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SCIENTIFIC OPINION

Safety of monosodium salt of L-5-methyltetrahydrofolic acid as a novel food pursuant to Regulation (EU) 2015/2283 and the bioavailability of folate from this source in the context of Directive 2002/46/EC, Regulation (EU) No 609/2013 and Regulation (EC) No 1925/2006

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on monosodium salt of L-5-methyltetrahydrofolic acid (5-MTHF) as a novel food (NF) pursuant to Regulation (EU) 2015/2283 and to address the bioavailability of folate from this source in the context of Directive 2002/46/EC, Regulation (EU) No 609/2013 and Regulation (EC) No 1925/2006. The NF is produced by chemical synthesis and consists of at least 95% (w/w) of 5-MTHF and 4%–5% (w/w) of sodium. It is proposed to be used as a partial or complete substitute to folic acid and other sources of added folate in a number of food categories. The production process, composition, specifications and stability of the NF do not raise safety concerns. When used as an ingredient in different food matrices, proper processing/storage conditions need to be considered to preserve the stability of the NF. Regarding bioavailability, the Panel considers that the NF readily dissociates into Na and L-methylfolate ions, which subsequently are absorbed and enter the circulation. Thus, the bioavailability of 5-MTHF from the NF is comparable to that of other currently authorised salts of 5-MTHF. The Panel considers that the consumption of the NF is not nutritionally disadvantageous as long as the combined intake of the NF and the other supplemental forms of folate under their authorised conditions of use is below the ULs established for the different age groups of the general population. The Panel concludes that the NF is safe under the proposed conditions of use. The Panel also concludes that the NF is a source from which folate is bioavailable.

KEYWORDS

bioavailability, folate, folic acid, food for specific groups, monosodium salt of L-5-methyltetrahydrofolic acid, novel food, nutrient source, safety

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Background

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 (EU) 2015/2283 of the European Parliament and of the Council on novel foods.[1]
- Directive 2002/46/EC of the European Parliament and of the Council lays down requirements on food supplements.[2]
- Regulation (EU) No 609/2013 of the European Parliament and of the Council on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control.[3]
- Regulation (EC) 1925/2006 on the addition of vitamins and mineral and of certain other substances to foods.[4]

On 12 November 2020, the company Merck & Cie submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[5] to authorise the placing on the Union market of monosodium salt of L-5-methyltetrahydrofolic acid as a novel food.

The applicant requests authorisation of the novel food in a number of foods, including food supplements, food intended for infants and young children (infant formula and follow-on formula; processed cereal-based food and baby food), food for special medical purposes, total diet replacement for weight control, and fortified foods.

The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

Terms of Reference

The Commission is of the opinion that the novel food, monosodium salt of L-5-methyltetrahydrofolic acid should be considered as a source of folate in the context of the relevant legislation.

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks EFSA to provide a scientific opinion:

– By carrying out the assessment for monosodium salt of L-5-methyltetrahydrofolic acid as a novel food in accordance with Article 10(3) of Regulation (EU) 2015/2283;
– Following the outcome of the novel food assessment, by evaluating the safety and bioavailability of the novel food when added for nutritional purposes as a source of folate to food supplements, food intended for infants and young children (infant formula and follow-on formula; processed cereal-based food and baby food), food for special medical purposes, total diet replacement for weight control, fortified foods and food for the general population, in the context of Directive 2002/46/EC, Regulation (EU) No 609/2013 and Regulation (EC) No 1925/2006.

The Commission also asks EFSA to evaluate and inform the Commission as to whether and if so, to what extent, the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled in elaborating its opinion on monosodium salt of L-5-methyltetrahydrofolic acid regarding the proprietary data for which the applicant is requesting data protection.

1.2 | Information on existing evaluations and authorisations

Various synthetic forms of folate have been authorised for use in foods in the EU:

– Pteroylmonoglutamic acid (thereafter called folic acid) and calcium L-methylfolate for addition to foods (Annex II of Regulation (EC) No 1925/2006),

[1]Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (OJ L 327. 11.12.2015, p. 1).

[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).

[3]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009 (OJ L 181, 29.6.2013, p. 35).

[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).

[5]Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (OJ L 327, 11.12.2015, p. 1).

- Folic acid, calcium L-methylfolate and (6S)-5-methyltetrahydrofolic acid, glucosamine salt in food supplements (Annex II of Directive 2002/46/EC),
- And folic acid and calcium L-methylfolate in all foods for special groups as per Regulation (EU) No 609/2013.

EFSA has set dietary reference values for folate (EFSA NDA Panel, 2014). For infants aged 7–11 months, an adequate intake (AI) for folate was set at 80 µg dietary folate equivalents (DFE)/day.[6] Population reference intakes (PRIs) were set for children ranging from 120 (1–3 years) to 330 µg DFE/day (15–17 years). For adults, an average requirement (AR) of 250 µg DFE/day and a PRI of 330 µg DFE/day were set. An AI was set for pregnant women at 600 µg DFE/day and a PRI of 500 µg DFE/day was established for lactating women.

In its recent assessment of tolerable upper intake levels (UL) for folate (EFSA NDA Panel, 2023), the Panel retained the previously established ULs for supplemental intake of 200 µg/day (1–3 years), 300 µg/day (4–6 years), 400 µg/day (7–10 years), 600 µg/day (11–14 years), 800 µg/day (15–17 years), 1000 µg/day (all adults including pregnant and lactating women) (SCF, 2000). The Panel also set a UL of 200 µg/day for infants aged 4–11 months. These ULs apply to the combined intake of folic acid and currently authorised forms of folate for addition to food and use in food supplements [(6S)-5-methyltetrahydrofolic acid glucosamine and L-5-methyltetrahydrofolic acid calcium salts].

As summarised in the previous opinion on calcium L-methylfolate (EFSA NDA Panel, 2020): *'In 2004, the European Food Safety Authority (EFSA) issued an opinion on calcium L-methylfolate (*EFSA AFC Panel, 2004*) concluding that its use as a source of folate in foods for particular nutritional uses, food supplements and foods intended for the general population, at level of 1 mg/adult person/day does not raise safety concerns in line with the tolerable upper level (UL) for folic acid for adults defined by the* Scientific Committee on Food *(*2000*). Additionally, in 2013 EFSA issued another opinion on (6S)-5-methyltetrahydrofolic acid (5-MTHF), glucosamine salt as an alternative source of folate added for nutritional purposes to food supplements. The Panel concluded that the proposed use levels (up to 1.8 mg/day, which equates to 1 mg 5-MTHF and 0.8 mg glucosamine) are not of safety concern (*EFSA ANS Panel, 2013*)'*. The same opinion was dealing with the extension of use of calcium L-methylfolate as a source of folate added for nutritional purposes to infant and follow-on formula, baby food and processed cereal-based food (EFSA NDA Panel, 2020). The Panel considered *'that calcium L-methylfolate is a source from which folate is bioavailable'* and concluded that *'calcium L-methylfolate is safe under the proposed uses and use levels*[7] *for infants and young children'*.

In addition, EFSA was recently asked to assess the bioavailability of various forms of folate added to food and establish the conversion factor to express their amount into DFE (EFSA NDA Panel, 2022). The derived DFE equations were:

- DFE = natural food folate + 1.7 × folic acid (FA) + 1.7 × 5-MTHF for fortified foods and food supplements providing intakes < 400 µg/day;
- DFE = natural food folate + 1.7 × FA + 2.0 × 5-MTHF for food supplements providing intakes ≥ 400 µg/day.

2 | DATA AND METHODOLOGIES

2.1 | Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[8].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: analytical reports and methods validation regarding the NF identification, characterisation, solubility, particle size and distribution, dissolution study, stability studies, study on bioavailability, genotoxicity studies and HACCP plan.

2.2 | Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions

[6]Dietary folate equivalents (DFE) are defined as follows: 1 µg DFE = 1 µg food folate = 0.6 µg folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a folic acid supplement taken on an empty stomach; for combined intakes of food folate and folic acid, DFEs can be computed as follows: µg DFE = µg food folate + (µg folic acid × 1.7) (EFSA NDA Panel, 2014).

[7]Formula for infants below 16 weeks of age may contain calcium L-methylfolate up to 21.6 µg/100 mL, while processed cereal-based food and baby food for infants and young children may contain up to 56 µg/100 kcal.

[8]Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

for the assessment of novel foods are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469. The legal provisions for the assessment of food intended for infants and young children, food for special medical purposes and total diet replacement for weight control are laid down in Regulation (EU) 609/2013 and, respectively, in Commission Delegated Regulation 2017/1798 (total diet replacement for weight control), in Commission Delegated Regulation (EU) 2016/128 (food for special medical purposes) and in Commission Delegated Regulation (EU) 2016/127 (as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit. This assessment also is not an assessment on whether the NF is suitable as stipulated by Regulation (EU) No 609/2013[9].

The evaluation of the bioavailability of the nutrient (folate) from the source (monosodium salt of L-5-methyltetrahydrofolic acid) was conducted in line with the principles contained in the 'Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources' (EFSA ANS Panel, 2018).

The assessment of small particles in the NF was conducted in line with the principles of the 'Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021).

3 | ASSESSMENT

3.1 | Introduction

The NF, which is the subject of the application, is the monosodium salt of L-5-methyltetrahydrofolic acid, denominated as Arcofolin®. The NF is produced by chemical synthesis and consists of at least 95% weight for weight (w/w) of 5-MTHF. The NF is proposed to be used as a source of folate in all categories of foods excluding foods for infants and young children (except where specifically provided for) 'as an alternative for folic acid' (see Section 3.8 *Proposed uses and use levels*). The NF falls under the following categories, as defined in Art. 3 of Regulation (EU) 2015/2283: (i) food with a new or intentionally modified molecular structure, where that structure was not used as, or in, a food within the Union before 15 May 1997; (ix) vitamins, minerals and other substances used in accordance with Directive 2002/46/EC, Regulation (EC) No 1925/2006 or Regulation (EU) No 609/2013.

3.2 | Identity of the NF

The NF, i.e. the monosodium salt of L-5-methyltetrahydrofolic acid (Table 1 and Figure 1) has two chiral carbon atoms: the α-C-atom in the L-glutamic acid moiety and the C-atom in position 6 of the pteroyl moiety. According to the applicant, the NF can be described by the following IUPAC name: (2S)-2-[[4-[(((6S)-2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl)amino]benzoyl]amino]pentanedioic acid monosodium salt. However, after the Panel's suggestion to be in line with nomenclature of the previously authorised calcium L-methylfolate (EFSA AFC Panel, 2004; EFSA NDA Panel, 2020), the applicant accepted the designation of the NF as (2S,6S)-2-[[4-[(2-amino-5-methyl-4-oxo-3,6,7,8-tetrahydropteridin-6-yl) methylamino]benzoyl]amino] pentanedioic acid monosodium salt.

TABLE 1 Chemical identity of the NF.

Chemical substance	
Chemical name	N-[4-[[(2-amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-(6S)-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid
IUPAC name	(2S,6S)-2-[[4-[(2-amino-5-methyl-4-oxo-3,6,7,8-tetrahydropteridin-6-yl)methylamino]benzoyl] amino] pentanedioic acid monosodium salt
Common name	Monosodium salt of L-5-methyltetrahydrofolic acid
Other names: Synonyms, trade names, abbreviations	Arcofolin® Monosodium L-mefolinate Monosodium L-mefolate Monosodium L-5-methyltetrahydrofolate L-Methylfolate L-5-Methyltetrahydrofolic acid, monosodium salt L-5-MTHF-Na (6S)-Methyltetrahydrofolic acid, monosodium salt (6S)-5-MethylTHF-Na

[9]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35.

Chemical substance	
CAS Number:	2246974-96-7
Molecular formula:	$C_{20}H_{24}N_7NaO_6$
Molecular weight	481.44 g/mol

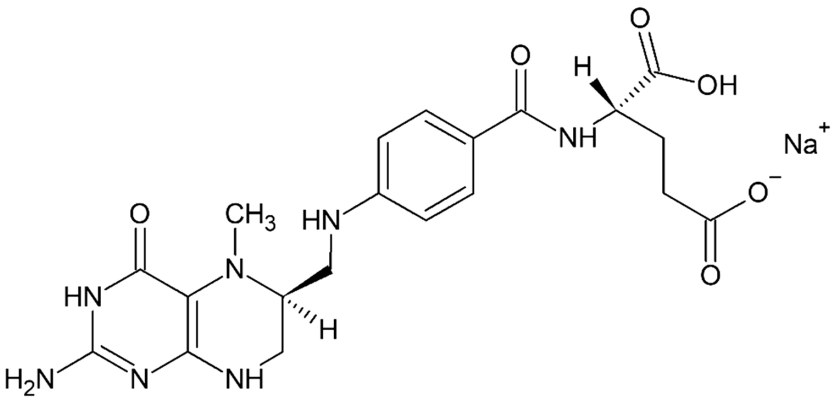

FIGURE 1 Structural formula of the NF.

Upon request of the Panel, the applicant characterised the NF by means of: infra-red (IR) spectroscopy, nuclear magnetic resonance spectroscopy (NMR), massspectrometry (MS), high-performance liquid chromatography with ultra-violet and mass spectrometric detection (HPLC–UV/MS), elemental analysis, specific optical rotation testing and diastereoisomer purity test (by HPLC–UV). In addition, the applicant provided X-ray diffractometry data.

3.2.1 | Particle size and solubility of the NF

Following a request of the Panel, the applicant performed solubility testing of the NF in water (pH = 5.8) and in artificial gastric juice (without pepsin, at pH = 2), in accordance with OECD TG 105 (flask method) (OECD, 1995) and compared it with the solubility of calcium L-methylfolate (Table 2).

TABLE 2 Solubility of NF in different media versus Ca L-methylfolate.

Parameter	NF (L-5-MTHF-Na) (n = 6)	Ca L-methylfolate (n = 3)
Mean water solubility (mg/mL)	26.5	9.7
Mean solubility in artificial gastric juice (mg/mL)	22.9	15.2
Mean solubility in ethanol (µg/mL)	4.2[a]	–

[a]Tested in 1 batch in duplicate.

In addition, the applicant provided information on particle size, shape and distribution on two batches of the NF, by automated optical microscopy-light microscopy, which is not considered as an appropriate technique by EFSA guidance document (EFSA Scientific Committee, 2021).

Since the NF solubility in water was lower than 33.3 g/L, the applicant was requested to perform the subsequent steps and to investigate and characterise the presence of small particles, i.e. either to test the dissolution kinetics in water or to demonstrate that particles < 500 nm are less than 10% in number. Thus, the applicant performed scanning electron microscopy (SEM) analysis on three batches of the NF. The results showed that the NF contains 40% of particles by number that are < 500 nm in diameter, and approx. 30% by number < 250 nm.

Consequently, the applicant was requested to further investigate the nature of the NF constituting particles by providing data on the dissolution rate, i.e. the kinetics of dissolution in water (EFSA Scientific Committee, 2021). The dissolution study was performed in 85 mmol/L $NaHCO_3$ and 40 mmol/L NaCl at pH of 3 and 7, up to 120 min and by using ultrafiltration and ultra performance liquid chromatography with ultra-violet detection (UPLC–UV) in three batches of the NF. Complete dissolution was observed at both pH values. The Panel concludes that the consumers will not be exposed to small particles since the NF will dissolve before uptake by intestinal cells.

3.3 | Production process

The NF is produced in line with Good Manufacturing Practice for medicinal products (GMP) and the Hazard Analysis Critical Control Points (HACCP) principles. The production process consists of three main steps (all conducted under nitrogen atmosphere), starting from commercially available folic acid.

Initially, folic acid is hydrogenated in the presence of platinum (IV) oxide hydrate catalyst to give a mixture of (6S)- and (6R)-tetrahydrofolic acid. The mixture is then isolated by filtration and crystallisation with benzenesulfonic acid and an intermediate, (6S)-tetrahydrofolic acid benzenesulfonate (LTBH), is formed.

In the second step, LTBH is treated with formaldehyde to give (6R)-5,10-methylenetetrahydrofolic acid which is subsequently reduced with sodium borohydride to give (6S)-5-methyl tetrafolate, which is subsequently acidified with HCl to form a second intermediate, (6S)-5-methyltetrahydrofolic acid (LMSR).

The last step includes addition of purified aqueous-alcoholic solution, 4-(2-hydroxyethyl)morpholine (HEM) and aqueous sodium hydroxide solution to LMSR, which then undergoes crystallisation to produce the final product (6S)-methyltetrahydrofolic acid, monosodium salt, i.e. the NF.

The Panel considers that the production process is sufficiently described. Further considerations on the safety of the residual amounts of platinum, formaldehyde, ethyl esters and HEM are reported in Section 3.7.2 ('Estimate of exposure to undesirable substances').

3.4 | Compositional data

The NF consists of L-5-methyltetrahydrofolic acid (≥ 95% w/w), sodium (4%–5% w/w), residual solvents such as ethanol and isopropanol (< 1% w/w) and other impurities such as residual starting material, by-products and degradation products of the production process (< 2.5% w/w) (Table 3).

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for five independently produced batches of the NF (Table 3). Batches 1 and 2 were presented as '*trial batches*' by the applicant, while the batches 3–5 were '*production scale batches*'. The analyses were performed by the applicant's internal laboratory, which has the GMP certificate.

TABLE 3 Batch to batch analysis of the NF.

Parameter (unit)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Method of analysis
Appearance colour	Beige	Beige	Beige	Beige	Beige	Visual
Appearance texture	Powder	Powder	Powder	Powder	Powder	Visual
Identity	Method at the time not yet in place	Method at the time not yet in place	Ok	Ok	Ok	IR-Spectrometry ATR
Water (% w/w)	0.6	0.5	0.2	0.2	0.2	Karl Fischer titration (USP 921, Method Ic)
Identity retention time (sodium)	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	IC (in-house methods)
Sodium (% w/w)	4.3	4.6	4.5	4.5	4.7	
4-(2-Hydroxyethyl) morpholine (HEM) (% w/w)[a]	< 0.001%	< 0.001%	< 0.001%	< 0.001%	< 0.001%	
Identity retention time (mefolate)	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	HPLC–UV USP DS Ca-L-MeTHFA
Mefolinate, as free acid (% w/w)	95.0	93.8	95.2	95.4	94.3	
Residual solvents						
Ethanol (% w/w)	0.2	0.2	0.06	0.07	0.06	GC-Headspace (USP 467)
Isopropanol (% w/w)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	
Heavy metals[b]						
Boron (mg/kg)	< 0.5	< 0.5	< 0.3	< 0.3	< 0.3	ICP-OES (USP 233)
Platinum (mg/kg)	< 0.4	< 0.4	< 0.3	< 0.3	< 0.3	ICP-MS (USP 233)
Arsenic (mg/kg)	< 0.2	< 0.2	< 0.3	< 0.3	< 0.3	
Cadmium (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Lead (mg/kg)	< 0.1	< 0.1	< 0.2	< 0.2	< 0.2	
Mercury (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	

(Continues)

TABLE 3 (Continued)

Parameter (unit)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Method of analysis
Other impurities						
4-Aminobenzoylglutamic acid (ABGA) (% w/w)	0.08	0.07	0.06	0.09	0.06	HPLC–UV USP DS Ca-L-MeTHFA
Hydroxymethyl-THFA (HOMeTHFA) (%w/w)	0.26	0.23	0.11	0.29	0.11	
Mefox (%w/w)	0.05	0.04	0.02	0.02	0.02	
Tetrahydrofolic acid (THFA) (%w/w)	0.09	0.08	0.07	0.07	0.1	
7,8-Dihydrofolic acid (DHFA) (%w/w)	0.27	0.22	0.01	0.01	0.01	
Folic acid (FA) (%w/w)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
Methylenetetrahydrofolic acid (CH2THFA) (%w/w)	0.02	0.02	0.02	0.02	0.02	
Methyletetrahydropteroic acid (MeTHPA) (%w/w)	0.12	0.11	0.11	0.12	0.13	
Dimethyl-THFA (DiMeTHFA) (%w/w)	0.09	0.08	0.05	0.05	0.06	
Sum of all related compounds (%w/w)	1.1	1.0	0.62	0.85	0.65	
Diastereomeric purity						
(6R)-Mefolinate (% area)	0.9	0.8	0.3	0.3	0.3	HPLC–UV USP DS Ca-MeTHFA
Microbial contaminants						
Microbial Count (TAMC), (CFU/g)	< 10	< 10	< 10	< 10	< 10	USP 61
Microbial Count (TYMC), (CFU/g)	< LOQ (10)	< LOQ (10)	< LOQ (10)	< LOQ (10)	< LOQ (10)	
Escherichia coli	Absence in 1 g	Absence in 1 g	Absence in 1 g	Absence in 1 g	Absence in 1 g	USP 62

Abbreviations: ABGA, 4-Aminobenzoylglutamic acid; ATR, Attenuated total reflection; CFU, colony forming unit; DHFA, dihydrofolic acid; FA, folic acid; GC, gas chromatography; HPLC, high-performance liquid chromatography; IC, ion chromatography; ICP-MS, inductively coupled plasma mass spectrometry; IR, infra-red; LOQ, limit of quantification; OES, optical emission spectroscopy; TAMC, total aerobic microbial count; THFA, tetrahydrofolic acid; THPA, tetrahydropteroic acid; TYMC, total yeasts & moulds count; USP DS, United States pharmacopeia dietary supplements; UV, ultra-violet; w/w, weight for weight.

[a]CoAs for additional three batches were provided with the same results.

[b]Different LOQs reported are due to slight modifications in the analytical method used: generic method was used for batches 1 and 2, while method specific to calcium L-methylfolate was used for batches 3, 4 and 5.

The Panel considers that the compounds and their respective amounts reported in Table 3 under 'Other impurities' are expected impurities that originate from the residual starting material, by-products and degradation products and as such are consistent with previous risk assessments of calcium L-methylfolate and 5-MTHF glucosamine salts (EFSA AFC Panel, 2004; EFSA ANS Panel, 2013; EFSA NDA Panel, 2020).

The Panel considers that the information provided on the composition and reported impurity profile of the NF is sufficient for characterising the NF and does not raise safety concerns.

3.4.1 | Stability of the NF

3.4.1.1 | *Stability of the NF*

The applicant performed stability tests with three independently produced batches and one trial batch of the NF (which contains more impurities in comparison to the production batches) in powder form and in its packaging (polyethylene bags and heat-sealed aluminium composite foil). The tests were carried out at refrigerated conditions (5°C), at room temperature (25°C and 60% RH) for a period of 36 months and under accelerated conditions (40°C and 75% RH) for a period of 12 months. The batches were analysed for compliance with the specifications for the content of the 5-MTHF compound ('MeTHFA, as free acid'), water content and the content of by-products and degradation products of the production process (as listed in Table 3, under 'Other impurities'). Results for all parameters showed consistency and compliance with the limits set in the specifications (see Section 3.5 Specifications).

Upon a request, the applicant provided stability data of the microbiological parameters (total aerobic microbial count, TAMC and total yeast/moulds count, TYMC) in the same three batches of the NF used in the stability tests reported above. The testing was conducted at month 46 (25°C and 60% RH) and both microbiological parameters were below the LOQ (10 CFU/g).

The shelf-life proposed by the applicant for the NF is 24 months at 5°C or 25°C.

The Panel considers that the data provides sufficient information with respect to the stability of the NF for 24 months.

3.4.1.2 | *Stability of the NF in different food matrices*

Initially, the applicant did not provide any stability data of the NF in different food matrices but instead referred to the stability data of calcium L-methylfolate (EFSA AFC Panel, 2004) arguing its comparability. The Panel disagreed with the applicant's conclusion due to the difference between monovalent and divalent compounds. Furthermore, the provided solubility study (see Section 3.2 Identity of the NF) reported that the stability of the NF in water is low, even after 26 h, which was reflected by the fact that up to 7% of degradation products were detected. Thus, the applicant was asked to investigate the stability of the NF in three food matrices (aqueous solution, infant formula and liquid food) and during bread making, in line with the data available on the stability of calcium L-methylfolate (EFSA AFC Panel, 2004).

In their reply, the applicant provided stability data of the NF in tablets, capsules, powdered infant formula, reconstituted infant formula, spring water, vitamin B12 solution, energy drink and bread (sponge, dough and baked bread).

Studies in tablets and capsules (both as mono-vitamin preparations) were conducted with one batch of the NF for each product, under three different storage conditions (5°C; 25°C and 60% RH; 40°C and 75%), over a period of 12 months and following the ICH Q1A guidelines. Purity assay (as 5-MeTHFA, by HPLC) showed no significant changes over time, indicating the stability of the NF in these products. The Panel noted the increase in the average weight of tablets (approx. +8%) under all three storage conditions, which was explained by the applicant to be most likely due to retention of moisture, while there was no increase in weight in capsules. The Panel notes that the NF in the form of tablets (not film-coated) is susceptible to water retention.

A stability study in powdered infant formula was conducted with one batch of the NF in order to compare stability data with calcium L-methylfolate, under the same three storage conditions as for tablets and capsules and over the period of 10 months. The content of 5-MTHF was assessed with LC–MS/MS (accredited method based on AOAC 2013.13). At T0, measurements were repeated nine times (due to the challenges with the mixing process and obtaining the target concentration of the NF) eventually reaching an overage of 46% of the target value, while at every consecutive time point, measurements were repeated only once. The Panel notes that, although the concentration of 5-MTHF did not decline at the end of the testing period, remarkable fluctuations in both directions were observed throughout different time points in comparison to the starting concentration (i.e. up to −24% to +50%) making it difficult to draw conclusions on the stability of the NF in this food matrix. The applicant further provided a signed statement from the contracted laboratory claiming that the measurement uncertainty is ±25%.

The stability study in reconstituted infant formula was conducted with the same batch of the NF, using the same accredited analytical method as above (AOAC 2013.13) where the content of 5-MTHF was measured at baseline and after 1 h. The results confirmed the stability of the NF in this food matrix.

For the stability in liquid foods, the applicant chose spring water, vitamin B12 solution and energy drink as representative food matrices. Stability in both spring water and vitamin B12 solution was studied over a period of 12 months (25°C and 60% RH, and 40°C and 75% RH), while the stability in energy drink was studied over a period of 4 months (25°C and 60% RH). Analytical method was LC–MS/MS which was not accredited for liquid food. In all instances, the applicant added antioxidants to improve the stability of the NF (Table 4). The concentrations of 5-MTHF in spring water, at the end of the testing period, were 81% (at room temperature) and 55% (under accelerated conditions) of the starting concentration. Results in vitamin B12 solution showed a reduction in the content of 5-MTHF after month 3 (down to 51% of the starting value at month 6 under accelerated conditions), which was partially recovered at the end of the testing period (93% at room temperature and 77% under accelerated conditions, respectively). Finally, the results in energy drink showed the highest stability of the NF, which remained at 96.1% of its starting concentration at the end of the testing period (month 4).

TABLE 4 Antioxidant use in stability studies with the NF.

Food matrix/category	Antioxidant(s) added	Amounts of antioxidant added
Spring water	Sodium ascorbate (E301) and rosmarinic acid (E392)	1 mg/mL
Vitamin B12 solution	E301	1 mg/mL
	Grape seed extract	0.02% (w/w)
Energy drink	E301	1 mg/mL
	EGCG	2 mg/mL
Breadmaking (sponge, dough and bread)	E301	0.1% (w/w)

Abbreviation: EGCG, epigallocatechin gallate.

Stability study during breadmaking was conducted in three batches of the NF in order to test how heat-treatment influences its stability. Samples were prepared using the 'AACC approved method 10-11' and the NF was tested by the accredited method for this food matrix (AOAC 2013.13). Mean values of 5-MTHF in sponge, dough and bread were 123%, 116% and 117% of the target concentration, respectively.

The Panel considers that the stability of the NF in different food matrices has only been demonstrated in the presence of added antioxidants.

3.5 | Specifications

The specifications of the NF are indicated in Table 5.[10]

TABLE 5 Specifications of the NF.

Test	Specification
Appearance	White to yellow or beige powder
Identity/IR	Conforms to reference
Water	≤ 1.0%
Residual solvents	
Ethanol	≤ 0.5%
Isopropanol	≤ 0.5%
Cations	
Identity retention time Sodium	Conforms to reference
Assay Sodium	4.0 to 5.0%
Elemental impurities	
Boron	≤ 10 mg/kg
Platinum	≤ 10 mg/kg[a]
Arsenic	≤ 1.5 mg/kg
Cadmium	≤ 0.5 mg/kg
Lead	≤ 1.0 mg/kg
Mercury	≤ 1.5 mg/kg[b]
Assay & related compounds	
Assay 5-MeTHFA-Na on dry basis	> 95%
Identity retention time Mefolinate	Conforms to reference
Sum of folate related substances	≤ 2.5
Diastereomeric purity	
(6R)-Mefolinate	≤ 1.0% area
Microbiological contaminants	
Total aerobic microbial count	≤ 100 CFU/g
Total yeast and moulds count	≤ 100 CFU/g
E. coli	Not detected in 10 g

Abbreviations: CFU, colony forming unit; IR, infra-red; MeTHFA, methyltetrahydrofolic acid.

[a]For foods intended for infants and young children, then ≤ 2 mg/kg.

[b]For foods intended for infants and young children, then ≤ 1 mg/kg.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6 | History of use of the NF

The NF has no history of use in Europe.

[10]Equals EU Purity on dry basis.

According to the applicant, the NF has a self-determined GRAS status in the USA. The applicant initiated the authorisation process for placing on the market the NF in South Korea, but had withdrawn the application due to simultaneous authorisation process initiated for the calcium L-methylfolate which was considered as priority.

3.7 | Proposed uses and use levels

The applicant states that the NF is intended to be used as a partial or complete substitute for folic acid and other sources of added folate. The proposed uses and use levels correspond to those of calcium L-methylfolate,[11] which is currently authorised to be used in the food categories listed in Table 6.

TABLE 6 Proposed uses and use levels of the NF.

Food category	Maximum levels
Foods for special medical purposes and total diet replacement for weight control as defined in Regulation (EU) No 609/2013	In accordance with Regulation (EU) No 609/2013
Infant formulae and follow-on formula as defined by Regulation (EU) No 609/2013	In accordance with Regulation (EU) No 609/2013
Processed cereal-based foods and baby foods for infants and young children as defined by Regulation (EU) No 609/2013	In accordance with Regulation (EU) No 609/2013
Food supplements as defined in Directive 2002/46/EC	In accordance with Directive 2002/46/EC
Food fortified in accordance with Regulation (EC) No 1925/2006	In accordance with Regulation (EC) No 1925/2006

3.7.1 | Target population

The target population proposed by the applicant is the general population.

3.7.2 | Estimate of exposure to undesirable substances

Since hydroxyethylmorpholine (HEM) and formaldehyde are used during the production process (see Section 3.3 Production process), the applicant was asked to quantify residual amounts and conduct the risk assessment accordingly.

The applicant provided analytical data (using an in-house method based on ion exchange chromatography) on eight samples of the NF and the content of residual HEM was below the LOQ of 0.001% w/w in all samples. The applicant also searched the PubMed database and additional authoritative databases to identify peer-reviewed articles and data that address the safety of HEM. Since no relevant toxicity data were retrieved, the applicant performed a read-across from the molecule morpholine (CAS 110-91-8), for which Health Canada has established an acceptable daily intake (ADI) of 0.48 mg/kg bw per day (Health Canada, 2002). Assuming that the daily intake of the NF is at the level of ULs for supplemental folates for infants (200 µg/day) and adults (1000 µg/day) (EFSA NDA Panel, 2023) and based on the LOQ established for HEM analysis in the batches of the NF (0.001% w/w), the daily exposure to HEM for infants (5 kg bw) and adults (70 kg bw) would be 0.0004 and 0.000014 mg/kg bw, respectively, which are orders of magnitude lower than the ADI.

By using HPLC–UV method, the applicant also confirmed that no quantifiable amounts of formaldehyde are present in the final product by providing CoAs for six batches of the NF (< 20 mg/kg or 0.002% w/w).

The applicant also provided analytical data by HPLC–UV/MS for six batches of the NF for the presence of monoethyl esters (two isomers) and the diethyl ester versus a reference material for L-5-methyltetrahydrofolic acid diethylester (that also contained the two monoethylesters), and concentrations of esters in the samples were below the limit of detection of 0.004% w/w.

Finally, residual levels of platinum may be present due to the use of platinum (IV) oxide hydrate during the production process. Based on the specification limit of 10 mg platinum/kg set for foods that are not intended for infants and young children (see Section 3.5 Specifications) and assuming that the daily intake of the NF would be at the level of UL for supplemental folate for adults (1000 µg/day), the daily exposure for adults would be 10 ng platinum per day, which is below the permitted daily exposure (PDE) set by EMA (2016) of 1.08×10^5 ng of platinum per day. The Panel notes that the proposed specification limit of 2 mg platinum/kg set for the NF when used in foods intended for infants and young children (Section 3.5 Specifications) is in line with the specification limit set for calcium L-methylfolate (EFSA NDA Panel, 2020). Taking into account the proposed conditions of use, intake estimates and mentioned specification limits, the Panel considers that the platinum exposure through this NF is of no toxicological concern for the target population groups.

[11]Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, p. 72–201.

3.8 | Absorption, distribution, metabolism and excretion (ADME)

Due to the concerns that the NF is unstable at low pH values, which would lead to the conversion of the NF into oxidation products and to a reduction of the active form of folate, the Panel requested the applicant to perform an in vitro gastric digestion study in order to characterise the extent of the gastric degradation and the proportion of formed oxidation products. As requested, such study was performed by studying the short-term stability of the NF (without added antioxidants) for 3 h in a phosphate-buffered solution at pH 2.0 and 37°C. The results showed that the 5-MTHF content was stable under the given conditions. In addition, the amounts of tested impurities were in line with those observed in the batch to batch analyses (Table 3) and specification limit for the sum of impurities (Table 5, parameter 'Sum of folate related substances').

The applicant provided a number of studies investigating the bioavailability of food folates, folic acid and calcium L-methyfolate or unspecified form of L-5-MTHF, which could not be used to assess the bioavailability of the NF.

In a randomised, double-blind, monocentric, placebo-controlled, cross-over study, 24 healthy adults (12 women and 12 men, approx. 30 years of age) were randomised to receive a single oral dose of 436 µg of the NF (i.e. 416 µg of 5-MTHF) and an equimolar oral dose of folic acid (400 µg) after overnight fasting (≥ 10 h) on 2 days with a 2 weeks washout period (Obeid et al., 2020; Unpublished study report, 2019a). Blood was taken at baseline (pre-dose) and at nine-time points up to 8 h after administration of the test substances. The area under the curve ($AUC_{0\text{-}8h}$), C_{max} and T_{max} of plasma 5-MTHF, total folate and unmetabolised folic acid were measured. Safety-related parameters were also recorded (adverse events, tolerability, haemogram and vital signs; see Section 3.10.5 Human data). The study was conducted in accordance with the ICH-GCP guidelines and was registered at the German Register for Clinical Studies. Sample size calculation was based on the findings of an RCT of a similar design conducted by the applicant to test 5-MTHF-Ca in a sample of women.

Relative to baseline (T_0), mean (SD) plasma 5-MTHF and total folate concentrations increased by 33.2 (10.2) nmol/L and 20.8 (7.3) nmol/L at $T_0 + 1$ h (T_{max}) after the consumption of the NF dose, and gradually decreased back to near baseline concentrations at $T_0 + 8$ h. The corresponding $AUC_{0\text{-}8h}$ were 126 (33.6) nmol/L*h and 89.2 (19.1) nmol/L*h, respectively. This indicates an efficient absorption of 5-MTHF from the NF, which is then available in circulation and can contribute to the 5-MTHF body pool.

Consistent with the different kinetic behaviour of 5-MTHF salts and folic acid (EFSA NDA Panel, 2022; Wright et al., 2003), lower $AUC_{0\text{-}8h}$ and C_{max}, and longer T_{max} were observed upon administration of the equimolar dose of folic acid. The Panel, however, notes that acute studies are not suitable to quantify the relative bioavailability of 5-MTHF salts and folic acid; rather longer-term measures of biomarkers of folate status (e.g. measures of RBC folate concentrations and plasma total folate in repeated-dose study) are required (EFSA NDA Panel, 2022).

In addition, Obeid et al. (2020) performed an indirect comparison of the $AUC_{0\text{-}8h}$ for 5-MTHF between the NF and calcium L-methylfolate by using data from a previous study (Prinz-Langenohl et al., 2009). Plasma 5-MTHF $AUC_{0\text{-}8h}$ in response to calcium L-methylfolate was re-calculated by applying a correction based on the comparison of the $AUC_{0\text{-}8h}$ for 5-MTHF in response to folic acid in the respective studies. The authors concluded that $AUC_{0\text{-}8h}$ for 5-MTHF of the NF and calcium L-methylfolate were similar. The Panel, however, notes that the kinetic parameters were measured in separate studies, which may limit the validity of quantitative comparisons, in spite of recalculations applied to address differences between studies.

In its previous assessment of the conversion factors to express the amount of currently authorised salts (calcium L-methylfolate and 5-MTHF glucosamine salt) into DFE, the NDA Panel considered that the nature of the cation associated with 5-MTHF is not likely to greatly affect its bioavailability even though differences in solubility may exist, depending on the cation. The Panel thus concluded that the conversion factors proposed for calcium L-methylfolate and 5-MTHF glucosamine salt could also be applied to 5-MTHF associated with other cations (EFSA NDA Panel, 2022). The Panel notes that similarities in the absorption and clearance pattern of the NF reported in the pharmacokinetic study from Obeid et al. (2020) with that observed in response to a calcium L-methylfolate dose (Prinz-Langenohl et al., 2009), notwithstanding methodological limitations of this comparison, is consistent with the previous Panel's conclusion.

Thus, the Panel considers that L-methylfolate from the NF is bioavailable in all age groups of the general population.

3.9 | Nutritional information

The NF, which is composed of at least 95% 5-MTHF on a dry weight basis and up to 5% sodium, is proposed to provide an alternative source of folate in place of folic acid. L-methylfolate is the predominant natural form of folates found in food and breast milk and the major form in human plasma (EFSA NDA Panel, 2014). Folate belongs to the group of B-vitamins and is a generic term used for a family of compounds which act as cofactors for enzymes involved in one-carbon metabolism. As such, folate is essential for the synthesis of RNA and DNA and consequently for cell division and tissue growth, methylation reactions and amino acid metabolism (EFSA NDA Panel, 2014). Naturally occurring food folates are a mixture of reduced mono- and polyglutamates, whereas synthetic folic acid, which is considered to have similar biological activity, is a fully oxidised monoglutamate arising in the diet only through ingesting fortified foods or food supplements.

Folate in food is mainly in polyglutamate forms. Before absorption, polyglutamated folate forms must be hydrolysed by conjugases to form folate monoglutamates. Upon absorption, monoglutamate forms are then formylated or methylated in the enterocytes. Reduced forms of folate are transferred faster from the intestine to the circulation than folic acid

(Darcy-Villon et al., 1988; Halstead, 1998; Herbert, 1998). This is in line with the results of the human oral bioavailability study which was provided by the applicant, that aimed to compare the NF with folic acid (confidential) (Unpublished study report, 2019a, i.e. Obeid et al., 2020, see Section 3.8) and which show a significantly higher plasma $AUC_{0\text{-}8h}$ and C_{max} of (6S)-5-MTHF and the maximum concentration was reached significantly faster than with folic acid.

As mentioned under Section *1.2 Information on existing evaluations and authorisations*, the NDA Panel (EFSA NDA Panel, 2023) reiterated the previously established ULs for supplemental folate of: 200 µg/day (1–3 years), 300 µg/day (4–6 years), 400 µg/day (7–10 years), 600 µg/day (11–14 years), 800 µg/day (15–17 years), 1000 µg/day (all adults including pregnant and lactating women) (SCF, 2000), while adding the UL of 200 µg/day for infants aged 4–11 months. These ULs apply to the combined intake of folic acid and currently authorised forms [(6S)-5-methyltetrahydrofolic acid glucosamine and L-5-methyltetrahydrofolic acid calcium salts] under their authorised conditions of use.

The sodium moiety represents a small fraction of the NF (up to 5% w/w). The Panel notes that the sodium intake resulting from the consumption of 1000 µg/day of the NF (worst-case scenario) would be negligible (50 µg/day) in the context of current sodium intakes (EFSA NDA Panel, 2019).

The Panel notes that the applicant proposes to use the NF at maximum levels which are compliant with Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control, Directive 2002/46/EC on food supplements and Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods (Section 3.7).

The Panel notes that a maximum permitted level (MPL) of 47.6 µg DFE/100 kcal (11.4 µg DFE/100 kJ) is established for infant and follow-on formula (Regulation (EU) 2016/127[12]) and an MPL of 50 µg/100 kcal (as folic acid) is established for processed cereal-based foods and baby foods (Directive 2006/125/EC[13]). The Panel considers that the use of the NF in infant and follow-on formula under the proposed conditions of use is not nutritionally disadvantageous.

The Panel also notes that, to date, no MPLs for folate in total diet replacement for weight control, food supplements and other foods are available in the above-mentioned regulations. The Panel notes that the applicant proposes to use the NF as a partial or complete substitute for folic acid and other sources of added folate (Section 3.7). The Panel considers that the use of the NF in these food categories is not nutritionally disadvantageous as long as the combined intake of the NF and the other supplemental forms of folate under their authorised conditions of use is below the ULs established for the different age groups of the general population (EFSA NDA Panel, 2023).

3.10 | Toxicological information

The applicant provided three toxicological studies with the NF, which were conducted in compliance with OECD principles of GLP (OECD, 1998) and in accordance with the test guidelines (TG) No 471, 473 and 487. These studies are listed in Table 7.

TABLE 7 List of toxicological studies with the NF.

Reference	Type of study	Test system	Dose
Unpublished study report, 2018	Bacterial reverse mutation test (GLP, OECD TG 471, 1997)	*S. typhimurium* TA98, TA100, TA1535 and TA1537, *E. coli* WP2 uvrA	Up to 5000 µg/plate (absence and presence of S9 mix)
Unpublished study report, 2020	In vitro micronucleus test (GLP, OECD TG 487, 2016b)	Human lymphocytes	Up to 2121 µg/mL
Unpublished study report, 2019b	In vitro chromosome aberration test (GLP, OECD TG 473, 2016a)	Human peripheral blood lymphocytes	Up to 2000 µg/mL with and without S9 (5 h exposure) and up to 1000 µg/mL with S9 (29 h exposure)

Abbreviations: GLP, good laboratory practice; OECD, Organisation for Economic Co-operation and Development; TG, test guideline.

The applicant did not conduct any repeated-dose toxicity study, but rather provided the unpublished study reports already addressed by the AFC Panel in 2004 when assessing calcium L-methylfolate. The AFC Panel concluded that '*sub-chronic and embryotoxicity/teratogenicity studies in rats with L-5-MTHF at doses that were at least 20,000 times higher than the tolerable upper intake level (i.e. 1 mg/adult person/day) for folic acid, did not reveal any adverse effects*'.

The Panel considers repeated-dose toxicity studies with the NF as not necessary and that a read-across approach from calcium L-methyfolate to the NF can be applied for the safety assessment. Both the Na- and Ca-salts of 5-MTHF are expected to readily and completely dissociate in an aqueous environment into Na- (or Ca-) and 5-MTHF ions. Once 5-MTHF from the NF is absorbed and has entered the circulation, it will have the same fate as 5-MTHF derived from any other source (i.e. natural folate sources, other 5-MTHF salts or folic acid). In addition, the production process of the NF is similar to that of

[12]Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding (Text with EEA relevance) *OJ L 25, 2.2.2016, p. 1–29*.

[13]Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children (Codified version) (Text with EEA relevance). *OJ L 339, 6.12.2006, p. 16–35*.

calcium L-methylfolate already assessed and no substance of toxicological concern is used and present in the NF at levels of concern.

3.10.1 | Genotoxicity

The NF was tested in a bacterial reverse mutation test (Unpublished study report, 2018) at concentrations of 0 (water or water with sodium ascorbate; negative control) and up to 5 mg/plate in the absence and presence of S9-mix. The plate incorporation test was applied and results were obtained in triplicates (OECD TG 471). The NF did not induce any biologically relevant increases in the frequency of revertant colonies for any of the bacterial strains, at any dose level, either with or without metabolic activation. Appropriate positive control compounds confirmed the sensitivity of the assay and efficacy of the S9-mix. The Panel concludes that the NF was not mutagenic in these bacterial systems.

The NF was tested in an in vitro micronucleus test (Unpublished study report, 2020) in human lymphocytes. The duration of exposure was 4 h in the absence and presence of S9-mix and 20 h in the absence of S9-mix at the concentrations of 0 (negative control: ultrapure water, 10% v/v), 0 [solvent control: ultrapure water supplemented with 1 mg/mL sodium-L(+)-ascorbate, 10% v/v], 693, 1212 and 2121 µg/mL. The positive controls used were mitomycin C (4 h, –S9), cyclophosphamide (4h, +S9) and demecolcine (40 h, –S9). Two parallel cultures in each experimental group were treated with cytochalasin B. At least 1000 binucleate cells per culture were scored for cytogenetic damage on coded slides. The highest tested concentration of 2121 µg/mL was chosen with regard to the molecular weight and purity (94.3%) of the test material and with respect to OECD TG 487. No cytotoxicity was observed up to this concentration. The NF did not induce micronuclei under the tested conditions up to the highest concentration tested. There was one statistically significantly increased value in the experiment with 4 h exposure in the absence of S9-mix at the highest concentration when compared to the concurrent solvent control. As this value was well within the historical control range and as there was no dose-dependency by trend test, the Panel considered this single increased value as biologically not relevant.

The applicant also conducted an in vitro chromosome aberration test with the NF in human lymphocytes (Unpublished study report, 2019b), based on the OECD TG 473. In the experiment with 5 h exposure, in the absence and presence of S9-mix, the following concentrations were investigated: 0 (control 1: ultrapure water), 0 (control 2: ultrapure water supplemented with 1 mg/mL sodium-L(+)-ascorbate), 250, 500, 1000, 2000 µg NF/mL. Positive controls were mitomycin C (–S9 mix) and cyclophosphamide (+S9 mix), respectively. In the experiment with 29 h exposure (–S9 mix), the following concentrations were investigated: 0 (control 1: ultrapure water), 0 (control 2: ultrapure water supplemented with 1 mg/mL sodium-L(+)-ascorbate), 125, 250, 500, 1000 µg NF/mL. The positive control used was mitomycin C. Colchicine was added for mitosis arrest. Results were provided for duplicate cultures, with controls as four replicates. The NF was soluble in ultrapure water supplemented with 1 mg/mL sodium-L(+)ascorbate up to the highest concentration. However, after 15 min, turbidity occurred resulting in a homogenous suspension at concentrations ≥ 250 µg/mL in the solvent. The NF showed precipitation at the beginning of exposure at concentrations ≥ 1000 µg/mL (5 h, +S9 mix) and ≥ 2000 µg/mL (5 h, –S9 mix) or ≥ 500 µg/mL (29 h, –S9 mix) in the culture medium. No precipitation was seen at the end of the exposure. At the highest tested concentration in cultures exposed for 29 h, cytotoxicity (low cell density) was seen and a clear reduction of the mitotic index (51% of control 2). The Panel considered that no biologically relevant increase in the numbers of chromosomal aberrations, endomitotic or polyploid cells was observed.

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2 | Human data

As a part of Obeid et al. (2020) study, safety endpoints were also investigated [see Section 3.8 Absorption, distribution, metabolism and excretion (ADME)]. No serious adverse events were reported during intervention days. Three subjects reported headaches on day one after intake of the NF, which were rated as unrelated to the study product. Overall, both folate forms were rated as 'well tolerated'. Blood routine parameters were in accordance with reference values.

In addition to Obeid et al. (2020) study, the applicant provided the search strings of literature search undertaken in PubMed for clinical studies with folic acid and L-5-MTHF, for which 119 hits were identified, but none of these studies was conducted with the NF.

The Panel notes that a more comprehensive review of the available studies was done recently in the context of the opinion on ULs for folate (EFSA NDA Panel, 2023) and that the human data submitted under this NF application do not provide additional evidence for the risk assessment.

3.11 | Allergenicity

The Panel considers that, owing to the absence of protein, the NF is unlikely to trigger allergic reactions in the target population under the proposed conditions of use.

4 | DISCUSSION

This opinion deals with the safety and bioavailability of the monosodium salt of L-5-methyltetrahydrofolic acid as a NF and as a new source of folate to be added to food supplements, food intended for infants and young children (infant formula and follow-on formula; processed cereal-based food and baby food), food for special medical purposes, total diet replacements for weight control and fortified foods. Other salts of 5-MTHF (calcium L-methylfolate and (6S)-5-methyltetrahydrofolic acid, glucosamine salt) were previously assessed by EFSA as sources of folate under the same or more limited[14] conditions of use as this NF. These earlier assessments concluded that the use of such salts of 5-MTHF is not of safety concern if the intake levels are in line with the established ULs for folates for different population groups.

The NF is produced by a three-step chemical synthesis starting from folic acid. The amounts of residual solvents, processing aids, impurities originating from the starting material and formed as by-products of the production process were analysed in several batches of the NF. No safety concerns are identified considering the reported amounts of such substances and proposed conditions of use of the NF.

Based on the nature of the NF and the results of stability studies conducted with the NF when added to certain food matrices, the Panel considers that the NF may be an unstable source of folate and the use of proper processing/storage conditions (e.g. antioxidants and adequate packaging) is needed to preserve its stability.

Regarding the bioavailability of the NF, the Panel reiterates the conclusion in the opinion on DFE (EFSA NDA Panel, 2022) that the influence of the cation on the bioavailability of L-methylfolate is likely to be minor and the bioavailability of the NF is comparable to that of calcium L-methylfolate. Therefore, the Panel considers that L-methylfolate from the NF is bioavailable under the proposed conditions of use.

The Panel considers that a read-across approach from calcium L-methylfolate to the NF can be applied for the safety assessment. Therefore, the Panel considers that the existing ULs for supplemental folate, which have been established for the combined intake of folic acid and currently authorised salts, also apply to the NF. The Panel notes that the applicant proposes to use the NF as a substitute for currently authorised sources of folate. The Panel considers that the NF does not raise safety concerns under the proposed conditions of use, as long as the combined intake of the NF and the other authorised sources of folate considering their authorised conditions of use is below the ULs established for the different age groups of the general population.

5 | CONCLUSIONS

The Panel considers that the NF is a source from which folate is bioavailable.

The Panel concludes that the NF, monosodium salt of L-5-methyltetrahydrofolic, is safe under the proposed conditions of use.

5.1 | Protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant (analytical reports and methods validation regarding the NF identification, characterisation, solubility, particle size and distribution, dissolution study, stability studies, study on bioavailability, genotoxicity studies and HACCP plan).

6 | STEPS TAKEN BY EFSA

1. On 28/06/2021 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of the monosodium salt of L-5-methyltetrahydrofolic acid as a novel food pursuant to Article 10 of Regulation (EU) 2015/2283. Ref. Ares(2021)4198609.
2. On 28/06/2021, a valid application on monosodium salt of L-5-methyltetrahydrofolic acid, which was submitted by Merck & Cie, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/2160) and the scientific evaluation procedure was initiated.
3. On 14/09/2021, 28/01/2022, 09/03/2022, 04/11/2022, 07/02/2023, 26/05/2023 and 11/07/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 10/01/2022, 17/02/2022, 02/09/2022, 07/02/2023, 03/05/2023, 15/06/2023 and 09/08/2023 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5. During its meeting on 26/10/2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of monosodium salt of L-5-methyltetrahydrofolic acid as a NF pursuant to Regulation (EU) 2015/2283 and the bioavailability

[14]Current authorisation of (6S)-5-methyltetrahydrofolic acid glucosamine salt includes only the use in food supplements as defined in Directive 2002/46/EC as a source of folate.

of folate from this source in the context of Directive 2002/46/EC, Regulation (EU) No 609/2013 and Regulation (EC) No 1925/2006.

ABBREVIATIONS

5-MTHF	(6S)-5-methyltetrahydrofolic acid
AACC	American Association of Cereal Chemists
ABGA	4-Aminobenzoylglutamic acid
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AI	adequate intake
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
AR	average requirement
ATR	attenuated total reflection
AUC	area under the curve
bw	body weight
CaLMF	calcium L-methylfolate
CAS	Chemical Abstracts Service
CFU	colony forming unit
CI	confidence interval
C_{max}	maximum concentration
CoA	certificate of analysis
DFE	dietary folate equivalent
DHFA	dihydrofolic acid
EGCG	Epigallocatechin gallate
EMA	European Medicines Agency
FA	folic acid
GC	gas chromatography
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRAS	generally recognised as safe
HACCP	Hazard Analysis Critical Control Points
HEM	hydroxyethylmorpholine
HPLC–UV	high-performance liquid chromatography with ultra-violet detector
HPLC–UV/MS	high-performance liquid chromatography with ultra-violet and mass spectrometric detection
IC	ion chromatography
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP–MS	inductively coupled plasma mass spectrometry
IR	infra-red
IUPAC	International Union Of Pure And Applied Chemistry
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LMSR	(6S)-5 methyltetrahydrofolic acid
LOQ	limit of quantification
LTBH	L-Tetrahydrofolic acid benzenesulfonic acid salt
MPL	maximum permitted level
NDA	EFSA panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NMR	nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
OES	optical emission spectroscopy
PDE	permitted daily exposure
PRI	population reference intake
RCT	randomised controlled trial
RH	relative humidity
SCF	Scientific Committee on Food
SD	standard deviation
SEM	scanning electron microscopy
TAMC	total aerobic microbial count
TG	test guideline

THFA	tetrahydrofolic acid
THPA	tetrahydropteroic acid
T_{max}	time to reach C_{max}
TYMC	total yeast and mould count
UL	tolerable upper intake level
UPLC–UV	Ultra performance liquid chromatography with ultra-violet detector
USP DS	United States pharmacopeia dietary supplements
UV	ultra-violet
v/v	volume for volume
w/w	weight for weight

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

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