European Food Safety Authority

EFSA Journal 2013;11(10):3358

SCIENTIFIC OPINION

Scientific Opinion on (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt as a source of folate added for nutritional purposes to food supplements[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver an opinion on the safety of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt (5MTHF-glucosamine), when added for nutritional purposes to food supplements as a source of folate, and on the bioavailability of folate from this source. 5-Methyltetrahydrofolic acid, calcium salt (5MTHF-Ca), has previously been authorised as a source of folate. 5MTHF-glucosamine is proposed as an alternative source of folate to be used in the manufacture of food supplements and it is proposed to be used at up to 1.8 mg/day, which equates to 1 mg 5MTHF and 0.8 mg glucosamine. A crossover comparative bioavailability study was performed in human volunteers and the Panel concluded that folate from 5MTHF-glucosamine exhibited a similar bioavailability to folate from 5MTHF-Ca. The Panel considered that 5MTHF-glucosamine will readily dissociate to 5MTHF and glucosamine in the aqueous environment of the digestive tract. The 5MTHF component will therefore be expected to be absorbed across the small intestine in a similar manner as 5MTHF originating from dietary sources and 5MTHF-Ca. In vitro genotoxicity studies with 5MTHF-glucosamine have been performed and the Panel concluded that the 5MTHF-glucosamine source did not raise concerns with respect to genotoxicity. The Panel therefore concluded that the proposed use and use levels of 5MTHF-glucosamine as an alternative source of folate to be used for the manufacture of food supplements is not of safety concern. The Panel does not conclude on the safety of 5MTHF-glucosamine in terms of the amount of folate that may be consumed, since this is outside the remit of the ANS Panel; however, the Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) will advise on population reference intakes for folate by 2015 (EFSA-Q-2011-01212).

KEY WORDS

folic acid, 5-methyltetrahydrofolic acid, folate, glucosamine

[1] On request from the European Commission, Question No EFSA-Q-2012-00843, adopted on 11 September 2013.

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[3] The Panel wishes to thank the members of the Working Group A on Food Additives and Nutrient Sources: Pierre Galtier, Rainer Guertler, Ursula Gundert-Remi, Claude Lambré, John Christian Larsen, Jean-Charles Leblanc, Pasquale Mosesso, Dominique Parent-Massin, Ivan Stankovic, Christina Tlustos and Matthew Wright for the preparatory work on this scientific opinion.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2013; Scientific Opinion on (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt, as a source of folate added for nutritional purposes to food supplements. EFSA Journal 2013;11(10):3358. [20 pp.] doi:10.2903/j.efsa.2013.3358.

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission (EC) to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt (5MTHF-glucosamine), when added for nutritional purposes to food supplements as a source of folate, and on the bioavailability of folate from this source. The safety of folate, in terms of the amounts that may be consumed, is outside the remit of the ANS Panel. The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) will advise on population reference intakes for folate by 2015 (EFSA-Q-2011-01212).

5MTHF-glucosamine is obtained synthetically from folic acid by chemical reduction and condensation with formaldehyde. Purification of the racemic mixture is performed by crystallisation processes, yielding pure (6*S*)-5-methyltetrahydrofolic acid, with which the glucosamine salt is formed.

The stability of 5MTHF was determined when incorporated in vitamin tablets and shown to be stable over a period of 18 months and 12 months in vitamin tablets at room temperature.

5MTHF-glucosamine is proposed as an alternative source of folate to be used in the manufacture of food supplements according to Directive 2002/46/EC and it is proposed to be used at up to 1.8 mg/day, in vitamin, multivitamin, and vitamin and mineral tablets as a source of folate, which equates to 1 mg 5MTHF and 0.8 mg glucosamine.

The calcium salt of (6*S*)-5-methyltetrahydrofolic acid (5MTHF-Ca) has previously been authorised as a source of folate under the EU food supplement Directive 2002/46/EC as amended by Commission Regulation (EC) No 1170/2009.

No data were submitted by the applicant regarding the bioavailability of folate from 5MTHF-glucosamine in experimental animal studies. A crossover comparative bioavailability study was performed in human volunteers, comparing the bioavailability of folate from 5MTHF-glucosamine with that of folate from 5MTHF-Ca after a single oral exposure, both at 400 µg as free folates (and both with co-administration of 400 µg of folic acid). No significant differences in the plasma levels of folate between participants given 5MTHF-glucosamine and those given 5MTHF-Ca were observed. The Panel considered that the bioavailability of folate from 5MTHF-glucosamine is similar to the bioavailability of folate from 5MTHF-Ca in humans.

The Panel considered that 5MTHF-glucosamine will readily dissociate to 5MTHF and glucosamine in the aqueous environment of the digestive tract. The 5MTHF component will therefore be expected to be absorbed across the small intestine in a similar manner as 5MTHF originating from dietary sources, 5MTHF-Ca and folic acid.

No toxicological data on 5MTHF-glucosamine, except for genotoxicity data, have been provided by the applicant. The Panel noted that 5MTHF-glucosamine dissociates to its respective individual ions, and therefore it is acceptable to approach the toxicity assessment of 5MTHF-glucosamine based on the individual components (5MTHF and glucosamine).

To determine whether the 5MTHF-glucosamine, meeting the specifications as proposed by the applicant, is of genotoxic concern, a variety of tests were performed: Ames tests using five tester strains - *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2 uvrA; testing for induction of 5-trifluorothymidine-resistant mutants in mouse lymphoma L5178Y cells; and testing for chromosomal aberrations in Chinese hamster ovary cells. Overall, the Panel considered that the 5MTHF-glucosamine source did not raise concerns with respect to genotoxicity.

5MTHF-Ca was previously evaluated by the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) in 2004 and it was reported that in aqueous media 5MTHF-Ca dissociates readily and completely into calcium and 5MTHF ions.

5MTHF-Ca was shown to be non-genotoxic. Subchronic and developmental toxicity studies with 5MTHF-Ca in rats utilising maximum daily doses up to 400 and 1 000 mg/kg body weight (bw), respectively, did not reveal any adverse effects. The AFC Panel concluded that the use of 5MTHF-Ca "as a source of folate in foods for particular nutritional uses, food supplements and foods intended for the general population, with a tolerable upper level of 1 mg/adult person/day is not of concern from a safety point of view". This conclusion was based on the assumption that the tolerable upper intake level for folic acid of 1 mg/adult person/day established by the Scientific Committee on Food (SCF, 2000) would also be applied to the combined intake of folic acid and 5MTHF-Ca (expressed as folic acid).

The commonly recommended daily intake of glucosamine in food supplement form is 1 500 mg/day (25 mg/kg bw/day). Human trials have shown no adverse effects in long-term efficacy studies in healthy individuals or in short-term trials with diabetic subjects.

In 2009, the EFSA NDA Panel published an opinion on the safety of glucosamine hydrochloride from *Aspergillus niger* as a food ingredient and concluded that glucosamine hydrochloride from *Aspergillus niger* is safe as a food ingredient for adult consumers at the proposed intake level of 750 mg of glucosamine/day, but that consumers with diabetes mellitus or glucose intolerance should be advised to seek medical advice before consumption. In 2011, an NDA Panel opinion drew attention to the fact that patients taking coumarin anticoagulants constitute a further risk group. The ANS Panel noted that, at the proposed uses and use levels, exposure to glucosamine from 5MTHF-glucosamine is 0.8 mg/day. The Panel noted that the maximum exposure to glucosamine in this opinion resulting from the proposed uses and use levels is negligible compared to the exposure given in the NDA opinion on glucosamine from *Aspergillus niger*.

The Panel concluded that the proposed use and use levels of 5MTHF-glucosamine, when added for nutritional purposes to food supplements as a source of folate, is not of safety concern.

Given the manufacturing process, the Panel concluded that specifications for 5MTHF-glucosamine should include an indication for the absence of mycotoxins.

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BACKGROUND AS PROVIDED BY THE EUROPEAN 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 (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt added for nutritional purposes to food supplements. The relevant Union 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[4].

- Regulation (EC) No 258/97 of the European Parliament and of the Council concerning novel foods and novel food ingredients[5].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to:

- carry out the additional assessment for (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt, as a source of folate added for nutritional purposes to food supplements, in the context of Regulation (EC) No 258/97, and

- provide a scientific opinion on the safety of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt, when added for nutritional purposes to food supplements as a source of folate and on the bioavailability of folate from this source.

[4] OJ L 183, 12.7.2002, p. 51.
[5] OJ L 43, 12.2.97, p. 1.

ASSESSMENT

1. Introduction

The present opinion deals with the safety of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt (5MTHF-glucosamine), when added for nutritional purposes to food supplements as a source of folate, and on the bioavailability of folate from this source. The safety of folate itself in terms of amounts that may be consumed is outside the remit of the ANS Panel. The NDA Panel will advise on population reference intakes for folate by 2015 (EFSA-Q-2011-01212). The safety of 5MTHF-glucosamine in the context of being a novel food in accordance with Regulation (EC) No 258/97[6] has been considered by the ANS Panel. This is because 5MTHF-glucosamine fully dissociates in the gastrointestinal tract into the two components (6*S*)-5-methyltetrahydrofolic acid and glucosamine, which are not novel foods.

2. Technical data

2.1. Identity of the substance

(6*S*)-5-Methyltetrahydrofolic acid, glucosamine salt (5MTHF-glucosamine), has the chemical name *N*-[4-[[[(6*S*)-2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl]methyl]amino]benzoyl]-L-glutamic acid, glucosamine salt. It has the molecular formula $C_{32}H_{51}N_9O_{16}$, an anhydrous molecular weight of 817.80 g/mol and its Chemical Abstracts Service (CAS)_ Registry Number is 1181972-37-1.

Synonyms for 5MTHF, glucosamine salt include L-glutamic acid, *N*-[4-[[[(6*S*)-2-amino-3,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl]methyl]amino]benzoyl]-, compound with 2-amino-2-deoxy-D-glucose (1:2); (6*S*)-L-5-methyltetrahydrofolic acid, glucosamine salt, and (6*S*)-5-methylfolate, glucosamine salt. Its chemical structure is given in Figure 1.

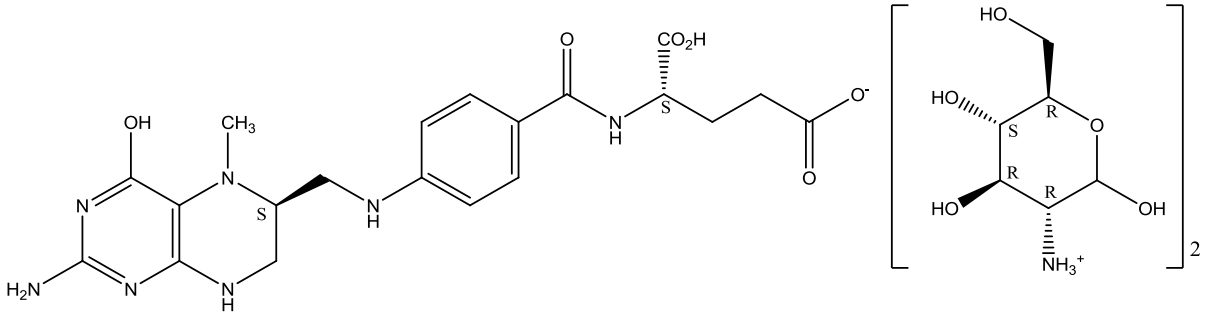


Figure 1: Chemical structure of 5MTHF-glucosamine

5-Methyltetrahydrofolic acid has two chiral carbon atoms: the C-atom in position 6 of the pteroyl moiety and the α-C atom in the glutamic acid moiety. The naturally occurring isomers of tetrahydrofolic acid and its 5-substituted derivatives are the (6*S*,α*S*) and (6*R*,α*S*) diastereoisomers. The natural form of the reduced folates is thought to be mainly the (6*S*) diastereoisomer, which has a greater biological activity than the (6*R*) isomer (SCF, 2000). 5MTHF-glucosamine, according to the specifications, has the configuration 6*S*,α*S*.

5MTHF-glucosamine is a creamy to light-brown powder that is very soluble in water at 25 °C, soluble in dilute acid or dilute alkali and insoluble in organic solvents.

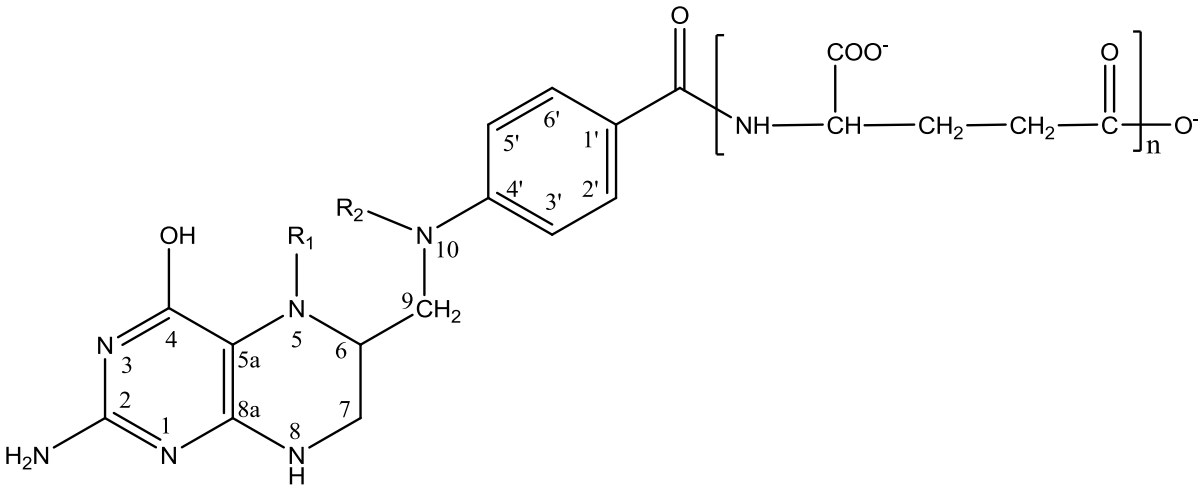
Infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR) and mass spectrometry (MS) spectra which allow the identification of the substance were provided by the applicant.

[6] 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, 14.2.97, p. 1.

2.2. Definition of folate(s)

In this opinion, the term "folate(s)" refers to all stereoisomers of tetrahydrofolic acid and all naturally occurring N5 and/or N10 substituted (methyl, methylene, methenyl, formyl and formimino) tetrahydrofolic acid compounds, in addition to substances metabolised to these substances (e.g. folic acid).

The general structural formula of the folates is given in Figure 2.



	R_1	R_2
Tetrahydrofolic acid	H	H
5-Methyltetrahydrofolic acid	$-CH_3$	H
5,10-Methylenetetrahydrofolic acid (R_1–R_2 ring closure)	$-CH_2-$	
5,10-Methenyltetrahydrofolic acid (R_1–R_2 ring closure)	-CH-	
5-Formyltetrahydrofolic acid	-HC=O	H
10-Formyltetrahydrofolic acid	H	-HC=O
5-Formiminotetrahydrofolic acid	-HC=NH	H

Figure 2: General structural formula of folates

Folates belong to the group of the B vitamins.

2.3. Specifications

Table 1 provides the specifications for 5MTHF-glucosamine as proposed by the applicant.

The analyses of three batches of 5MTHF-glucosamine showed that the material produced by the manufacturing process described below is consistent and complies with the proposed specifications.

Table 1: Specifications as proposed by the applicant of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt (5MTHF-glucosamine)

Parameter	Limits
Appearance	Creamy to light-brown powder
Identification (IR)	Positive
Water content	≤ 8.0 %
Glucosamine assay (HPLC)	34 - 46 % d.b.
5-Methyltetrahydrofolic acid assay (HPLC)	54 - 59 % d.b.
(6*S*)-5-Methyltetrahydrofolic acid (HPLC)	≥ 99.0 %
Total impurities (HPLC)	≤ 2.5 %
Related substances (HPLC):	
4-Aminobenzoylglutamic acid (ABGA)	≤ 0.3%
4α-Hydroxy-5-methyltetrahydrofolic acid (HOMeTHFA)	≤ 1.0%
(6*S*)-Pyrazino-s-triazine derivative [(6*S*)-Mefox]	≤ 0.3 %
5-Methyltetrahydropteroic acid (MeTHPA)	≤ 0.3 %
Any other individual impurity (HPLC)	≤ 1.0 %
Ethanol content*	≤ 0.5 %
Lead	≤ 2.0 mg/kg
Cadmium	≤ 1.0 mg/kg
Mercury	≤ 0.1 mg/kg
Arsenic	≤ 2.0 mg/kg
Boron	≤ 10 mg/kg
Microbiological criteria	
Total aerobic microbial count	≤ 10^2 CFU/g
Total combined yeast and moulds	≤ 10^2 CFU/g
E. coli	Absent/10 g

*Ethanol content is determined only when the product is isolated by precipitation in ethanol.

HPLC, high-performance liquid chromatography; d.b, dry basis; CFU, colony-forming unit

Given the manufacturing process, the Panel noted that specifications for 5MTHF-glucosamine lack an indication for the absence of mycotoxins.

2.4. Manufacturing process

5MTHF-glucosamine is chemically synthesised from folic acid (CAS Registry Number 59-30-3) by reduction with sodium tetrahydroborate, condensation of the resulting tetrahydrofolic acid with formaldehyde and reduction of the formed 5,10-methylene-tetrahydrofolic acid to (6*R,S*)-5-methyltetrahydrofolic acid with sodium tetrahydroborate. Purification of the racemic mixture is performed by crystallisation processes, yielding pure (6*S*)-5-methyltetrahydrofolic acid. Glucosamine hydrochloride is used to form the glucosamine salt. The final product is obtained either by lyophilisation or by precipitation in ethanol.

According to the applicant, the glucosamine component of 5MTHF-glucosamine originates from glucosamine hydrochloride (CAS Registry Number 66-84-2) derived from chitin sourced from a specific strain of *Aspergillus niger* and is United States Pharmacopeia (USP)/National Formulary (NF) grade. The applicant claims that this is the strain evaluated by the NDA Panel (EFSA, 2009), which does not produce ochratoxin A.

The content of (6*S*)-5-methyltetrahydrofolate (5MTHF) and glucosamine in preparations of 5MTHF-glucosamine, and the diastereoisomeric purity of 5MTHF, can be determined by three separate high-performance liquid chromatography (HPLC) methods. Results from three independent production batches were submitted by the applicant.

The Panel noted that the assay methodology was appropriate and the final product meets the specifications proposed by the applicant (Table 1).

2.5. Methods of analysis in food

The contents of 5MTHF and glucosamine in food supplements (tablets) are determined by separate laboratory-validated HPLC/UV methods. The assay for 5MTHF is also suitable for detection of folate degradation products.

2.6. Reaction and fate in food to which the source is added

The stability of 5MTHF and glucosamine in 5MTHF-glucosamine preparations alone, or when 5MTHF-glucosamine was incorporated into a multivitamin tablet preparation, was determined by the applicant. At room temperature, both 5MTHF and glucosamine were stable over a period of at least 18 months as 5MTHF-glucosamine alone and at least 12 months when incorporated into a multivitamin tablet.

2.7. Case of need and proposed uses

5MTHF-glucosamine is proposed as an alternative source of folate to be used in the manufacture of food supplements (tablets) according to Directive 2002/46/EC.[7] It is proposed to be used at up to 1.8 mg/day, in vitamin, multivitamin, and vitamin and mineral tablets, as a source of folate.

According to the applicant, the 5MTHF content of the 5MTHF-glucosamine is up to 59 % by weight. In order to receive the same molar dose of folate (5MTHF) from folic acid and 5MTHF-glucosamine, 5MTHF-glucosamine will be used at 185 % of the levels used for folic acid on a weight for weight basis. Using the recommended dose level of 400 µg folic acid/day, this is equivalent to 416 µg of 5MTHF or 740 (741) µg of 5MTHF-glucosamine. Based on the different molecular weights of the two sources, 1.8 mg of 5MTHF-glucosamine corresponds to 1 mg folic acid.

2.8. Information on existing authorisations and evaluations

5MTHF-glucosamine has not been previously evaluated, with the exception of the Food Safety Authority of Ireland (FSAI), as a novel food.

The FSAI considered it a novel food ingredient class 1.1. This class comprises foods and food components that are single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified and the source of the novel food has a history of food use in the Community (Commission Recommendation 97/618/EC[8]).

5MTHF (when used as the calcium salt) is authorised for use as a food supplement (Directive 2002/46/EC as amended by Commission Regulation (EC) No 1170/2009[9]).

The safety of (6*S*)-5-methyltetrahydrofolic acid, calcium salt (5MTHF-Ca), termed "calcium-L-methylfolate", was evaluated by the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) in 2004 (EFSA, 2004) and was included as a new source of folate in Annex II of Directive 2002/46/EC as amended by Commission Regulation (EC)

[7] 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.

[8] 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, 16.9.97, p. 1.

[9] Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.

No 1170/2009. 5MTHF-Ca was also approved for use in foods for particular nutritional uses, dietetic foods only (Commission Regulation (EC) No 953/2009).[10]

The AFC Panel concluded that the use of 5MTHF-Ca as a source of folate in foods for particular nutritional uses, food supplements and foods intended for the general population is not of concern from a safety point of view (EFSA, 2004). This conclusion was based on the assumption that the tolerable upper intake level for folic acid of 1 mg/adult person/day established by the SCF (2000) would also be applied to the combined intake of folic acid and 5MTHF-Ca (expressed as folic acid). No intake estimates for 5MTHF-Ca resulting from the intended levels of use were reported and instead it was stated that 5MTHF-Ca was intended to be used as a partial or complete substitute for folic acid, and indicated that the authorisation of 5MTHF-Ca as an alternative form of folate will, therefore, not increase the total intake of supplemental folates (EFSA, 2004).

The safety of 5MTHF-Ca was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006). JECFA evaluated the intended use of 5MTHF-Ca as a substitute for folic acid but did not evaluate the safety of folate fortification and supplementation. The Committee reported that it had no concerns about the safety of the proposed use of 5MTHF-Ca in dry crystalline or microencapsulated form as an alternative to folic acid in dietary supplements, foods for special dietary uses and other foods.

The safety of 5MTHF-Ca was also evaluated by Food Standards Australia New Zealand. It was concluded that the use of 5MTHF-Ca for the fortification of certain foods would not raise public health or safety concerns (FSANZ, 2008).

Glucosamine sulphate is listed in the EC Novel Food Catalogue[11] as a product used only as or in food supplements before 15 May 1997.

In June 2009, EFSA published an Opinion from the Panel on Dietetic Products, Nutrition and Allergies (NDA) on the safety of glucosamine hydrochloride from *Aspergillus niger* as a novel food ingredient. The NDA Panel concluded at this time that RGHAN (glucosamine hydrochloride from *Aspergillus niger*) was safe as a food ingredient for adult consumers at the proposed intake level of 750 mg of glucosamine per day, but that consumers with diabetes or glucose intolerance should be advised to seek medical advice before consumption (EFSA, 2009).

In 2011, EFSA published a statement from the NDA Panel on the safety of glucosamine for patients receiving coumarin anticoagulants. The NDA Panel concluded that there was evidence of an interaction between glucosamine and coumarin anticoagulants in some individuals that could lead to increased risk of haemorrhage. The level of risk could not be ascertained because of insufficient data (EFSA NDA Panel, 2011).

2.9. Exposure

According to the applicant, 5MTHF-glucosamine is proposed as an alternative source of folate in vitamin, multivitamin and vitamin and mineral tablets. The proposed maximum daily intake of 5MTHF-glucosamine in food supplements is 1.8 mg per person, which equates to 1 mg 5MTHF per person and 0.8 mg glucosamine per person. A tolerable upper intake level for folic acid of 1 mg/person/day for adults has been established by the SCF (SCF, 2000).

[10] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.

[11] http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/index.cfm?action=mod_search.details&seqfce=300, http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/whatimage.cfm?img=

3. Biological and toxicological data

3.1. Bioavailability

3.1.1. 5MTHF-glucosamine

No data were submitted by the applicant regarding the bioavailability of folate from 5MTHF-glucosamine in experimental animal studies.

The Panel noted that studies on folate bioavailability in animals may be complicated by the effects of both coprophagy and dietary constituents (Abad and Gregory, 1987), and therefore, without controlling for these factors, results from animal studies with folates may not be relevant for humans.

However, Bhandari and Gregory (1992) reported studies using 200 g male Crl:CDR BR rats receiving an oral administration of tritiated folic acid, 5MTHF or 5-formyl-tetrahydrofolate. The animals were starved overnight and faeces were collected for analysis, therefore significantly reducing coprophagic complications. Each compound underwent nearly complete absorption within 8 hours, and there was no significant difference in the excretion kinetics in relation to the form of folate administered. A biphasic pattern of excretion was observed over the following 8 days. Both urine and faeces were important excretory routes. The rapid phase of total isotopic excretion (urinary and faecal) exhibited a half-life ($t_{1/2}$) of 0.11 - 0.12 days, whereas the $t_{1/2}$ of the slower phase was 13.4 - 15.9 days. Isotopic distributions and the pattern of labelled folates in urine and tissues were similar regardless of the form administered. According to the authors, folic acid, 5MTHF and 5-formyl-tetrahydrofolate exhibited equivalent intestinal absorption, metabolism and in vivo kinetics.

Gregory et al. (1992) examined the oral bioavailability in man of the monoglutamyl forms of folic acid, tetrahydrofolate, 5-formyl-tetrahydrofolate, 10-formyl-tetrahydrofolate and 5-MTHF using stable-isotope labelling methods. Fasting adult males (n = 7) on a folate saturation regimen (2 mg/day for 7 days before each study, employed, according to the authors, to enhance the excretion of labelled folates), were given a single oral dose of each of the folates in apple juice (at a concentration not expected to saturate the luminal uptake mechanism), as well as an intravenous injection of folic acid as a control. The authors concluded that, in humans, differences exist in the bioavailability of monoglutamyl folates. However, the Panel noted that the mean urinary excretion (0 - 48 hours post dose) of folic acid and 5MTHF was not significantly different.

Venn et al. (2002) examined folate levels in women of childbearing age (18 - 49 years) in a randomised, placebo-controlled, double-blind trial. The effects of (6*S*)-5-MTHF and folic acid supplementation for 24 weeks on plasma folate and red cell folate (RCF) were compared. Women (n = 104) were randomly assigned to receive a supplement containing (6*S*)-5-MTHF (113 µg/day), folic acid (100 µg/day) or placebo. The mean estimated linear increase in plasma folate concentration was 0.3 (95 % confidence interval (CI) 0.1 - 0.5) and 0.4 (95 % CI 0.2 - 0.6) nmol/(l. week) in the (6*S*)-5-MTHFand folic acid groups, respectively. The mean estimated linear increase in RCF was 7.4 (95 % CI 4.5 - 10.3) and 8.3 (95 % CI 4.4 - 12.3) nmol/(l. week) in the (6*S*)-5-MTHF and folic acid groups, respectively. After 24 weeks, estimated mean increases in plasma folate concentrations were 6.9 (95 % CI 1.7 - 12.2) and 9.2 (95 % CI 3.3 - 15.1) nmol/l, and in RCF, 251 (95 % CI 143 - 360) and 275 (95 % CI 148 - 402) nmol/l, in the (6*S*)-5-MTHF and folic acid groups, respectively, relative to the placebo group. The authors concluded that low dose (6*S*)-5-MTHF and folic acid supplementation increased blood folate indices to a similar extent.

JECFA (2006) reported a study in humans in which ^{3}H- and ^{14}C-folic acid were given orally and the bioavailability of synthetic folic acid was estimated to be 90 - 95 %.

JECFA (2006) also reported a study with 5MTHF-Ca in humans and concluded that, after absorption, 5MTHF is indistinguishable from other absorbed and metabolised natural folates or from 5MTHF formed from synthetic folic acid. The bioavailability of 5MTHF-Ca and synthetic folic acid

(400 µg/day per person as folate) was compared in a randomised, double-blind, crossover study in 21 healthy women and reported to be similar.

JECFA concluded that the bioavailability of folate from 5MTHF-Ca was similar to the bioavailability of folate from folic acid in man and that synthetic 5MTHF-Ca had the same metabolic fate as other absorbed natural folates (JECFA, 2006).

A crossover comparative bioavailability study was performed in human volunteers, comparing the bioavailability of folate from 5MTHF-glucosamine with that of folate from 5MTHF-Ca after a single oral exposure (and both with co-administration of 400 µg of folic acid). A dose of 400 µg of 5MTHF-glucosamine or 5MTHF-Ca (expressed as free 5MTHF) was chosen as it represents the daily dose recommended as a dietary supplement (Technical dossier, 2012).

Twenty-four healthy males and females (one participant dropped out owing to consent withdrawal approximately one hour after the first period of administration) between 18 and 55 years of age and with a body mass index (BMI) between 18 and 30 were randomly assigned to each arm of the study before at least a 7-day washout period and crossover to the other arm of the study. Plasma folate levels were determined for up to 12 hours after each oral administration.

Mean and standard deviation C_{max} for folates after 5MTHF-glucosamine administration was 80.40 ± 18.694 nmol/l compared to 85.29 ± 28.457 nmol/l after administration of 5MTHF-Ca. The mean plasma half-life of folates after 5MTHF-glucosamine administration was 8.41 ± 3.887 hours, compared to 7.53 ± 3.805 hours after administration of 5MTHF-Ca.

No significant differences in the plasma levels of folate between participants given 5MTHF-glucosamine and those given 5MTHF-Ca were observed (Technical dossier, 2012).

The Panel considered that the bioavailability of folate from 5MTHF-glucosamine is similar to the bioavailability of folate from 5MTHF-Ca in humans.

The Panel also considered that 5MTHF-glucosamine will readily dissociate to 5MTHF and glucosamine in the aqueous environment of the digestive tract. The 5MTHF component will therefore be expected to be absorbed across the small intestine in a similar manner as 5MTHF originating from dietary sources and 5MTHF-Ca.

3.1.2. Glucosamine

Glucosamine-6-phosphate can be produced endogenously from fructose-6-phosphate and glutamine. In humans, the endogenous production of glucosamine is in the range of 4 - 20 g/day, with median values of ~ 14 g/day or 230 mg/kg bw/day, for a 60 kg adult (EFSA, 2009).

Data from studies in rats (Aghazadeh-Habashi and Sattari, 2002), dogs (Setnikar et al., 1986) and human volunteers (Setnikar et al., 1993; Setnikar and Rovati, 2001) suggest that glucosamine bioavailability is comparable between species and that glucosamine is metabolised via analogous pathways. According to the authors, rats and dogs therefore represent appropriate models for establishing the safety of glucosamine in humans (Setnikar and Rovati, 2001).

After oral administration, glucosamine is detectable in most tissues examined, including the liver, kidney and joint cartilage. Approximately 90 % of orally administered glucosamine is absorbed, with a significant fraction undergoing first-pass metabolism in the liver. Blood levels of glucosamine after oral glucosamine administration were 20 % of those achieved by intravenous administration (Setnikar and Rovati, 2001; Aghazadeh-Habashi and Sattari, 2002; IOM, 2003).

3.2. Toxicological data

No toxicological data on 5MTHF-glucosamine, except for genotoxicity data, have been provided by the applicant.

From the bioavailability data, the Panel considered that 5MTHF-glucosamine likely dissociates to its respective individual ions, and therefore it is acceptable to approach the toxicity assessment of 5MTHF-glucosamine based on the individual components (5MTHF and glucosamine).

3.2.1. Genotoxicity of 5MTHF-glucosamine

In vitro genotoxicity studies with 5MTHF-glucosamine have been performed by the applicant to test for genotoxicity of impurities that might have been formed during the manufacturing process.

5MTHF-glucosamine was tested for mutagenic activity in Ames tests using five tester strains - *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, and *Escherichia coli* WP2 uvrA - in either the absence or presence of metabolic rat liver S9 activation in compliance with OECD Guideline 471. Genotoxicity was examined using a plate incorporation assay and pre-incubation assay. No cytotoxicity was observed at any dose level with any tester strain in the absence or presence of S9 metabolism. Genotoxicity of 5MTHF was examined at the following concentrations: 313, 625, 1 250, 2 500 and 5 000 µg/plate. 5MTHF-glucosamine did not induce reverse mutation in *S. typhimurium* or *E. coli* under the assay conditions employed (Technical dossier, 2012).

5MTHF-glucosamine was tested for mutagenic activity by assaying for the induction of 5-trifluorothymidine-resistant mutants in mouse lymphoma L5178Y cells, in the absence and presence of S9 metabolic activation in compliance with OECD Guideline 476. A preliminary cytotoxicity assay was performed. Both in the absence and in the presence of S9 metabolism, no cytotoxicity was observed after 3 hours up to the maximum concentration of 5 000 µg/ml. After 24-hour treatment in the absence of S9 metabolic activation, no cells survived at 5 000 µg/ml and toxicity was observed at 2 500 µg/ml and 1 250 µg/ml, reducing survival to 15 % and 38 % respectively. Based on the toxicity results obtained in the preliminary trial, two independent assays for mutation to 5-trifluorothymidine resistance were performed using a variety of dose levels between 78.1 and 5000 µg/ml, with higher concentrations examined at 3 hours only. No relevant increases in mutant frequencies were observed following treatment with 5MTHF-glucosamine, in the absence or presence of S9 metabolism. 5MTHF-glucosamine therefore did not induce mutation in mouse lymphoma L5178Y cells in the absence or presence of S9 metabolic activation, under the assay conditions employed (Technical dossier, 2012).

The ability of 5MTHF-glucosamine to cause chromosomal aberrations was examined in Chinese hamster ovary cells in the absence and presence of S9 metabolic activation at 39.1, 78.1, 156, 313, 625, 1 250, 2 500 and 5 000 µg/ml in compliance with OECD Guideline 473. Cells were treated for 3 hours and harvested after 20 hours, or continually treated for 20 hours in the absence of S9. One hundred metaphase spreads from high-dose groups were scored for chromosomal aberrations. 5MTHF-glucosamine caused slight increases in the numbers of cells bearing aberrations, including and excluding gaps, but without reaching a statistical significance in cells treated for 3 hours without S9. No increase in the incidence of aberrant cells (including or excluding gaps) was observed at any dose level selected for scoring in the presence of S9 metabolism or with the continuous 20-hour treatment in the absence of S9 metabolism. Increases in the number of endoreduplicated cells over the controls were observed in the presence of S9 metabolism at the 5 000 µg/ml dose level. 5MTHF-glucosamine did not induce chromosomal aberrations in Chinese hamster ovary cells after in vitro treatment, under the assay conditions employed (Technical dossier, 2012).

Overall it can be concluded that the 5MTHF-glucosamine source did not raise concerns with respect to genotoxicity.

3.2.2. Toxicological data on the individual components

3.2.2.1. 5MTHF

The Panel noted that the EFSA AFC Panel evaluated the use of 5MTHF-Ca in food for particular nutritional uses, food supplements and foods intended for the general population (EFSA, 2004). In aqueous media, 5MTHF-Ca dissociates readily and completely into calcium and 5MTHF ions (EFSA, 2004). As for 5MTHF-Ca, the Panel considered that 5MTHF-glucosamine will be fully hydrolysed to release 5MTHF ions in the gastrointestinal tract.

The AFC Panel has previously evaluated unpublished subchronic and developmental toxicity studies with 5MTHF-Ca (EFSA, 2004). In a subchronic toxicity study, Wistar rats (10 animals per sex per group, age not reported) were orally dosed with 5MTHF-Ca at either 0, 25, 100 or 400 mg/kg bw/day for 13 weeks. No treatment-related effects were observed (Hamman et al., 2001). In a developmental study, female Wistar rats (25 rats per group) were treated orally with either 0, 100, 300 or 1 000 mg 5MTHF-Ca/kg bw/day between gestation days 5 and 19. No fetal, maternal or developmental toxicity was observed (Schubert and Jacobs, 2003).

3.2.2.2. Glucosamine

In June 2009, EFSA published an opinion of the NDA Panel on the safety of glucosamine hydrochloride from *Aspergillus niger* as a novel food ingredient (EFSA, 2009). In an acute oral toxicity study using male and female rats, glucosamine hydrochloride (99 % purity) did not induce adverse effects after administration at a dose of 5 000 mg/kg bw. The No-Observed-Adverse-Effect Level (NOAEL) identified for free-base glucosamine in a 52-week rat study was 2 130 mg/kg bw/day. The NDA Panel noted that the commonly recommended daily intake of glucosamine in food supplement form is 1 500 mg/day (25 mg/kg bw/day), and human trials have shown no adverse effects in long-term efficacy studies in healthy individuals or in short-term trials with diabetic subjects (EFSA, 2009).

A subchronic toxicity study with *N*-acetylglucosamine (GlcNAc), a monomeric form of chitin, was conducted in groups of 10 male and 10 female F344 rats fed pelleted diets containing 0, 0.625, 1.25, 2.5 or 5 % concentrations for 13 weeks (Lee et al., 2004). All animals survived until the end of the experiment. Slight, non-significant increases in body weights were observed in males receiving 0.625, 1.25 or 2.5 % GlcNAc from week 4 until the end of the experiment, at which point, body weight of animals at sacrifice was found to be significantly increased in males receiving 0.625, 1.25 or 2.5 % GlcNAc (resulting in decreased relative weights of many organs). However, there were no obvious indications of toxicity in any group receiving GlcNAc in terms of clinical signs, food intake, haematology, serum biochemistry and histopathological findings. The authors concluded that orally administered GlcNAc exerts no obvious toxicity in F344 rats at concentrations up to 5 % in the diet for 13 weeks, and identified a NOAEL of 5 %, equivalent to 2 476 and 2 834 mg/kg bw/day for male and female rats, respectively.

4. Discussion

Following a request from the European Commission to EFSA, the ANS Panel was asked to provide a scientific opinion on the safety of 5MTHF-glucosamine when added for nutritional purposes to food supplements as a source of folate and on the bioavailability of folate from this source. The safety of folate, in terms of the amounts that may be consumed, is outside the remit of this Panel. The safety of 5MTHF-glucosamine in the context of being a novel food in accordance with Regulation (EC) No 258/97 has been considered by the ANS Panel. This is because 5MTHF-glucosamine fully dissociates in the gastrointestinal tract into the two components (6*S*)-5-methyltetrahydrofolic acid and glucosamine, which are not novel foods.

In this opinion, the term "folate(s)" refers to all stereoisomers of tetrahydrofolic acid and all naturally occurring N5 and/or N10 substituted (methyl, methylene, methenyl, formyl and formimino) tetrahydrofolic acid compounds, in addition to substances metabolised to these substances. Folates are

present in the normal diet and are found at high levels in liver, lentils and leafy green vegetables such as spinach (EGVM, 2003). Folates consist of three constituents – a substituted pteridine, ρ-aminobenzoate and glutamate. Mammals are unable to synthesise the pteridine rings. Folates function as a biochemical carrier of ^{1}C-methyl groups in a variety of oxidation states. Folate deficiency results in inhibition of nucleic acid synthesis (and the synthesis of some amino acids), and a dietary deficiency is notably associated with adverse effects during fetal development (Eichholzer et al., 2006). Only the safety of 5MTHF-glucosamine when added for nutritional purposes to food supplements as a source of folate, and the bioavailability of folate from this source, are addressed in this opinion.

5MTHF-glucosamine is chemically synthesised from folic acid and glucosamine hydrochloride. Analytical assays are used to confirm the identity of the constituents and contaminants. Several contaminants of 5MTHF-glucosamine are identified as folate-related substances.

Glucosamine is a well-characterised amino monosaccharide in which a hydroxyl group (-OH) is replaced with an amino group (-NH_2) (2-amino-2-deoxy-D-glucose). Glucosamine sulphate, when extracted from shellfish, has an established history of consumption in the EU prior to 15 May 1997. According to the EFSA Panel on Biological Hazards (BIOHAZ Panel) opinion, *Aspergillus niger* is not recommended for inclusion in the qualified presumption of safety (QPS) list (EFSA BIOHAZ, 2012). However, according to the applicant, the glucosamine component of 5MTHF-glucosamine originates from glucosamine hydrochloride derived from chitin sourced from the same strain of *Aspergillus niger* evaluated by the NDA Panel (EFSA, 2009). The Panel noted that the NDA Panel concluded that "*strains not producing ochratoxin A would be safe production strains. The strain used to produce RGHAN (glucosamine hydrochloride from Aspergillus niger) is not an ochratoxin A producer*" (EFSA, 2009). Given the manufacturing process, the Panel noted that specifications for 5MTHF-glucosamine should include an indication for the absence of mycotoxins.

5MTHF-glucosamine is proposed as an alternative source of folate to be used in the manufacture of food supplements in accordance with Directive 2002/46/EC, and it is proposed to be used at up to 1.8 mg 5MTHF-glucosamine/day, in vitamin, multivitamin, and vitamin and mineral tablets, as a source of folate, which is considered by the applicant biologically equivalent to 1 mg 5MTHF/day and 0.8 mg glucosamine/day.

The Panel noted that an earlier application for authorisation of 5MTHF-glucosamine was submitted to the FSAI. The FSAI considered it a novel food ingredient class 1.1. This class comprises foods and food components that are single chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified and the source of the novel food has a history of food use in the community (Commission Recommendation 97/618/EC).

Folic acid is authorised as a food supplement in the EU (Directive 2002/46/EC). However, folic acid does not occur in foods in significant amounts. 5MTHF is the predominant natural form of folate in many foods and also the essential form in which folates occur and are stored in the human body (Shane, 2000; Scott, 2001).

The population reference intake for folic acid set by the SCF is 200 μg/day for adults, and 400 μg/day in pregnancy (SCF, 2000). In European countries in the 1990s, the average folate intake in adults was found to be around 300 μg/day in adult males, and 250 μg/day in adult women (de Bree et al., 1997). For pregnant women and women intending to become pregnant, intake > 400 μg/day is considered protective against neural tube defect (SCF, 2000).

A tolerable upper intake level for folic acid of 1 mg/day/person was set on the basis of findings in patients suffering from pernicious anaemia and treated with high doses of folic acid (SCF, 2000). The SCF (2000) stated that there is no evidence of risk associated with high intakes of natural, reduced folates, and thus no data to set a tolerable upper intake level for natural folates. However, they also

stated that the safety and efficacy of synthetic reduced folates, i.e. 5MTHF, as an alternative for folic acid, needs further study. In any case, 5MTHF-Ca was included as a new source of folate in Annex II of Directive 2002/46/EC as amended by Commission Regulation (EC) No 1170/2009.

Natural food folates are predominantly polyglutamates with a variable number of glutamate residues (Shane, 2000). Folates, including folic acid, are primarily absorbed in the proximal small intestine (Shane, 2000) and absorption depends on glutamate residues. Because only the monoglutamate form is absorbed, the polyglutamyl folates must be deconjugated by the intestinal brush border enzyme γ-glutamyl hydrolase to the monoglutamyl form (Gregory et al., 1992; Shane, 2000). Before monoglutamated forms of folic acid can enter circulation, they are reduced to tetrahydrofolate and then either methylated or formylated during passage through mucosal cells in the jejunum (Shane, 2000). Thus, the absorption efficiency of folates depends on the length of the glutamyl side-chain, with the polyglutamate chain shown to reduce bioavailability relative to monoglutamyl folic acid (Melse-Boonstra et al., 2004). Given the hydrophilic nature of folates, they are unlikely to cross cell membranes by diffusion (Selhub and Rosenberg, 1981; Hou et al., 2005). The absorption primarily occurs via a highly specific reduced folate carrier (Hou et al., 2005).

No data were submitted by the applicant regarding the bioavailability of folate from 5MTHF-glucosamine in experimental animal studies. The applicant has reported a crossover comparative bioavailability study in human volunteers, comparing the bioavailability of folate from 5MTHF-glucosamine with that of folate from 5MTHF-Ca after a single oral exposure, both at 400 µg as free folates (and both with co-administration of 400 µg of folic acid). No significant differences in the plasma levels of folate between participants given 5MTHF-glucosamine and those given 5MTHF-Ca were observed. The Panel considered that the bioavailability of folate from 5MTHF-glucosamine is similar to the bioavailability of folate from 5MTHF-Ca in humans.

No adverse effects have been associated with the consumption of excess folate from foods (Butterworth and Tamura, 1989). Adverse effects were exclusively reported for excessive intakes of folic acid (SCF, 2000).

No toxicological data on 5MTHF-glucosamine, except for genotoxicity data, have been provided by the applicant. The Panel noted that 5MTHF-glucosamine dissociates in the gastrointestinal tract to its respective individual ions, and therefore it is acceptable to approach the toxicity assessment of 5MTHF-glucosamine based on the individual components, 5MTHF and glucosamine.

To determine whether the 5MTHF-glucosamine, meeting the specifications as proposed by the applicant, is of genotoxic concern, a variety of tests were performed: Ames tests using five tester strains - *S. typhimurium* TA1535, TA1537, TA98 and TA100, and *E. coli* WP2 uvrA; testing for induction of 5-trifluorothymidine-resistant mutants in mouse lymphoma L5178Y cells; and testing for chromosomal aberrations in Chinese hamster ovary cells. Overall, the Panel concluded that the 5MTHF-glucosamine source did not raise concerns with respect to genotoxicity.

5MTHF-Ca was previously evaluated by the AFC Panel and it was reported that in aqueous media 5MTHF-Ca dissociates readily and completely into calcium and 5MTHF ions (EFSA, 2004). As for 5MTHF-Ca, 5MTHF-glucosamine is obtained synthetically from folic acid, and the Panel considered that 5MTHF-glucosamine will be fully hydrolysed to release 5MTHF ions in the gastrointestinal tract. 5MTHF-Ca has been reported to be non-genotoxic (EFSA, 2004). Subchronic and developmental toxicity studies in rats with 5MTHF-Ca utilising maximum daily doses of up to 400 and 1 000 mg/kg bw, respectively, did not reveal any adverse effects. The AFC Panel therefore concluded that the use of 5MTHF-Ca as a source of folate in food supplements used as a replacement for folic acid (with a tolerable upper intake level of 1 mg folic acid/adult person/day) is not of concern from a safety point of view.

Glucosamine sulphate is listed in the "Novel Food catalogue" with "FS-status". The Panel noted that, since glucosamine hydrochloride is used as the source of glucosamine in 5MTHF-glucosamine,

glucosamine as contained in the product may be considered a novel substance to the population of the EU.

In 2009, the NDA Panel published an opinion on the safety of glucosamine hydrochloride from *Aspergillus niger* as a food ingredient and concluded that glucosamine hydrochloride from *Aspergillus niger* is safe as a food ingredient for adult consumers at the proposed intake level of 750 mg of glucosamine/person/day, but that consumers with diabetes mellitus or glucose intolerance should be advised to seek medical advice before consumption (EFSA, 2009). In 2011, an NDA Panel opinion drew attention to the fact that patients taking coumarin anticoagulants constitute a further risk group (EFSA NDA Panel, 2011).

The Panel noted that, although the exact dose-response relationship cannot be determined, the case reports as mentioned in the NDA opinion indicate that glucosamine intakes would be several hundreds mgs/day and above (EFSA NDA Panel, 2011).

The Panel noted that under the proposed uses and use levels, maximum exposure to glucosamine from 5MTHF-glucosamine is 0.8 mg/day. The Panel noted that the maximum exposure to glucosamine in this opinion resulting from the proposed uses and use levels is negligible compared to the exposure given in the NDA opinion on glucosamine from *Aspergillus niger*.

CONCLUSIONS

The Panel concluded that the bioavailability of folate from 5MTHF-glucosamine would be comparable to that of folate from 5MTHF-Ca after oral exposure in humans.

The Panel concluded that the folate source 5MTHF-glucosamine did not raise concerns with respect to genotoxicity.

The Panel concluded that the proposed use and use levels of 5MTHF-glucosamine, when added for nutritional purposes to food supplements as a source of folate, is not of safety concern.

Given the manufacturing process, the Panel concluded that specifications for 5MTHF-glucosamine should include an indication for the absence of mycotoxins.

DOCUMENTATION PROVIDED TO EFSA

1. Application for the placing on the market of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt as novel food ingredient according to Regulation (EC) No 258/97. June 2011. Submitted by GNOSIS S.p.A., Desio, Italy.

2. Technical dossier (2012). Request for the authorisation of (6*S*)-5-methyltetrahydrofolic acid, glucosamine salt for inclusion in Directive 2002/46/EC. July 2012. Submitted by GNOSIS S.p.A., Desio, Italy.

3. Additional data follow-up EFSA letter on 12 April 2013. Data provided on 25 April 2013.

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ABBREVIATIONS

AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
BIOHAZ	EFSA Panel on Biological Hazards
BMI	body mass index
CAS	Chemical Abstracts Service
EFSA	European Food Safety Authority
EU	European Union
FSAI	Food Safety Authority of Ireland
GlcNAc	*N*-acetylglucosamine
HPLC	High-performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
IR	infrared
MS	mass spectrometry
5MTHF-Ca	(6*S*)-5-methyltetrahydrofolic acid, calcium salt
5MTHF-glucosamine	(6*S*)-5-methyltetrahydrofolic acid, glucosamine salt
NDA	Panel on Dietetic Products, Nutrition and Allergies
NMR	nuclear magnetic resonance
NOAEL	No Observed-Adverse-Effect Level
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
RCF	red cell folate
RGHAN	glucosamine hydrochloride from *Aspergillus niger*
SCF	Scientific Committee on Food
USP/NF	United States Pharmacopeia/National Formulary
UV	ultraviolet