number of repeated dosing experiments using different animal species (calves, pigs and rats). These data are summarised below.

The effect of ch-OSA supplementation in young calves was studied. Calves (1 week old) were supplemented for 23 weeks with ch-OSA (n=30) or a placebo (n=30). The mean administered ch-OSA dose in this study was 0.3 mg silicon/kg bw/day. A placebo group received a solution of choline chloride in ultra pure water (dose level not given).

The results showed a comparable growth of the supplemented animals compared to non-supplemented controls without ch-OSA related side effects. The silicon concentration in serum of supplemented calves was significantly higher compared to controls (70 %), after 23

weeks of supplementation, indicating a high bioavailability of silicon from ch-OSA. The supplementation with ch-OSA did not interfere with the calcium, phosphorus and magnesium levels in the serum (Calomme and Vanden Berghe, 1997).

The efficacy of ch-OSA was investigated in a supplementation study with pigs. A group of 21 sows were randomized in two groups. Both groups were similar regarding the number of previous litters. One group was supplemented with ch-OSA (0.3 mg silicon/kg bw) during both gestation (16 weeks) and lactation (4 weeks) resulting in a total supplementation period of 20 weeks. The supplementation started 3 days prior to artificial insemination.

There was no statistical difference between supplemented sows and controls regarding the total number of piglets per litter, the number of living piglets per litter (at birth), the number of dead piglets per litter (at birth) and the bodyweight at birth. No specific side effects were observed due to ch-OSA supplementation in sows and their offspring (piglets).

The authors concluded that the dose of ch-OSA was considered to be safe for the foetus (Vanden Berghe, 2004).

Vanden Berghe (2004) also performed three subchronic dosing experiments with Wistar SPF HAN rats. The age of the rats at the start of each experiment was 9.5 months (first and third experiment) and 8.5 months (second experiment). The ch-OSA dose was given in a 0.1 % citric acid solution (5 mL) prepared daily in ultra pure water. All the rats were housed individually in cages at 25 ºC with a 12h/12h light/dark cycle. Urine was collected in metabolic cages (24 h collection). At the end of each experiment, rats were sacrificed by cardiac puncture to collect serum.

- In the first experiment two doses of ch-OSA were evaluated, i.e. daily oral administration of 1 and 3 mg silicon/kg bw for 32 weeks. Histology of the kidney was performed in addition to the analysis of several biochemical parameters in both urine and serum.
- In the second experiment two doses of ch-OSA were evaluated, i.e. daily administration of 0.2 and 1 mg silicon/kg bw for 29 weeks. An additional group was administered 23.3 mg choline/kg bw which is equivalent to the choline dose present in 1 mg silicon/kg bw from ch-OSA. Several biochemical parameters were analysed both in serum and urine.
- In the third experiment one dose of ch-OSA was evaluated, i.e. daily administration of 1 mg silicon/kg bw for 29 weeks. Several biochemical parameters were analysed both in serum and urine.

The authors concluded that in adult rats (aged 34-38 weeks), when supplemented for 8.5 - 9.5 months with ch-OSA, no specific ch-OSA related side effects were observed for any of the tested dosages in any of the experiments. The bodyweight remained similar for supplemented rats compared to controls of the same age. ch-OSA supplementation resulted in significant higher silicon serum levels and silicon urinary excretion compared to controls. Of all the measured serum parameters only aspartate-amino-transferase (ASAT) and lipase were found to be decreased compared to controls. The authors state that this can be explained by the metabolism of choline, which is present in ch-OSA, since choline supplementation resulted in a similar change in ASAT and lipase. Choline was reported to be involved in lipid metabolism (Zeisel, 2000; Blusztajn, 1998).

Histology of the kidney was evaluated as renal clearance is known to be a major route for silicon excretion, but no signs of nephrotoxicity were found. None of the biochemical

parameters measured in urine was found to be different for supplemented rats compared to age-matched controls.

3.3.2. Human data

A 5-week supplementation study was conducted with 3 healthy volunteers (2 men, 1 woman) to investigate the safety of liquid ch-OSA (Vanden Berghe, 2003). The administered ch-OSA dose was in the range of 0.364-0.256 mg silicon/kg bw. Several haematology (11 parameters) and serology parameters (24 parameters) were measured at baseline and after 5 weeks of supplementation.

Following a 5-week supplementation, all parameters remained within the normal range except for total and HDL cholesterol which were both already higher than the upper limit of the normal range at baseline. Of all the evaluated serum parameters only the magnesium concentration was found significantly lower compared to the baseline level but still within the normal range. No ch-OSA related side effects were reported during this period.

Spector (2003) conducted another supplementation study in 184 women (mean age ± SD: 61 ± 10.4) with documented osteopenia (DEXA of the hip: T-score ≤ -1.5). The study population was randomized in four groups. For a period of 12 months, the study subjects were daily supplemented as follows:

- ***Placebo group:*** calcium/vitamin D_3 (1000 mg calcium as calcium carbonate + 20 µg vitamin D3) + choline (liquid containing 47% choline chloride) as placebo.
- ***ch-OSA dosing group 1:*** Calcium/vitamin D_3 + 3 mg silicon as ch-OSA liquid
- ***ch-OSA dosing group 2:*** Calcium/vitamin D_3 + 6 mg silicon as ch-OSA liquid
- ***ch-OSA dosing group 3:*** Calcium/vitamin D_3 + 12 mg silicon as ch-OSA liquid

Several biochemical parameters were analysed in serum (26 items) and urine (17 items) at baseline and after 12 months treatment. An increase in sodium concentration was found after a 12-month ch-OSA supplementation (3 mg silicon/day) but within the normal range for serum sodium concentration. Women who were supplemented with 6 mg silicon, as ch-OSA, showed a significant increase in copper and magnesium concentration, but within the normal concentration range for these elements in the serum. As a result of the daily vitamin D_3 supplementation a significant increase but within the normal range was found for 25-OH-vitamin D_3 in all groups.

Baseline values of total- and LDL-cholesterol were higher than the upper limit of the normal range in both the placebo and the three ch-OSA groups. The mean serum amylase concentration was outside the normal range in the ch-OSA group receiving 3 mg silicon both at baseline and after 12 months of treatment. The remaining parameters were found within the normal range at baseline and after a 12-month treatment in all groups. No significant differences were found between the groups for urine parameters.

From these studies the authors concluded that oral intake of relatively high doses of ch-OSA during a 5–week period and intake of ch-OSA over a long period (20 weeks to 12 months) did not result in a significant change in biochemical parameters measured in the blood. In these two randomised, placebo-controlled clinical trials, no serious adverse events were observed that were related to ch-OSA.

3.3.3. Toxicological data on choline chloride

Choline chloride does not show a mutagenic, clastogenic or DNA damaging potential when tested *in vitro*; furthermore it has no structural alerts. There is therefore no indication of a genotoxic potential *in vivo*. No developmental toxic effects were observed in mice after oral doses of 1250 mg/kg bw/day on gestation days 1 to 18. Doses above the levels recommended currently (4160 mg/kg bw/day), and associated with maternal toxicity, did produce developmental toxic effects, but these were secondary to the maternal toxicity at the excessive doses used (OECD, 2004).

4. Discussion

Silicon occurs naturally in foods as silicon dioxide (SiO_2) and silicates. High levels of silicon are found in foods derived from plants, and in particular cereals. However, silicon levels are lower in foods from animal sources.

Orthosilicic acid [$Si(OH)_4$] is the major silicon species present in drinking water and other liquids, including beer, and is the most readily available source of silicon to man.

The bioavailability of silicon under the species of orthosilicic acid has been proven for choline-stabilised orthosilicic acid.

According to the petitioner, the proposed dosage of ch-OSA to be used in food supplements is 5-10 mg silicon/day (equivalent to 0.083-0.17 mg silicon/kg bw/day for a 60 kg person). The equivalent intake of choline amounts to 101-203 and 117-234 mg choline/day for the ch-OSA liquid and pellets, respectively. This results in an intake of 135-272 mg choline chloride/day for ch-OSA liquid and of 157-314 mg choline chloride/day for ch-OSA pellets.

CONCLUSIONS

The present opinion deals only with the safety of choline-stabilised orthosilicic acid, in both liquid and pellets form, as source of silicon (Si) and with the bioavailability of silicon from these sources. The safety of silicon itself, in terms of amounts that may be consumed and the consideration of silicon as a nutrient are outside the remit of this Panel.

The Panel concludes that silicon is bioavailable from this source and that its use in supplements, at the proposed use levels of the source, is of no safety concern provided that the upper level for choline is not exceeded.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on choline-stabilised orthosilicic acid proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council relating to Food Supplements. June 2005. Submitted by Bio Minerals n.v., Destelbergen, Belgium.

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GLOSSARY / ABBREVIATIONS

ANS	Panel on Food Additives and Nutrient Sources added to Foods
ASAT	Aspartate Amino-transferase
AUC	Area Under Curve
bw	body weight
CAS	Chemical Abstracts Service
ch-OSA	choline-stabilised orthosilicic acid
EC	European Commission
EFSA	European Food Safety Authority
ETAAS	Electrothermal Atomic Absorption Spectrometry
EVM	Expert group on Vitamins and Minerals
FDA	US Food and Drug Administration
FNB	Food and Nutrition Board
GRAS	Generally Recognized as Safe
IOM	Institute of Medicine
LC-MS	Liquid Chromatography - Mass Spectrometry
LDL	Low Density Lipoprotein
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50 % of treated animals
SCF	Scientific Committee on Food
UL	Tolerable Upper Intake Level