SCIENTIFIC OPINION

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Safety of organic silicon (monomethylsilanetriol, MMST) as a novel food ingredient for use as a source of silicon in food supplements and bioavailability of orthosilicic acid from the source

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

Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to food (ANS) was asked to deliver an opinion on the safety of organic silicon (monomethylsilanetriol, MMST) as a novel food ingredient for use as a source of silicon in food supplements, and on the bioavailability of silicon from this source. MMST is proposed to be used as an aqueous solution at a concentration of 4.1 mM, corresponding to 115 mg Si/L. The anticipated intake of silicon from the proposed uses and use levels of 60–90 mL/day of MMST solution corresponds to approximately 7–10 mg Si/day. Data from *in vitro* and *in vivo* tests showed no genotoxic effect of MMST. From a 90-day toxicity study in rats a no observed adverse effect level (NOAEL) was set at the concentration of 20.5 mM of MMST, corresponding to 232 mg/kg body weight (bw) per day of MMST, the highest dose tested and technically achievable. The Panel considered these data as fulfilling the requirements for the evaluation of the safety of the novel food ingredient and did not request additional testing neither for chronic toxicity and carcinogenicity nor for reprotoxicity and developmental toxicity. The Panel therefore concluded that the proposed use and use levels of MMST as a source of silicon to be used in food supplements is not of safety concern. The conversion of MMST to orthosilicic acid (OSA), the form under which silicon is typically present in food, could not be directly measured, and therefore, the Panel based its opinion on the indirect evidence provided and concluded that OSA is released from MMST and silicon bioavailable from the source. The Panel did not conclude on the safety of MMST in terms of the amount of silicon that may be consumed, as this is outside the remit of the ANS Panel.

Keywords: organic silicon, monomethylsilanetriol, MMST, nutrient source, novel food ingredient, food supplements

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

Panel members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Agneta Oskarsson, Dominique Parent-Massin, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes

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Summary

Following a request from the European Commission (EC) to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the assessment of organic silicon (monomethylsilanetriol; MMST) as a novel food ingredient in the context of Regulation (EC) No 258/97. Following the outcome of the novel food assessment, the Panel was also asked to evaluate the safety of MMST when added for nutritional purposes to food supplements as a source of silicon, and on the bioavailability of silicon from this source in the context of Directive 2002/46/EC. The safety of silicon, in terms of the amounts that may be consumed, is outside the remit of the ANS Panel.

Data from literature have established that silicon is typically present in food in the form of orthosilicic acid (OSA) and as such is readily absorbed from the gastrointestinal tract in humans and then readily excreted in urine. Therefore, in order to address the Terms of Reference of the current mandate, and consistently with previous scientific opinions issued on other sources of silicon, the Panel considered that bioavailability of OSA liberated from MMST as the basis for the evaluation of the bioavailability of silicon from that source.

The present assessment is based on the initial dossier submitted by the applicant and on additional data generated upon request from the Panel.

The ANS Panel assessed the safety of MMST as a novel food ingredient in line with the principles laid down in Commission Recommendation 97/618/EC. For what concerns the data requirements for the safety evaluation of MMST, the Panel followed the approach envisaged in the Guidance for submission for food additive evaluations issued by the Panel in 2012 since this was considered applicable to this assessment. Dietary exposure was estimated based on the proposed use in food supplements and the proposed use levels as reported in the dossier.

The organic silicon preparation subject of this opinion is an aqueous solution of MMST at 4.1 mM, corresponding to 115 mg Si/L. The pH of the solution is 6.6. As other monomeric silicon species, including naturally occurring OSA, MMST undergoes spontaneous self-association in a concentration-dependent fashion and this limits the solubility because at elevated concentrations, solid-phase polymers will be formed. The applicant identified a limit concentration of 21 mM above which MMST in aqueous solution at 21°C undergoes irreversible polymerisation.

According to the applicant, the synthesis of the MMST solution is a two-step process. In a first step, potassium methylsiliconate (called 'the raw material') is synthesised under strong alkaline conditions. This raw material is then diluted and pH adjusted to form the MMST supplement solution as sold. As a result of the manufacturing process applied, methanol may be present as an impurity although no limit for it has been proposed by the applicant in the technical specifications.

The stability and species distribution of MMST were investigated in long-term (91-week) studies and at different temperatures. Based on these data, the applicant concluded that the product should be sold for use at refrigerated (i.e. > 4°C) or room temperature only.

MMST is proposed as an alternative source of silicon to be used in the manufacture of food supplements according to Directive 2002/46/EC and it is proposed to be used in the form of an aqueous solution at a concentration of 4.1 mM. The recommended intake is 60–90 mL/day, which corresponds to an intake of silicon from the source equal to approximately 7–10 mg/day.

In 2004, EFSA estimated typical dietary intake of silicon of 20–50 mg Si/day and concluded that this level of intake was unlikely to cause adverse effects. No suitable dose–response data were available at that time to establish a tolerable upper level (UL) for silicon.

With respect to the bioavailability of silicon following oral administration of MMST, the applicant anticipated that in order for it to be achieved, MMST should undergo conversion to OSA. This postulated mechanism could not be directly proven as the analytical technique used in the majority of the studies can only provide a quantification of total silicon and does not allow identification of the specific form under which it is present. The Panel therefore based its conclusions on a mode of action analysis providing plausible and detailed explanation on the conversion of MMST to OSA *in vivo*. Data

from human studies provide further evidence on the bioavailability of silicon from MMST as being comparable if not higher than oral OSA itself.

Although there was some evidence that MMST was absorbed prior to conversion to OSA, the Panel considered that this was part of the mechanism by which bioavailability of the nutrient was achieved and there was evidence that MMST was not present systemically.

With respect to the toxicological data provided by the applicant, *in vitro* and *in vivo* testing demonstrated that MMST was not genotoxic. From a 90-day study in the rat with MMST supplementation in drinking water, a no observed adverse effect level (NOAEL) of 20.5 mM MMST (equal to 232 mg/kg body weight (bw) per day of MMST) was derived at the highest feasible concentration to prevent polymerisation of MMST, which is fivefold higher the intended use level. In another 90-day study in rats, levels of silicon in serum, bone and cartilage were measured following administration of MMST in drinking water at doses of 0, 115 and 575 mg/L (corresponding to 0, 10.35 and 51.75 mg/kg bw per day). In female animals only, the serum and cartilage levels showed a dose related increase whereas bone showed an increase at the mid dose but not at the high dose which the authors ascribed to feedback control of bone silicon levels. The Panel noted that the apparent increase in silicon levels in serum and ear cartilage was caused by the nutrient (OSA) but not by the nutrient source (MMST), and the Panel was unable to determine the long-term consequences from possible increases in cartilage silicon level. However, this is part of the evaluation of safety of the nutrient (silicon) itself, which is outside the remit of the Panel.

In addition to the results from the studies conducted with MMST, the Panel considered additional supporting evidence generated for other siloxanes. In a chronic toxicity study with polymerised liquid forms of siloxanes administered to rats at a dose level of 3,000 mg active substance/kg diet, no detrimental effects were observed.

Based on the above considerations, the Panel considered that there were insufficient triggers for requesting additional toxicological testing.

The Panel concluded that it was unlikely that there would be toxicity from the proposed use and use levels based on these being a fifth of the highest feasible dose for the toxicity studies and the absence of toxicity at this highest feasible dose. The Panel concluded that the nutrient is released from this source and was likely to have comparable or higher bioavailability than from other sources. The Panel concluded that there was sufficient evidence to that the nutrient source was likely to be safe as a novel food at the proposed uses and use levels.

Given the relatively small margin between the proposed use level and the highest feasible dose tested in the toxicological studies performed, the Panel recommends that higher use level should not be permitted. In addition, the Panel recommends that if in future the polymerisation can be prevented potentially allowing use of higher concentrations a new assessment should be carried out.

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1. Introduction

The present scientific opinion deals with the evaluation of the safety of organic silicon (monomethylsilanetriol, MMST) as a novel food ingredient to be used in food supplements as a source of silicon, and with the bioavailability of silicon from the 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.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

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

The relevant Union legislative measures are:

- Regulation (EC) No 258/97 of the European Parliament and of the Council concerning novel foods and novel food ingredients[1].
- 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]

The dossier relating to organic silicon as a source of silicon has been submitted to the Food Safety Authority of Ireland (FSAI), the competent authority for novel food in Ireland, for an initial assessment under Article 6(2) of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients.

On 17 April 2013, FSAI forwarded to the Commission the initial assessment report, concluding that an additional assessment by the European Food Safety Authority (EFSA) is required in line with Article 6(3) of that Regulation.

The petitioner LLR-G5 Ltd has also submitted to the Commission a dossier relating to organic silicon, monomethylsilanetriol (MMST), to be used as a source of silicon in food supplements.

1.1.2. Terms of Reference as provided by the European Commission

In accordance with Article 29 (1)(a) of Regulation (EC) No 178/2002[3], the European Commission asks the European Food Safety Authority to provide one scientific opinion:

- by carrying out the additional assessment for organic silicon as a novel food ingredient in the context of Regulation (EC) No 258/97, and
- following the outcome of the novel food assessment by evaluating the safety of organic silicon, MMST, when added for nutritional purposes to food supplements as a source of silicon and on the bioavailability of silicon from this source, in the context of Directive 2002/46/EC.

1.1.3. Interpretation of Terms of Reference

The Panel is aware that the mineral silicon is included in the positive lists of minerals that can be added to foods, including food supplements, as defined by Annex I to Regulation (EC) No 1925/2006[4] and Annex I to Directive 2002/46/EC, respectively. Data from literature have however established that

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.
[2] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.
[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.
[4] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26–38.

silicon is typically present in food in the form of orthosilicic acid (OSA) and as such is readily absorbed from the gastrointestinal tract in humans and then readily excreted in urine. Therefore, in order to address the Terms of Reference of the current mandate, and consistently with previous scientific opinions issued on other sources of silicon, the Panel considered bioavailability of OSA from MMST, should be the basis for the evaluation of the bioavailability of silicon from that source.

1.2. Information on existing evaluations and authorisations

With respect to the substances currently authorised for use in the manufacture of food supplements as sources of silicon and listed in annex II of Directive 2002/46/EC, there are choline-stabilised orthosilicic acid (ChOSA), silicon dioxide and silicic acid in the form of gel.

The use of MMST in food supplements was permitted under national legislation until 31st December 2009. MMST has been lawfully used in Member States until that date. MMSTs in drinking food supplements were marketed in France and Belgium since 1998 and in Spain since 2003.

In 2009, the ANS Panel first evaluated MMST as a source of silicon for nutritional purposes but given the absence of adequate data on the bioavailability of silicon from MMST and the absence of data on the toxicity of MMST, the ANS Panel could not assess the safety of the source and the bioavailability of silicon from the source (EFSA, 2009a).

In 2010, the ANS Panel re-evaluated the safety of organic silicon based on newly submitted data. However, the Panel noted that the newly submitted information was related to the bioavailability and toxicology of silicon from a source substance designated as monomethylsilanetriol orthohydroxybenzoate sodium salt (MSS). The Panel therefore concluded that the newly submitted data were insufficient to fill the data gaps on the bioavailability of silicon from MMST and on the toxicity of MMST as expressed in the 2009 opinion, and would therefore not justify the re-evaluation of MMST added for nutritional purposes in food supplements (EFSA ANS Panel, 2010).

The Panel noted that the essentiality of silicon for man has not been established, and a functional role for silicon has not been identified (EFSA, 2004). A recommended intake for silicon has not been set (SCF, 1993; IOM, 2001).

In 2004, EFSA concluded that there were no suitable dose–response data to establish an upper level for silicon (EFSA, 2004) and also the Institute of Medicine (IOM) reported that due to lack of data indicating adverse effects of silicon it was not possible to establish a UL (IOM, 2001).

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

The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) estimated that the typical dietary intake of 20–50 mg Si/day was unlikely to cause adverse effects (EFSA, 2004).

The NDA Panel has also evaluated a number of health claims related to silicon pursuant to Article 13(1) of Regulation (EC) No 1924/2006[5] (i.e. protection against aluminium accumulation in the brain; 'cardiovascular health'; forming a protective coat on the mucous membrane of the stomach; neutralisation of gastric acid; contribution to normal formation of collagen and connective tissue; maintenance of normal bone; maintenance of normal joints; maintenance of normal appearance and elasticity of the skin; and contribution to normal formation of hair and nails). On the basis of the data presented, the NDA Panel concluded that a cause and effect relationship had not been established between the consumption of silicon and any of the health claims proposed (EFSA NDA Panel, 2011).

[5] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

2. Data and methodologies

2.1. Data

The present evaluation is based on the data on MMST in a newly submitted dossier by the applicant. Additional data were generated by the applicant upon request from the ANS Panel during the assessment of the dossier. The request for additional data was discussed at a technical hearing held during a meeting of the Standing Working Group on Applications on 2 October 2014[6].

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The ANS Panel assessed the safety of MMST as a novel food ingredient in line with the principles laid down in Commission Recommendation 97/618/EC[7]. In particular, where it is stated that 'Most of the defined chemical substances can probably be tested for their safety similarly to food additives by utilizing conventional methods of safety evaluation as described in the SCF Report No 10.', the Panel considered that to reflect state of the art scientific knowledge and welfare considerations the reference to SCF Report No 10 should be replaced by the latest existing guidance on the safety evaluation of food additives, namely the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

With respect to the evaluation of bioavailability of the nutrient (silicon) from the source MMST, the principles contained in the 'Guidance on submissions for safety evaluation of nutrients or of other ingredients proposed for use in the manufacture of foods' (SCF, 2001) were followed.

Dietary exposure to the nutrient source was estimated based on the proposed uses and use levels. As the only proposed use of MMST is in food supplements, the Panel did not undertake any other exposure scenarios (see Section 3.3).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

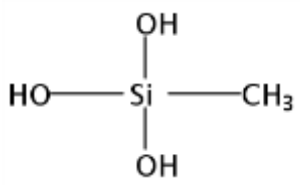
According to the information provided by the applicant, organic silicon is identified as follows:

Chemical name:	Silanetriol, 1-methyl-
CAS Number:	2445-53-6
EINECS Number:	219-489-9
Synonyms:	Silanetriol, methyl-(6CI,7CI,8CI,9CI); 1-methylsilanetriol; methaneorthosiliconic acid; methylsilanetriol; methyltrihydroxysilane; trihydroxy(methyl)silane; methylsilanetriol (REACH, EINECS); silanetriol, methyl-(AICS); methaneorthosiliconic acid; methyltrihydroxysilane; silanetriol, 1-methyl-; trihydroxy(methyl)silane
Trade name:	Monomethyl silanetriol (MMST)
Chemical formula:	CH_6O_3Si

[6] http://www.efsa.europa.eu/sites/default/files/applicationswg.pdf

[7] Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253, 16.9.1997, p. 1–36.

Structural formula:



Molecular weight: 94.14 g/mol

Organic silicon preparation is supplied commercially as an aqueous solution of MMST at 4.1 mM (115 mg Si/L). The pH of the solution is 6.6 (i.e. $[H^+]$ concentration of 2.5×10^{-7} M and $[OH^-]$ concentration of 4.0×10^{-8} M).

In general, monomeric methyl silanols $[(CH_3)_n–Si(OH)_{4-n}$, where n=1–3] are highly water-soluble (> 1,000 mg/kg at 25°C in the case of the mono- and dimethylated compounds), and although they have a tendency to form oligomers at concentrations much above 10 mM, they are stable below this minimum threshold level (Spivack and Dorn, 1994; Côté-Beaulieu et al., 2009).

The applicant indicated that the solubility of MMST in water at 21°C, up to 21 mM (equal to 588 mg Si/L), is without irreversible polymerisation after 2 months. It is further indicated that, as with all monomeric silicon species, including naturally occurring OSA $[Si(OH)_4]$, MMST undergoes spontaneous self-association in a concentration-dependent fashion and this limits the solubility because solid-phase polymers will be formed at elevated concentrations.

3.1.2. Specifications

The applicant submitted analytical results of seven batches of MMST for the specification of silicon and of two batches for the specification of heavy metals. The specification for the pH is based on data of at random in-house quality testing by the applicant.

The specifications for MMST as proposed by the applicant are listed in Table 1.

Table 1: Specifications for MMST as proposed by the applicant

Specification parameter	Specification value in the product as sold
Acidity (pH)	6.6 (Range 6.4–6.8)
Silicium	Not less than 100 mg Si/L; not more than 150 mg/L
Arsenic	Not more than 3 µg/L
Lead	Not more than 1 µg/L
Mercury	Not more than 1 µg/L
Cadmium	Not more than 1 µg/L

The Panel noted that the applicant did not propose a limit for methanol that may be present as an impurity resulting from the manufacturing process.

The applicant further indicates that in the MMST supplement solution as sold, the methanol concentration is always below the maximum residue limit allowed by the extraction solvent residues legislation (Directive 2009/32/EC[8]) of 10 mg/kg or 0.001%.

The microbiological specifications for MMST, as proposed by the applicant, are given Table 2. The values given in the table are the average values of a single batch analysed in quadruplicate.

[8] Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. OJ L 141, 6.6.2009, p. 3–11.

Table 2: Microbiological specifications for MMST as proposed by the applicant.

Specification parameter	Specification value in the product as sold
Total viable count at 22°C	< 1cfu/g
Total viable count at 37°C	< 1cfu/g
Yeasts	< 1cfu/g
Moulds	< 1cfu/g
Coliforms	< 1cfu/g
Escherichia coli	< 1cfu/g
Pseudomonas aeruginosa	< 1cfu/g
Staphylococcus Aureus	< 1cfu/g
Salmonella	Not detectable (not present)
Listeria	Not detectable (not present)

The Panel noted that the analytical data provided are sufficient to demonstrate conformity with the proposed specifications.

3.1.3. Manufacturing process

According to the applicant, the synthesis of the MMST solution is a two-step process. In a first step, potassium methylsiliconate (called 'the raw material') is synthesised under strong alkaline conditions. This raw material is then diluted and pH adjusted to form the MMST supplement solution as sold.

Based on detailed information provided by the applicant, the production process can be summarised as follows: in a reactor, high-purity precursor molecule is hydrolysed with a high-purity aqueous alkaline solution yielding a so-called 'raw material' of alkaline methylsiliconate. This intermediate is in equilibrium with its dimer, trimer, etc., in a concentration- and pH-dependent fashion.

The applicant provided data to show that methanol, a side product of the reaction, is removed to trace amount levels (concentration < 0.05%) to obtain a colourless concentrated and pure aqueous solution.

According to the applicant, in the second step of manufacturing, the concentrate is diluted to prepare the final MMST supplement solution as sold.

3.1.4. Methods of analysis in food

The applicant indicates that the total silicon content of the MMST solution is identified by inductively coupled plasma optical emission spectrometry (ICP-OES) at a wavelength of 251.611 nm. This is specific for silicon and has a limit of detection of around 1 ng/mL and a precision of ±5%. Proton nuclear magnetic resonance (^{1}H-NMR) provides specific identification of the source. MMST is specifically identified at a δ(1H) of -0.05 ppm as a sharp singlet. Just as for silicic acid, this is always in equilibrium with its dimer and minute amounts of trimer, through natural self-association in aqueous solutions.

3.1.5. Stability of the substance, and reaction and fate in food

Long-term stability

NMR spectrometry was used to determine long-term (i.e. 91 weeks) stability and species distribution of the active ingredient (4.1 mM MMST). Samples were stored at 21°C and analysed at room temperature. Immediately after preparation of the solution, in addition to the monomer, a high proportion of oligomeric MMST species (i.e. dimer, acyclic trimer and cyclic trimer) was detected. About 64% of the MMST existed as monomer, 20% as dimer and 16% as trimer (acyclic and cyclic). The monomeric MMST content rose to about 90% after the first week, and had effectively reached the long-term equilibrium level of 92.6 ± 0.2% by the second week. The total dissolved MMST content (4.1 mM MMST) remained constant over the 91-week test period.

Effect of temperature on stability

To study the effect of storage temperature on the stability and species distribution of MMST, aqueous solutions containing 6 up to 31 mM MMST were stored at temperatures of –22, 4 or 21°C for 37 weeks. Upon freezing, precipitation of a white silicon-like solid was seen in the solutions stored at –22°C. Therefore, the experiment was terminated at the end of week 1.

Of the samples stored at 4 or 21°C, those containing ≥ 25 mM MMST exhibited small amounts of precipitate by the end of week 1. All the others samples remained clear throughout the 37-week evaluation period.

NMR analysis revealed that, at any given time and temperature, the extent of MMST condensation increased with sample concentration. Immediately after preparation, the solutions contained proportionately less monomeric MMST than solutions which had been previously made from an older stock of methylsiliconate concentrate. This is in agreement with the results for long-term storage presented above.

By the end of the first week, however, the monomer content was comparable. Apparently, concentrated methylsiliconate begins to polymerise over time. Nevertheless, the dissolved fraction yields a normal equilibrium distribution of oligomers within a week or so of dilution. By contrast, the solid polymer that was formed when solutions were frozen at –22°C did not return to solution upon warming back to room temperature. The data provided further showed that the total dissolved MMST concentrations in the 6, 13 and 19 mM samples at both storage temperatures were effectively constant over the entire 37-week test period. The concentrations in all the 25, 29 and 31 mM samples decreased with time, levelling off at about 21.3 mM after 3 weeks at 21°C and after about 4 to 5 weeks at 4°C. Therefore, the maximum solubility of MMST appears to be about 21 mM.

Based on these data, the applicant considered that at temperatures lower than 4°C, irreversible self-association (polymerisation) may occur and that the reduced absorption and the nutrient content can be anticipated. Therefore, the applicant concluded that the product should be sold for use at refrigerated (i.e. > 4°C) or room temperature only.

3.2. Proposed uses and use levels

The applicant requests the acceptance of use of MMST as a silicon source in food supplements under the provisions of Directive 2002/46/EC. The MMST supplement solution is prepared according to the manufacturing process as described above and consists of an aqueous solution containing 4.1 mM MMST. The amount of silicon is 115 mg Si/L of MMST supplement solution. The maximum daily recommended quantity by the manufacturer is 90 mL of liquid, amounting to a maximum daily silicon intake from this source of 10.35 mg. A more typical daily quantity is 60 mL of supplement solution, yielding a daily intake of 6.9 mg silicon. According to the applicant, these intakes represent approximately 20–50% of typical daily dietary silicon intake for an adult, which is estimated on average to be 17–40 mg/day.

3.3. Exposure estimate

According to the applicant, MMST preparation is supplied commercially as an aqueous solution of MMST at 4.1 mM, which based on a molecular weight of 94.2 g/mol, would result in the solution containing 386 mg MMST/L, of which 115 mg is Si.

The applicant proposes a maximum daily use level of 90 mL of liquid, amounting to a maximum daily silicon intake from this source of approximately 10 mg, and a typical daily use level of 60 mL of supplement solution, yielding a daily intake of approximately 7 mg silicon.

Silicon occurs naturally in foods as silicon dioxide (silica) and silicates, and may also be added as an anticaking and antifoaming agent in the form of silica, silicates and dimethylpolysiloxane. Silicate-containing antacids have been widely used for a number of decades (EFSA, 2004). According to the applicant, daily dietary silicon intake for an adult is estimated on average to be 17–40 mg/day, which is in agreement with the estimated range of 20–50 mg Si/day reported by EFSA in 2004 (EFSA, 2004). Daily consumption of the supplement at the maximum recommended daily dose would result in an estimated total intake from supplement use and from the diet of between 30 and 60 mg Si/day.

3.4. Biological and Toxicological data on MMST

3.4.1. Introduction

Silicon occurs as silicon dioxide (SiO_2) or the corresponding silicic acids formed by the hydration of the oxide. OSA [$Si(OH)_4$] is the simplest acid and the main chemical species of silicon soluble in water (Carlisle, 1997).

OSA is accepted as being the natural biological form of silicon (Si) in humans and animals and plays a major role in delivering silicon to the living cells (Reffitt et al., 1999; Jugdaohsingh et al., 2000; Jugdaohsingh et al., 2002; Sripanyakorn et al., 2009; Jurkić et al., 2013). The availability of silicon from a given source depends on the solubility or speciation of the compound concerned (Van Dyck et al., 1999).

3.4.2. Bioavailability of Si from MMST

In the context of this opinion, the term bioavailability is used to indicate systemic availability of OSA from MMST (see Section 1.1.3.).

The applicant indicated that the conversion of MMST to OSA in mammalian samples at concentrations that are commensurate with bioavailability assessment cannot be 'directly' demonstrated. Indeed, ICP-OES, the analytical technique used in the majority of the studies that are publicly available, only measures 'total' silicon; the specific form under which silicon is present cannot be identified. Therefore, the applicant provided circumstantial evidence to postulate the conversion of MMST to OSA *in vivo*. This evidence is drawn from the results of a study by Varaprath et al. (2003). In this study, a ^{14}C-labelled linear siloxane (hexamethyldisiloxane, HMDS, $(CH_3)_3–Si–O–Si–(CH_3)_3$) and a ^{14}C-labelled cyclic siloxane (decamethylcylclopentasiloxane, D5, $[(CH_3)_2–Si–O–]_5$) were administered to female Fischer 344 rats (2 animals/substance) either by gavage or intravenously. After administration, urine samples were collected over a 24-h period and the siloxane metabolites were analysed. Several metabolites were detected, particularly following administration of HDMS; dimethylsilanediol (DMSD, $(CH_3)_2–Si–(OH)_2$) and $(CH_3)_3–Si–O–Si–(CH_3)_2OH$ in the case of HDMS, and in the case of D5, $CH_3–Si–(OH)_3$ (i.e. MMST) and $CH_3–Si–(OH)_2–O–Si–(OH)_3$. It was concluded by the applicant that the mechanistic basis for these *in vivo* metabolic transformations resulted from the much greater bond strength of Si–O bonds than Si–C bonds.

To assess the feasibility for demethylation of MMST and to evaluate whether MMST would be similarly metabolised, the applicant provided data on the Si–C bond energies of MMST compared with those of larger organosilicons (i.e. octamethylcyclotetrasiloxane (D4), $[(CH_3)_2–Si–O–]_4$) as well as the common intermediary metabolite of these larger organosilicons, namely DMSD (Documentation provided to EFSA n.3). These dissociation energies were very similar (i.e. 5.3987 eV for D4, 5.2362 eV for DMSD and 5.3846 eV for MMST) indicating that MMST had the potential to be demethylated *in vivo* to form OSA.

As regards to possible mode of action, the available data demonstrated that demethylation of the Si–CH_3 bonds occurs; that the CH_3-group is first hydroxylated followed by silyl group migration from the C-atom to the O-atom (methyl → hydroxyl bioconversion known as the 'Brook rearrangement') (Jung and Nichols, 1996), and finally, hydrolysis of the resulting methoxysilane following the reaction scheme: $R_3Si–CH_3 \rightarrow R_3Si–CH_2OH \rightarrow R_3Si–OCH_3 \rightarrow R_3Si–OH$.

Other data in literature indicated that methyl–silane bonds (i.e. the Si–CH_3 bonds) can be cleaved *in vivo* and converted to silanols (i.e. Si–OH bonds). This was found to occur in the stomach of mammals, such as dogs (Tacke and Linoh, 1989) and rats (Graiver et al., 2003), and presumably also in humans, or via enzymatic conversion as in the studies by Sabourin et al. (1996), Hirner et al. (2003) or Graiver et al.(2003).

Studies have been provided to support the *in vivo* availability of silicon from MMST.

Sripanyakorn et al. (2009) studied the comparative absorption of silicon from supplemental MMST and from other six high-silicon-containing sources (i.e. alcohol-free beer; bananas; green beans; OSA solution, supplemental ChOSA; supplemental colloidal silica; magnesium trisilicate).

Healthy volunteers (aged 19–40 years; 16 males; 16 females) with normal serum creatinine levels were recruited. Subjects who had been taking silicon supplements and/or medicines containing silicon were excluded. Subjects with a history of chronic illness were also excluded, as well as pregnant and lactating women. Age, sex, height, weight, BMI and serum creatinine were recorded for each subject. Subjects fasted overnight from 22.00 h and remained fasted until 14.30 h the following day (end of the study period), except for the ingestion of the test solutions or meals and the ultrahigh pure water that were supplied at 08.30 h and 11.30 h, respectively. Subjects were asked to avoid high-silicon-containing foods (i.e. beer, breakfast cereals, rice and certain vegetables and fruit, particularly bananas and green beans) 24 h before the start of the study.

Each of the fasting, healthy subjects (n = 3 for colloidal silica; n ≥ 5 for other substances) ingested two of the sources separated by a 1-week wash-out period. Blood and urine were collected and measured for total silicon concentrations by ICP-OES.

Fourteen healthy volunteers (6 males and 8 females) completed the absorption study for MMST. These test subjects ingested 60 mL MMST containing 6.9 mg Si.

The study showed that silicon absorption, based on urinary Si excretion, was highest for MMST and alcohol-free beer (64% of dose), followed by green beans (44%), OSA (43 %), ChOSA (17%), bananas and magnesium trisilicate (4%), and colloidal silica (1%). Peak serum concentrations occurred by 0.5 h for MMST and green beans, 1.5 h for OSA and alcohol-free beer, 2 h for ChOSA and colloidal silica, and 4 h for magnesium trisilicate. Area under the serum curves correlated positively with urinary Si output ($r = 0.82$; $P > 0.0001$). Absorption of Si from supplements and antacids was consistent with their known chemical speciation and kinetics of dissolution under simulated gastrointestinal conditions. Monomeric silicates were readily absorbed, while particulate silicates were decreasingly well absorbed with increasing polymerisation. The authors concluded this study indicated that silicon from MMST was very well absorbed; however, the Panel noted that the analytical method was not able to provide information on the form of silicon absorbed.

Jugdaohsingh et al. (2013) studied the metabolism and safety of the MMST in a 4-week human supplementation study. One objective of the study was to establish whether MMST could contribute to the body pool of silicon following sustained supplementation in humans. According to the authors, a rise in fasting serum silicon levels following mid- to long-term supplementation provides the best known indication of changes in the body pool of silicon (Sripanyakorn, 2005) and was used as a marker for supplementation efficacy. In the study, total silicon was determined by ICP-OES, while a subset of the fasting serum (n = 9) and urine samples (n = 10) following MMST supplementation were analysed by ^{1}H-NMR. Twenty-two healthy premenopausal women (ages 22–38 years) with no history of serious illness and not taking any medication or silicon-containing food supplements, were supplemented with MMST (10.5 mg Si/day, equivalent to 40% of total average daily dietary silicon intake in this population (Jugdaohsingh et al., 2002)) for 4 weeks in a double-blind, randomised, placebo-controlled, cross-over design (i.e. 8 weeks in total). The subjects were instructed to take 30 mL of either the MMST solution (3.5 mg silicon) or the placebo solution three times a day before meals. Fasting serum and urine samples were collected at baseline and at the end of the 4-week supplementation/placebo periods for analysis of total Si (by ICP-OES), MMST (by ^{1}H-NMR) and full serum biochemistry. Participants also reported (by questionnaire) on their health, well-being and quality of life at 0, 4 and 8 weeks.

The data showed that 4-weeks supplementation with MMST significantly increased total fasting silicon concentrations in serum (mean 272 µg/L vs baseline mean 173 µg/L ($p = 0.0002$ or placebo mean 191 µg/L ($p = 0.003$)) and urine (mean 17.0 mg/L vs baseline mean 8.5 mg/L ($p = 0.008$) or placebo mean 7.8 mg/L ($p = 0.007$)). MMST was semiquantifiable in serum and quantifiable in urine, but only accounted for approximately 50% and 10%, respectively, of the increased total-Si concentration. There were no reported adverse effects (i.e. changes to health and well-being) or serum biochemical changes with MMST versus placebo. The authors concluded from their data that oral MMST was safe and that MMST was absorbed and underwent sufficient metabolism *in vivo* to raise fasting serum silicon levels, consistent with other well absorbed forms of dietary silicon.

Comparing the fasting serum concentration in humans of metabolised OSA (Si) following a 4-week oral supplementation with non-alcoholic beer containing high levels of OSA (data from Sripanyakorn, 2005) or with oral supplementation of MMST (data from Jugdaohsingh et al., 2013), the applicant estimated the bioavailability of silicon from MMST to be 13%.

3.4.3. Absorption, distribution, metabolism and excretion

General information on the metabolic fate of silicon has been described earlier by the ANS Panel (EFSA, 2009b). For dietary silicon, there appear to be two distinct steps in its biodistribution following oral absorption, namely rapid urinary excretion for the majority of the silicon as detailed in Popplewell et al. (1998), Reffitt et al. (1999) and Jugdaohsingh et al. (2002), and tissue loading and/or cellular metabolism for a minor part, commensurate with physiological balance (Popplewell et al 1998). In a human study that utilised radioactive ^{32}Si, Popplewell et al. (1998) demonstrated that 90% of circulating OSA was rapidly excreted without any form of cellular Si processing. More recently, Pruksa et al. (2014) reported that in healthy subjects (presumably in Si balance), the ingestion of a soluble dose of dietary silicon resulted in the same quantity being excreted within 24 h.

With specific reference to MMST, rapid urinary excretion of a majority, but with long-term loading of a minority, also occurs. This and literature data would support bioconversion (metabolism) of MMST to OSA (dietary silicon).

To demonstrate the bioconversion of MMST to dietary silicon (OSA) in humans, the applicant referred to the data in the study by Jugdaohsingh et al. (2013). They argued that, if silicon loading (i.e. increase in fasting serum levels) is observed following subchronic ingestion of MMST in volunteers, as it was observed for other dietary sources of silicon (Calomme et al. 1998; Sripanyakorn 2005), quantification of MMST by ^{1}H-NMR compared with quantitative ICP-OES for the increase in total silicon would allow bioconversion to be detected. This approach was therefore applied to a subset of samples from the 4-week intervention study by Jugdaohsingh et al., (2013).
In the study, MMST was assessed in a subset of fasting serum (n = 9) and urine samples (n = 10), that showed an increase in total silicon above baseline to determine whether MMST could be detected, and to investigate whether by balance (i.e. comparison to total silicon) MMST was converted/metabolised to OSA. A small subset of the baseline samples (n = 3–5), with high silicon concentrations, and n = 3–4 placebo samples with the highest increase in total silicon (compared to baseline), were also analysed, as controls. ^{1}H-NMR was used to quantify the level of MMST in urine, which was then compared to the total silicon increase in urine (determined by ICP-OES) over and above baseline of the same paired samples. MMST was detected above the detection limit in all 10 fasting urine samples collected following supplementation with MMST.
The applicant argued that the increase in fasting serum levels and urinary levels of silicon following 4 weeks intervention with MMST, cannot be explained by a simple linear increase in MMST concentrations and, thus, implies bioconversion of MMST to OSA [$Si(OH)_4$] as previously proposed in other studies (Tacke and Linoh 1989; Sabourin et al. 1996; Hirner et al. 2003; Graiver et al. 2003; Varaprath et al., 2003). MMST is the smallest possible organosilicon molecule and its *in vivo* anabolism to form larger organosilicon species was neither expected nor detected by ^{1}H-NMR.

The Panel agreed that this explanation appeared plausible.

3.4.4. Acute toxicity

Acute oral toxicity of MMST was tested in SPF Sprague–Dawley albino rats (n = 3, females) at an oral dose of 5,000 mg MMST/kg bw. The test was carried out according to OECD guidelines. No mortality was observed 14 days after administration. Additionally, no significant differences were detected on the remaining indicators of toxicity. Liquid MMST was classified in the hazard category 5 or unclassified with a median lethal dose (LD_{50}) > 5,000 mg/kg in the rat (Documentation provided to EFSA n.1).

3.4.5. Short-term and subchronic toxicity

In a dose range-finding 90-day study, 30 Sprague–Dawley rats (n = 15 males; n = 15 females) were randomly assigned (n = 5 males and n = 5 females) to receive, ad libitum, MMST in the drinking water at levels 0 mM (control), 4.1 mM and 20.5 mM (calculated to be equivalent to 12 mg Si/kg bw per day for males or 19 mg Si/kg bw per day for females for the lower dose and to 58 mg Si/kg bw per day for males or 96 mg Si/kg bw per day for females at the highest). After 90 days, the rats were fasted for ≥ 15 h before blood sample collection for serum and plasma analysis and were then sacrificed for tissue collection. The MMST concentrations represented, respectively, 1x and 5x the dose concentrations of the MMST supplement solution as sold. It was pointed out by the applicant

that absolute exposures to MMST were much higher than this, because the MMST test items were consumed by the animals as a substitute for drinking water during the 90 days, whereas normal maximum human exposure would be ≤ 5% of daily fluid intakes. All animals maintained good health, with consistent group mean weight gain, for the duration of the study. There were no lesions observed at necropsy and there were no clinical pathology or histology findings considered to be related to the administration of MMST. In conclusion, all the animals were observed to maintain a good health, and the MMST showed no negative effects on the rat model tested. (Documentation provided to EFSA n.1)

In addition, plasma samples taken from 30 rats were analysed for thyroid-stimulating hormone (TSH) and C-reactive protein (CRP). To allow for three-way comparisons to be made, linear regression analyses were undertaken with plasma TSH or CRP levels from male and female rats combined. No significant differences in plasma TSH levels were found between treatments (P = 0.308, diluent control vs 4.1 mM MMST and P = 0.541, diluent control vs 20.5 mM MMST). Similarly, there were no significant differences in plasma CRP levels between treatments (P = 0.291, diluent control vs 4.1 mM MMST and P=0.578, diluent control and 20.5 mM MMST). (Documentation provided to EFSA n.1).

On request of EFSA, the applicant provided an additional 90-day toxicity study in the rat to assess the potential subchronic toxicity of MMST after oral administration (Documentation provided to EFSA n.3). The test was performed according to good laboratory practice (GLP) and according to Organisation for Economic Co-operation and Development (OECD) guidelines (OECD 408).

In this study, MMST was administered daily in drinking water to male and female Wistar rats (10 animals/sex per dose) at dose levels of 20.5 mM, 9.2 mM and 4.1 mM (equal to 232 mg MMST/kg bw per day, 104 mg MMST/kg bw per day, 46.7 mg MMST/kg bw per day, respectively) for 92 days. The vehicle control group of 10 male and 10 female rats received pure drinking water. The applicant indicated that, based on a preliminary solubility study, MMST needs to be dosed at a concentration < 24.99 mM to prevent irreversible polymerisation. Therefore in the 90-day study, 20.5 mM was chosen as the highest possible dose. A satellite group of 10 male and 10 female animals received daily the test item (at the highest concentration of 20.5 mM), by oral gavage, at a dose of 20 mL MMST/kg bw (equal to 38 mg/kg bw per day).

General clinical signs, viability, reactions to treatment and conspicuous behavioural traits of all experimental animals were monitored daily during the in-life phase. Group food consumption and individual body weight were recorded once weekly. Group water consumption was recorded twice weekly. At the end of the treatment period, blood samples from all animals were drawn to provide data on haematology, clinical biochemistry and blood clotting time. All animals were sacrificed after bleeding and examined for gross necropsy. Samples from the high-dose groups and the vehicle groups were processed for histopathological examination.

As regards body weight, food and water consumption, for both sexes and over the whole in-life phase, no abnormal differences were observed between the treated animals and the controls. Also, haematology and clinical biochemistry showed no relevant effects between the treated animals and the controls. At necropsy, there were no macroscopic findings and no significant changes in organ weights that could be considered as related to treatment. Histopathological examination did not reveal microscopic findings considered to be related to treatment. All microscopic findings recorded were considered to be spontaneous in nature and within the normal background pathology commonly seen in rats of this strain and age. Under the conditions of this study, the continuous oral administration of MMST up to 20.5 mM did not produce any evidence of pathomorphological findings related to the intake of MMST. The authors concluded the no observed adverse effect level (NOAEL) of the study to be 20.5 mM MMST in drinking water. The bolus oral application of MMST via gavage at the dose of 38 mg/kg bw per day did not show any toxic effects. The Panel noted that the highest dose (20.5 mM MMST) used was the highest that could be technologically achieved and was fivefold greater than the highest proposed administration.

The Panel noted that sternum rather than femur was investigated for the histopathology investigation because according to the authors this allowed the collection of adjoining connective tissues (cartilage). The Panel considered this could be a deviation from the guideline which clearly indicates the need for investigating target organs and that it would be unlikely that histopathological changes from accumulation of silicon would be observed in any bone.

However, the Panel also noted that bone and connective tissues would be a target for the nutrient (OSA) and not the nutrient source (MMST).

The Panel noted that, in another 90-day study in rats (Jugdaohsingh et al., 2015), levels of silicon in serum, bone and cartilage were measured following administration of MMST in drinking water at doses of 0, 115 mg/L and 575 mg/L (corresponding to 0, 10.35 and 51.75 mg/kg bw per day as calculated by the Panel using a default factor of 0.09 for subchronic studies in rats). In female animals only, the serum and cartilage levels showed a dose-related increase, whereas bone showed an increase at the mid dose but not at the high dose which the authors ascribed to feedback control of bone silicon levels. The Panel noted that the apparent increase in silicon levels in serum and ear cartilage was caused by the nutrient (OSA) but not by the nutrient source (MMST), and the Panel was unable to determine the long-term consequences from possible increases in cartilage silicon level. However, this is part of the evaluation of safety of the nutrient (silicon) itself, which is outside the remit of the Panel.

3.4.6. Genotoxicity

In vitro

MMST (20 mM aqueous solution as sold) was tested for mutagenic activity in the bacterial reverse mutation test in *Salmonella* Typhimurium TA 1535, TA 1537, TA 98, TA 100 and *Escherichia coli* WP2uvrA tester strains. Two independent experiments were conducted in triplicate without and with S9 metabolic activation using the standard plate assay. Concentrations used ranged from 58.75 to 1880 µg/plate. The highest concentration represents the maximum attainable in the test system (MMST 20 mM, 1 mL/plate). Concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the S9 mix. MMST was found to be not mutagenic under these test conditions (Documentation provided to EFSA n.1). The Panel noted that the study was compliant with the methods and recommendations indicated in the relevant OECD Guideline no. 471 and was carried out in compliance with GLP.

MMST (20 mM aqueous solution as sold) was tested in the mouse lymphoma assay (L5178Y cells) at the TK locus, formulated in a 10x concentration of the basic culture medium, to give the highest feasible concentration in the cells (approximately 7.2 mM, deemed the 100% treatment). Tests were conducted both in the absence and in the presence of S9 mix. The study was performed according to OECD Guideline No. 476 and Directive 2000/32/EC[9]. Biological relevance was given to any increase in mutation frequency greater than 126 mutants per million (global evaluation factor) above the concurrent control value. In addition, the results were analysed for comparison of the log mutation frequency between the vehicle controls and each concentration of MMST. In addition, all experiments were tested for dose-related trends in mutation frequency. No evidence of mutagenic activity was obtained either in the absence or in the presence of S9 mix, when tested to the highest practicable concentration of 7.2 mM (Documentation provided to EFSA n.1).

MMST (20 mM aqueous solution as sold) was assayed for the induction of chromosomal aberrations in cultured human peripheral lymphocytes, in two independent assays with a short treatment (5 h), both in the absence and in the presence of S9 metabolic activation and continuous treatment (25 h) in the absence of S9 metabolic activation up to dose level of 10 mM, the maximum dose level recommended by the relevant OECD Guideline. Cultures were harvested at 29 h following short and continuous treatments both in the absence and in the presence of S9 metabolic activation or 53 h following continuous treatment in the absence of S9 metabolic activation. Results obtained indicated that MMST did not induce structural chromosomal aberrations at any dose level and sampling time used both in the absence and in the presence of S9 metabolic activation. Furthermore, MMST did not induce any increase in polyploidy in the absence of S9 metabolism in cultures harvested at 53 h (Documentation provided to EFSA n.1). The Panel noted that the study was compliant with the methods and recommendations indicated in the relevant OECD Guideline no. 473, adopted 26 September 1997 and with GLP.

[9] Commission Directive 2000/32/EC of 19 May 2000 adapting to technical progress for the 26th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. OJ L 136, 8.6.2000, p. 1–89.

In vivo

The *in vivo* genotoxic potential of soluble and spray-dried (S-D) MMST was evaluated in a micronucleus test in bone marrow erythrocytes of young, male and female CD rats after three oral doses at 24 h intervals. Bone marrow samples were taken once at 24 h following the final treatment.

Preliminary range finder tests were undertaken to establish a suitable dose range for the micronucleus experiment.

In the micronucleus test, one group of 5 male CD rats was dosed with soluble MMST (20 mM solution as sold) at 10 mL/kg bw per day (equivalent to 18.8 mg/kg bw per day of MMST, the highest attainable dose level with this formulation). In addition, 2 groups were dosed with S-D MMST formulated in carboxymethylcellulose (medium viscosity) at 0.5% (w/v) and Tween 80 at 0.1% (w/v) in water at 1,000 mg/kg bw per day (5 male CD rats) and at 2,000 mg/kg bw per day (8 male and 8 female CD rats). Bone marrow samples were taken 72 h after the initial dose. Vehicle and positive-control animal groups were also included.

No increase in the frequency of micronuclei was detected in bone marrow polychromatic erythrocytes from rats dosed with either soluble or S-D MMST at any of the dose levels assayed. No substantial reduction of the ratio polychromatic/normochromatic erythrocytes was observed for both soluble and S-D MMST at any of dose levels assayed, possibly indicating that test compound could have not adequately reached the target tissue. However, the Panel considered that in the case of S-D MMST, the animals were treated at the limit dose of 2,000 mg/kg bw as indicated by the relevant OECD Guideline 474 (Documentation provided to EFSA n.1).

The Panel noted that the study was compliant with the methods and recommendations indicated in the relevant OECD Guideline and with GLP.

Overall, the Panel considered that on the basis of the data available there are no concerns with respect to the genotoxicity of MMST.

3.4.7. Chronic toxicity and carcinogenicity

The applicant indicated that as the dose of MMST is increased it will start to polymerise and form a methylpolysiloxane in equilibrium with a small amount of monomer MMST.

The applicant stated that chronic toxicity of unpolymerised MMST has not been evaluated directly, but referred to a study by Rowe et al (1948). In this study, polymerised liquid forms of siloxanes of the type $(CH_3)_3$–Si–O–$[(CH_3)_2$-Si-O$]_x$–$(CH_3)_3$ were tested for toxicity in different animals (rats, rabbits guinea pigs). More in particular, in a study in rats (25 males; 25 females; strain not given) octamethylcyclotetrasiloxane (Antifoam A; $[-Si(CH_3)_2O-]_4$; monomer content not stated) was administered in the diet (on continuous dosing) at a level of 3,000 mg active substance/kg diet for up to 400 days. No detrimental effect was observed on general health, growth, blood analysis or survival of the animals. From the overall results of their studies, the authors concluded that silicones as a group have a very low order of toxicity.

Carcinogenicity

The applicant argued that no *in vivo* carcinogenicity tests with MMST have been undertaken in the light of the full set of results in animal and human toxicity, mutagenicity, genetic toxicity and clastogenicity studies showing no adverse effects at all for MMST. The applicant further argued that in the assessment of other silicon sources, EFSA has accepted their safety in the absence of carcinogenicity testing.

3.4.8. Reproductive and developmental toxicity

The applicant again argued that no *in vivo* reproductive and developmental toxicity test with MMST have been undertaken in light of the full set of results in animal and human toxicity, mutagenicity, genetic toxicity and clastogenicity studies showing no adverse effects at all for MMST.

The Panel noted that another organic silicon source (i.e. organosilicon quaternary ammonium chloride, quaternary silsesquioxane) was tested for reproductive and developmental toxicity in CD rats by Siddiqui and York (1993).

In this study, groups of 25 pregnant CD rats were administered 100, 300 or 1,000 mg/kg bw per day of the test substance by gavage on days 6 through 15 of gestation. The control group received corn oil. Caesarean examinations were performed on all females on gestation day 20 followed by evaluation of the fetuses for teratogenicity. Maternal effects included a slight but statistically significant increase in relative liver weights at the 1,000 mg/kg bw per day dose level, and therefore, the maternal NOAEL for quaternary silsesquioxane was considered as 300 mg/kg bw per day. For the fetuses, there were no statistically significant differences between control and test groups in relation to number of corpora lutea, implantation sites, viable fetuses, early or late resorptions, fetal body weight, crown-rump length, and gravid uterine and corrected body weights. Similarly, no differences were observed in the occurrence of external and internal soft tissue malformations or in the incidence of skeletal malformations for both groups. Oral organic silicon, in the form of quaternary silsesquioxane, was not teratogenic and did not present any developmental toxicity in CD rats at doses up to 1,000 mg/kg bw per day.

The applicant argued that the results of this study are relevant for the safety evaluation of MMST because higher organosilicons will be metabolised to lower organosilicons (MMST being the lowest) and because MMST has been shown to lack any toxicity at all doses, whereas silsesquioxane shows some toxicity to liver weights at very high dose levels in adult rats. The applicant considered that the reproductive data for silsesquioxane can therefore be considered as a 'worst-case scenario' and can be used for read across to establish the safety of MMST.

3.4.9. Hypersensitivity, allergenicity and food intolerance

No data were provided

3.4.10. Other studies

Human studies

For the human study, it is referred to the 4-week human supplementation study by Jugdaohsingh et al. (2013) as described under Section 3.4.3.

The results revealed that there were no significant differences between the MMST intervention group and placebo in any of the biochemical or blinded measures of perceived well-being following 4 weeks of maximum MMST daily dosing (i.e. intake of 10.5 mg Si/day) in a double-blind and cross-over fashion. The biological parameters tested included serum creatinine, calcium, alkaline phosphatase, cholesterol, total bilirubin, triglycerides, TSH, glucose and phosphate. General well-being, quality of life and skin health were not affected by the intervention with organic silicon as per analysis of the participants' questionnaires.

3.5. Discussion

The European Commission asked EFSA to provide a scientific opinion on the safety of organic silicon (MMST) as a novel food ingredient, when added for nutritional purposes to food supplements as a source of silicon and on the bioavailability of silicon from this source. The safety of silicon in terms of the amounts that may be consumed is outside the remit of this Panel.

In 2004, EFSA concluded that there were no suitable dose–response data to establish an upper level for silicon and also the IOM reported that due to lack of data indicating adverse effects of silicon it was not possible to establish a UL.

EFSA estimated that a typical dietary intake of 20–50 mg Si/day per person would be unlikely to cause adverse effects (EFSA, 2004). The daily consumption of MMST at the maximum daily dose recommended by the applicant (10 mg/day of supplemental silicon) would result in an estimated total intake from supplement use and from the diet of roughly 30–60 mg silicon/day.

Methanol may be present in commercially supplied MMST as an impurity present in trace amounts resulting from the manufacturing process. The applicant did not propose a limit for methanol in the specifications.

The Panel noted that it was not currently technically feasible to directly measure OSA *in vivo* following MMST administration. However, based on:

- indirect evidence from the 4-week human supplementation study (Jugdaohsingh et al., 2013) demonstrating that the increase in fasting serum silicon levels could not be accounted for solely by systemic MMST;
- increased levels of silicon in bone following such administration showing that, unlike MMST, OSA can be deposited in bone at the molecular level;
- data in the literature on the metabolism of linear and cyclic siloxanes and on bond strengths, both indirect evidence of the presence of OSA;
- a plausible and credible mode of action for formation of OSA from MMST *in vivo*.

The Panel considered that there were no other viable reaction pathways for MMST under physiological conditions other than its conversion to OSA.

In vitro and *in vivo* testing demonstrated that MMST was not genotoxic. From data of a 90-day study in the rat with MMST supplementation in drinking water, a NOAEL of 20.5 mM MMST (equal 232 mg MMST/kg bw per day) was derived at the highest feasible concentration to prevent polymerization of MMST, which is fivefold higher the intended use level. In a chronic toxicity study with polymerised liquid forms of siloxanes ($[-Si(CH_3)_2O-]_4$) at a dose level of 3,000 mg active substance/kg, no detrimental effects were observed.

Although there was indirect evidence that MMST was absorbed prior to conversion to OSA, the Panel considered that this was part of the mechanism by which bioavailability of the nutrient was achieved and there was evidence MMST was not present systemically. The Panel further noted that systemic concentrations of MMST following oral administration would be limited by polymerisation in the gastrointestinal tract at higher concentrations.

Given these specific circumstances that the absence of toxicity and genotoxicity in tier 1 testing and lack of toxicity of polymerised siloxanes in chronic studies, the Panel considered that there were insufficient triggers for tier 2 testing and that there was no toxicological concern with MMST as a nutrient source at the proposed use levels.

When compared to the bioavailability of silicon from a recognised dietary source (non-alcoholic beer), the bioavailability of silicon from MMST was comparable and higher than for oral OSA itself.

The Panel noted that the apparent increase in silicon levels in serum and cartilage was caused by the nutrient (OSA) not the nutrient source (MMST) and the Panel was unable to determine the long-term consequences from possible increases in cartilage silicon level. However, this is part of the evaluation of safety of the nutrient (silicon) itself, which is outside the remit of the Panel.

4. Conclusions

The Panel considered that the fivefold margin between the proposed use level and the maximum feasible dose tested in the toxicological studies performed was low; nevertheless, the Panel concluded that MMST was unlikely to be of safety concern as a novel food ingredient at the single proposed use and use level.

The Panel concluded that OSA is released from MMST and was likely to have comparable or higher bioavailability from MMST than from other sources (non-alcoholic beer and OSA, respectively).

5. Recommendations

Given the relatively small margin between the proposed use level and the highest feasible dose tested in the toxicological studies performed, the Panel recommended that higher use level should not be permitted. To decrease the uncertainty associated with this small margin, the Panel recommended that if in future the polymerisation can be prevented potentially allowing use of higher concentrations in toxicity studies a new assessment should be carried out.

Given the manufacturing process, the Panel recommended that specifications for MMST should include an indication for the residual presence of methanol.

Documentation provided to EFSA

1. Application for the approval of organic silicon (MMST) as a source of silicon for use in the manufacture of food supplements, including Appendixes. December 2012. Submitted by LLR-G5 Ltd., Ireland.

2. Oral clarification provided by the applicant during a technical hearing held at the 1st meeting of the Standing Working Group on Applications. October 2014.

3. Additional information submitted to EFSA. Report: A Repeated Dose 90-day Oral Toxicity Study of Organic Silicon (MMST) and a Mode of Action Analysis to determine its Bioavailability as a Source of Silicon including Appendixes 1–3. October 2015. Submitted by LLR-G5 Ltd., Ireland.

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Abbreviations

ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
ChOSA	choline-stabilised OSA
CRP	C-reactive protein
D4	octamethylcyclotetrasiloxane
D5	decamethylcylclopentasiloxane
DMSD	dimethylsilanediol
EVM	UK Expert Group on Vitamins and Minerals
FSAI	Food Safety Authority of Ireland
GLP	good laboratory practice
HMDS	hexamethyldisiloxane
ICP-OES	inductively coupled plasma optical emission spectrometry
IOM	US Institute of Medicine
LD_{50}	median lethal dose
MMST	monomethylsilanetriol, organic silicon
MSS	monomethylsilanetriol orthohydroxybenzoate sodium salt
^{1}H-NMR	proton nuclear magnetic resonance
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
OSA	orthosilicic acid
SCF	Scientific Committee on Food
S-D MMST	spray-dried MMST
TSH	thyroid-stimulating hormone
UL	tolerable upper level