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Evaluation of di-calcium malate, used as a novel food ingredient and as a source of calcium in foods for the general population, food supplements, total diet replacement for weight control and food for special medical purposes

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Abstract

The present scientific opinion deals with the evaluation of the safety of di-calcium malate (DCM) proposed as a novel food ingredient and as a source of calcium for use in foods for the general population, food supplements, total diet replacement for weight control and food for special medical purposes (FSMP), and with the bioavailability of calcium from this source. The structural formula of the proposed complex is based on expert judgement and not supported by any analytical data. On the basis of the available data, the Panel concluded that there was insufficient scientific evidence of a difference between the proposed novel food ingredient named as di-calcium malate (DCM) and calcium malate already authorised as a source of calcium included in Annex II to Directive 2002/46/EC. Accordingly, the Panel was unable to assess the safety of DCM as a novel food ingredient. On the basis of the results provided, the Panel considered that DCM does not completely dissociate into calcium and malic acid. The Panel concluded that when DCM dissociates, calcium would be available following ingestion of DCM and the bioavailability would appear similar to values reported for other sources of calcium already permitted. Furthermore, the Panel concluded that on the basis of the information available it was not possible to calculate the exposure to DCM as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and FSMP.

Keywords: DCM, di-calcium malate, calcium, nutrient source, food supplements, food for special medical purposes, calcium malate

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Amendment: An editorial correction (edit EFSA question number, page 1) was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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Summary

The present scientific opinion deals with the evaluation of the safety of di-calcium malate (DCM) proposed as a novel food ingredient and as a source of calcium for use in foods for the general population, food supplements, total diet replacement for weight control and food for special medical purposes (FSMP), and with the bioavailability of calcium from this source. The safety of calcium itself, in terms of amounts that may be consumed, and the consideration of calcium as a nutrient are outside the remit of this Panel.

The Panel noted that the structural formula of the proposed complex, as indicated in the dossier, is based on expert judgement and not supported by any analytical evidence (e.g. X-ray crystallography). Following a request for additional information, the applicant did not provide any further data in support to the proposed structural formula of the proposed complex. The Panel noted that the applicant proposed a different chemical structure for DCM compared to that provided in the initial dossier. The Panel considered that the scientific evidence submitted by the applicant does not demonstrate the difference between 'dicalcium malate (DCM)', which is subject of the application under evaluation and 'calcium malate' which is already included in the Annex II to Directive 2002/46/EC as one of the mineral substances which may be added to food supplements, following a scientific opinion by (EFSA AFC Panel, 2006).

The applicant has proposed chemical and microbiological specifications for DCM and provided results from five non-consecutive batches. The Panel noted that some parameters in the proposed specifications were not commensurate with the structure proposed.

The maximum limits proposed for arsenic is higher than the values obtained in the five batches analysed. Based on the analytical results provided by the applicant, lower levels of the maximum limit for arsenic present as an impurity, should be considered in the proposed specifications. Furthermore, the Panel noted that the currently proposed specifications indicate a minimum limit for calcium and malic acid while no maximum limits are provided.

The applicant provided analytical data on particle size of five non-consecutive lots of DCM. However, the Panel considered that the described vibratory sieve testing method with the smallest sieve opening of 45 μm is not appropriate to determine nano-size particles. The Panel also noted that an average of 81.58% of the tested material passed through the smallest 45 μm sieve.

The Panel noted that the applicant did not submit any information on proposed uses and use levels for DCM in the initial application dossier. Further to a request for additional information, the applicant has submitted data on the proposed use and use levels of DCM as a source of supplemental calcium in foods using the FAIM template (version 1). However, the Panel noted that the use levels as indicated by the applicant in the FAIM template did not clearly refer to the maximum use levels, typical use levels or to the amount of calcium available from DCM. Despite a request for clarification, the applicant did not provide a reply.

Furthermore, the Panel noted that no information was provided for the typical and maximum daily dose of DCM and the resulting typical and maximum daily calcium intake which was intended to be achieved by the proposed use of the source for food supplements, total diet replacement products for weight control and for food for special medical purpose.

The Panel noted that, besides a bioavailability study conducted in human volunteers, an *in vitro* absorption study of calcium from DCM and an acute toxicity study performed in rats, no further biological and toxicological data on DCM were submitted by the applicant as part of the dossier. The applicant provided a justification for not complying with the Tier 1 requirements of the 2012 ANS Panel 'Guidance for submission for food additive evaluations' by stating that, following oral ingestion, DCM readily dissociated into calcium and malic acid. Both calcium and L-malic acid are natural constituents of the diet and also of the body, for which extensive data on their biological effects exist.

Following EFSA's request, the applicant submitted a study report designed to provide some evidence to support that DCM, when placed into acidic solution like that found in the stomach, dissociated into its individual components (calcium and malic acid). On the basis of the results provided, the Panel considered that DCM does not completely dissociate into calcium and malic acid.

On the basis of the available data, the Panel concluded that there was insufficient scientific evidence of a difference between the proposed novel food ingredient named as DCM and calcium malate already authorised as a source of calcium included in Annex II to Directive 2002/46/EC. Accordingly, the Panel was unable to assess the safety of DCM as a novel food ingredient.

The Panel concluded that based on the data provided DCM does not completely dissociate into calcium and malic acid.

The Panel concluded that when DCM dissociates, calcium would be available following ingestion of DCM and the bioavailability would appear similar to values reported for other sources of calcium already permitted.

Furthermore, the Panel concluded that on the basis of the information available it was not possible to calculate the exposure to DCM as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and FSMP.

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1. Introduction

The present scientific opinion deals with the evaluation of the safety of di-calcium malate (DCM) proposed as a novel food ingredient and as a source of calcium for use in foods for the general population, food supplements, total diet replacement for weight control and food for special medical purposes (FSMP), and with the bioavailability of calcium from this source. The safety of calcium itself, in terms of amounts that may be consumed, and the consideration of calcium as a nutrient are outside the remit of this Panel.

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

1.1.1. Background

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

The relevant Union legislative measures are:

- Regulation (EC) No 258/97 of the European Parliament and the Council concerning novel foods and novel food ingredients[1];
- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Members States relating to food supplements[2];
- Regulation (EC) 1925/2006 on the addition of vitamins and mineral and of certain other substances to food[3];
- Regulation (EU) No 609/2013 of the European Parliament and of the Council on the food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control.[4]

The dossier relating to DCM as a source of calcium has been submitted to the Food Safety Authority of Ireland (FSAI), the competent authority for novel food in Ireland, for an initial assessment under Article 6(2) of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients. The applicant has asked the authorisation for DCM as a source of calcium in the following food categories: foods for the general population (fortified food), food supplements, total diet replacement for weight control and food for special medical purposes.

On 23 October 2015, FSAI forwarded to the Commission the initial assessment report, concluding that an additional assessment by the European Food Safety Authority is required in line with Article 6 (3) of that Regulation (EC) No 258/97.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[5], the European Commission asks the European Food Safety Authority to provide a scientific opinion:

- By carrying out the additional assessment for DCM as a novel food ingredient in the context of Regulation (EC) No 258/97, and
- Following the outcome of the novel food assessment by evaluating the safety of DCM when added for nutritional purposes to food supplements as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and food for special medical purposes, and the bioavailability of calcium from this source, in the context of Regulation (EC) No 1925/2006, Directive 2002/46/EC and Regulation (EU) No 609/2013.

[1] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

[2] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

[3] Regulation (EC) No 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 183, 12.7.2002, p. 51.

[4] 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.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

1.1.3. Information on existing evaluations and authorisations

The Panel noted that the applicant provided information on the regulatory status of calcium and malic acid.

Calcium

In 2007, the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) evaluated calcium citrate malate as a source for calcium added to foods for Particular Nutritional Uses, food supplements and foods intended for the general population (EFSA AFC Panel, 2007). The AFC Panel concluded that the use of calcium citrate malate as source for calcium intended for use in foods for Particular Nutritional Uses (PARNUTS) and foods for the general population (including food supplements) is of no safety concern at the maximum levels estimated in this opinion. Based on a potential exposure from all these sources (PARNUTS), food supplements and foods intended for the general population, matching the tolerable upper intake level for calcium of 2,500 mg calcium/day (SCF 2003), the equivalent exposure to citric acid would be around 1,400 mg/day and the malic acid exposure would be around 500 mg/day.

In 2006, the AFC Panel evaluated calcium malate added to PARNUTS, food supplements and foods intended for the general population (EFSA AFC Panel, 2006). The AFC Panel concluded that the use of malates as sources for calcium is of no safety concern. However, it was stressed that for infants and young children, only L-malates should be used. The Panel concluded that the bioavailability of calcium malate is expected to be similar to the bioavailability from other dissociable calcium salts permitted to be used for food supplements.

Calcium malate is included in Annex II of Directive 2002/46/EC on vitamin and mineral substances which may be used in the manufacture of food supplements following a scientific opinion by EFSA AFC Panel (2006). Calcium malate is authorised as a food additive (E 352) in accordance with the Regulation (EU) No 1129/2011[6] amending Annex II to Regulation (EC) No 1333/2008[7] by establishing a Union list of food additives and may be added to foods following the *quantum satis* principle.

Furthermore, calcium malate, calcium citrate malate and calcium hydroxide are allowed to be used as food supplements in the EU as a source of calcium for dietetic foods (Regulation (EC) No 953/2009[8] on substances that may be added for specific nutritional purposes in foods for particular nutritional uses and for food supplements and fortified foods (Regulation (EC) No 1170/2009[9] of 30 November 2009 as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements.

Calcium hydroxide is authorised as a food additive (E 526) in accordance with Annex II to Regulation (EC) No 1333/2008 by establishing a Union list of food additives for the pH adjustment of processed cereal based foods and baby foods and dietary foods for infants for special medical purposes and special formulae for infants at the *quantum satis* level.

Malic acid

Malic acid and sodium-, sodium hydrogen-, potassium-, calcium- and calcium hydrogen-malate (E 296, E 350–352) are authorised food additives in the European Union (EU) according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives. Currently, their re-evaluation as food additives is still ongoing as foreseen in Regulation (EC) No 257/2010[10].

DL-Malic acid was first evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for use as an acidity regulator and flavouring agent at its 9th meeting in 1965 at which point the Committee did not specify an acceptable daily intake (ADI) level for the L-isomer given its well-established pathway of metabolism and daily consumption of malic acid-containing foods (JECFA, 1967). Conversely, since only limited data were available on the metabolic fate of the D-isomer, the

[6] Commission Regulation (EU) No 1129/2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 21.11.2013, p. 1–212.

[7] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 1–16.

[8] Commission Regulation (EC) No 953/2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 1-11. (No longer in force, date of end of validity: 19/7/2015, replaced by Regulation (EU) No 609/2013).

[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, 1–7.

[10] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00598

Committee set a conditional ADI of 100 mg/kg body weight/day and requested further data on the metabolism of D-malic acid in humans (JECFA, 1967). Furthermore, it was specified that with the exception of therapeutic purposes, neither DL-malic acid nor D-malic acid should be added to food intended for very young infants. Subsequently, based on additional data reviewed by the Committee, indicating that adults are capable of metabolising D-malic acid, the previously set conditional ADI for D-malic acid was withdrawn; however, the restriction with respect to foods for very young infants was maintained (JECFA, 1970). The L-form of malic acid was re-evaluated by the Committee in 1999 for use as a flavouring agent (JECFA, 2000). The Committee concluded that there was 'no safety concern at current levels of intake when (L-malic acid) used as a flavouring agent' (i.e. 16 and 158 mg/day in Europe and the US, respectively).

JECFA has also evaluated the calcium, potassium, and sodium salts of malic acid (JECFA, 1980; JECFA, 1986). Concluding that the salts of malic acid are freely ionisable, it was considered to be appropriate to establish ADIs on the basis of data on the corresponding anion (i.e. DL-malate). Thus, the previously set ADI of 'not specified' for DL-malic acid was extended to a group ADI for DL-malic acid and its calcium, potassium and sodium salts.

2. Data and methodologies

2.1. Data

The present evaluation is based on the data on DCM provided by the applicant in a dossier submitted in support of its application ('Documentation provided to EFSA' n. 1), an initial assessment performed by FSAI ('Documentation provided to EFSA' n. 2), the comments raised by the Member States during the assessment of DCM as a novel food ingredient ('Documentation provided to EFSA' n. 3).

Additional data were generated by the applicant upon request from the ANS Panel during the assessment of the dossier ('Documentation provided to EFSA' n. 4). The request for additional data was discussed at a technical hearing held during a meeting of the Standing Working Group on Applications on 29 January 2018[11] ('Documentation provided to EFSA' n. 5).

2.2. Methodologies

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

The ANS Panel assessed the safety of DCM as a novel food ingredient in line with the principles laid down in Commission Recommendation 97/618/EC.[12] In particular, where it is stated that 'Most of the defined chemical substances can probably be tested for their safety similarly to food additives by utilising conventional methods of safety evaluation as described in the SCF Report No 10.', the Panel considered that to reflect state of the art scientific knowledge and welfare considerations the reference to SCF Report No. 10 should be replaced by the latest existing guidance on the safety evaluation of food additives, namely the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

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

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

In the original application dossier, a DCM complex, dicalcium malate chelate with a molecular formula $C_8H_8O_{10}Ca_2$ was proposed ('Documentation provided to EFSA' n. 1).

[11] https://www.efsa.europa.eu/sites/default/files/wgs/food-ingredients-and-packaging/applicationswg.pdf

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

Further to a request for additional clarification by EFSA on the substance under evaluation, the applicant did not provide any additional data in support to the proposed structural formula of the proposed complex, however, submitted an amendment to the dossier with respect to the identity of the substance ('Documentation provided to EFSA' n. 5). According to the applicant, DCM is a salt made of two calcium, one malic acid and two hydroxyl ions, $C_4H_6Ca_2O_7$ as molecular formula, a molecular weight of 246.25 g/mol and CAS number 671197-49-2. The chemical structure as provided by the applicant is presented in Figure 1. The applicant stated that DCM is different from calcium malate (CAS 17482-42-7).

The Panel noted that the identity of the substance named DCM proposed in the original application dossier (dicalcium malate chelate ($C_8H_8O_{10}Ca_2$) ('Documentation provided to EFSA' n. 1) is different from DCM proposed in the amended dossier (dicalcium malate ($C_4H_6O_7Ca_2$) ('Documentation provided to EFSA' n. 5) and the Panel considered the latter as representing the substance under evaluation.

According to the applicant, the calcium content can be analysed via inductively coupled plasma (ICP) spectrometry and the quantity of malic acid in the product analysed via high-performance liquid chromatography (HPLC) analysis. The quantity of water in the product was calculated via moisture balance. The applicant compared the Fourier-transform infrared (FTIR) spectrum of DCM with the one from calcium hydroxide and DL-malic acid; a broad signal from 3,600 to 3,000 cm^{-1} is observed instead of the sharp peak at approximately 3,450 cm^{-1} for DL-malic acid; this shows coordination between carboxylate and the metal but the FTIR spectrum does not permit to reach any conclusion on the structure of DCM. No comparison with the FTIR spectrum for calcium malate (CAS 17482-42-7) was submitted to demonstrate that DCM is different from the already authorised calcium malate.

According to the information provided by the applicant ('Documentation provided to EFSA' n. 5), DCM is identified as follows:

Chemical name:	Di-calcium malate
Description:	White to off-white, fine powder, with no or a slight scent
Solubility:	Very slightly soluble in water; solubility increases in the presence of hydrochloric acid.
Average composition of each component:	Calcium: ≥ 29% (32.5–29.1%) Malic acid: 64% (56.6–53.0%) Moisture: ≤ 6% (2.95–0.84%)

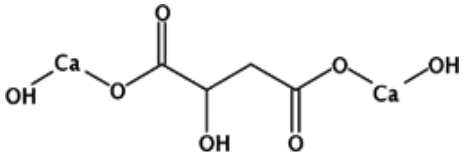

Figure 1: Chemical structure for DCM as proposed by the applicant following a request for additional information ('Documentation provided to EFSA' No. 5)

The applicant provided analytical data on particle size distribution of five lots of DCM ('Documentation provided to EFSA' n. 4) tested using a vibratory sieve testing apparatus. The Panel noted that the applicant provided a description of the method of analysis used for determining the particle size distribution. The Panel also noted that the results were reported in three different formats from the same data. The first format is as a percentage of the total mass retained above or on top of each sieve. The second is a cumulative percentage of the total mass retained above or on top of each sieve as a progression through the sieve nest. Finally, the data are reported as a percentage of the total mass that passed through each sieve. The data are summarised in Table 1.

Table 1: Results of particle size distribution analysis of five batches of di-calcium malate (DCM)

US Sieve No.	Opening (μm)	Percentage above (%)			Cumulative above (%)			Percentage below (%)		
		Ave	SD	RSD	Ave	SD	RSD	Ave	SD	RSD
35	500	0.10	0.03	31	0.10	0.03	31	99.90	0.03	0
45	355	0.29	0.20	69	0.39	0.22	57	99.61	0.22	0

US Sieve No.	Opening (μm)	Percentage above (%)			Cumulative above (%)			Percentage below (%)		
		Ave	SD	RSD	Ave	SD	RSD	Ave	SD	RSD
60	250	0.45	0.15	33	0.84	0.32	38	99.16	0.32	0
80	180	0.74	0.10	13	1.58	0.35	22	98.42	0.35	0
120	125	1.20	0.16	13	2.78	0.20	7	97.22	0.20	0
170	90	1.67	0.22	13	4.45	0.09	2	95.55	0.09	0
230	63	4.25	1.78	42	8.70	1.83	21	91.30	1.83	2
325	45	9.72	3.09	32	18.42	4.29	23	81.58	4.29	5
Pan	N/A	81.58	4.29	5	100.00	0.00	0	0.00	0.00	N/A

Ave: average; SD: standard deviation; RSD: residual standard deviation; N/A: not applicable.

On the basis of the data provided by the applicant, the Panel considered that the described vibratory sieve testing method with the smallest sieve opening of 45 μm is not appropriate to determine nano-size particles. The Panel also noted that an average of 81% of the tested material passed through the smallest 45 μm sieve.

The Panel considered that the scientific evidence submitted by the applicant (initial application dossier and additional data provided) does not demonstrate the difference between 'dicalcium malate (DCM)', which is subject of the application under evaluation and 'calcium malate' which is already included in the Annex II to Directive 2002/46/EC as one of the mineral substances which may be added to food supplements, following a scientific opinion by EFSA AFC Panel (2006).

3.1.2. Specifications

The specifications for DCM as proposed by the applicant are listed in Table 2.

Table 2: Specification for DCM as proposed by the applicant

Specification parameters	Specification value	Analytical method
Description	A white to off-white, fine powder, with no or slight scent	Visual and olfactory inspection
Identification		
Dicalcium malate	Passes test	FTIR analysis
Calcium	Passes test	Flame test for calcium
Assay		
Assay	NLT 88%	Potentiometric titration
Calcium content	NLT 29%	ICP-AES
Malic acid content	440–600 mg/g	HPLC
pH	11.5–12.5 (in water)	pH meter
Moisture/loss of drying	NMT 6.0%	Moisture balance
Fluoride	NMT 30 ppm	Ion-specific electrode
Fumaric acid	NMT 1.0%	HPLC
Maleic acid	NMT 0.05%	HPLC
Magnesium and alkali salts	NMT 4.8%	ICP-AES
Heavy metals		
Arsenic	NMT 3.0 ppm	ICP-AES
Lead	NMT 0.5 ppm	ICP-AES
Cadmium	NMT 0.5 ppm	ICP-AES
Mercury	NMT 0.1 ppm	ICP-AES
Microbiological specifications		
Total plate count	Less than 10,000	FDA BAM 8th Edition
Mould and yeast	Less than 1,000	FDA BAM 8th Edition
Coliform count	Less than 10[(a)]	FDA BAM 8th Edition
Salmonella	Negative	FDA BAM 8th Edition

Specification parameters	Specification value	Analytical method
Escherichia coli	Less than 10[(a)]	FDA BAM 8th Edition
Staphylococcus aureus	Less than 10[(a)]	FDA BAM 8th Edition

FTIR: Fourier-transforming infrared; NLT: not less than; ICP-AES: inductively coupled plasma atomic emission spectroscopy; HPLC: high-performance liquid chromatography; NMT: not more than; CFU: colony forming units; BAM: bacteriological analytical manual.
(a): Less than 10: detection limit for the test.

The Panel noted that the assay as proposed in the specifications relies exclusively on the analysis of calcium in the final product DCM. Additionally, the minimum amount proposed for malic acid (440 mg/g) would correspond to 26% of Ca that it less than the 29% proposed in the same specifications. Furthermore, the Panel noted that the applicant proposed limits for magnesium without justifying its presence in DCM.

The results of five non-consecutive batches analyses on DCM indicated that it is a consistent product compliant with some of the parameters of the proposed specifications, including those established for microbiological and heavy metals contaminations.

The maximum limits proposed for arsenic is higher than the values obtained in the five batches analysed (< 1 ppm). Based on the analytical results provided by the applicant, lower levels of the maximum limit for arsenic present as impurity, should be considered in the proposed specifications.

Furthermore, the Panel noted that the currently proposed specifications indicate a minimum limit for calcium and malic acid whilst no maximum limits are provided.

3.1.3. Manufacturing process

The Panel noted that the information on manufacturing process provided by the applicant in the dossier ('Documentation provided to EFSA' n. 1) is claimed as confidential.

According to the applicant, DCM is manufactured from DL-malic acid (FCC grade) and calcium hydroxide (FCC grade) under aqueous conditions in accordance with current Good Manufacturing Practices (GMP) and with the Hazard Analysis Critical Control Point (HACCP) system.

All the specification data sheets for the raw materials were provided by the applicant.

The Panel considered that the method outlined by the applicant sufficiently described the manufacturing process for a calcium malate, however, it does not demonstrate the specific production of DCM.

3.1.4. Methods of analysis in food

According to the applicant the amount of calcium may be determined by the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method and expressed as the level of DCM added to foodstuff; each milligram of calcium assayed is equivalent to 3.45 mg of DCM minimum. The Panel noted that the ICP-AES method determines the content of total calcium in the foodstuff and is not specific only for calcium from DCM.

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

According to the applicant, in the event of degradation of DCM, the expected degradation products would be calcium ions, hydroxide ions (OH-) and malic acid.

Six random lots of DCM were selected for stability testing. Products were tested at the time of release for calcium content and pH ('Documentation provided to EFSA' n. 1). The product was stored for 3 years in a polystyrene container at ambient temperature and controlled humidity. At the end of 3 years, the samples were tested again for calcium content and pH. Differences between initial and final results were calculated and evaluated, taking into account expected analytical variation. According to the applicant, the pH results would indicate that there is chemical stability, but there is a significant reduction of calcium content over 3 years. According to the applicant, evaluation of the data did not indicate any outliers that were causing bias of the data. Further evaluation of the physical specification yielded some insight. This product had a significant moisture gain over 3 years – 28% from initial values. The Panel noted that the moisture is calculated theoretically by 'moisture balance' (Section 3.1.2), and therefore, the explanation given by the applicant would not explain the loss of calcium content observed.

No stability studies for DCM in a food matrix were submitted in the dossier.

3.2. Proposed uses and use levels

The Panel noted that the applicant did not submit any information on proposed uses and use levels for DCM, in the initial application dossier ('Documentation provided to EFSA' n. 1).

Further to a request for additional information, the applicant has submitted data on the proposed use and use levels of DCM as a source of supplemental calcium in foods using the FAIM template (version 1) ('Documentation provided to EFSA' n. 4). However, the Panel noted that the use levels as indicated by the applicant in the FAIM template did not clearly refer to the maximum use levels, typical use levels or to the amount of calcium available from DCM. Despite a request for clarification, the applicant did not provide any additional data.

Furthermore, the Panel noted that no information were provided for the typical and maximum daily dose of DCM and the resulting typical and maximum daily calcium intake which was intended to be achieved by the proposed use of the source for food supplements, total diet replacement products for weight control and for FSMP ('Documentation provided to EFSA' n. 4).

3.3. Exposure data

The Panel noted that the applicant has provided an estimate of the exposure to DCM based on the output obtained using the FAIM model (version 1) ('Documentation provided to EFSA' n. 4).

However, the Panel considered that the data provided did not allow a reliable estimate of the exposure to DCM as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and FSMP.

3.4. Biological and toxicological data

The Panel noted that, besides a bioavailability study conducted in human volunteers, an *in vitro* absorption study of calcium from DCM and an acute toxicity study performed in rats, no further biological and toxicological data on DCM were submitted by the applicant as part of the dossier.

The applicant provided a justification for not complying with the Tier 1 requirements in the 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) by stating that, following oral ingestion, DCM readily dissociated into calcium and malic acid.

Both calcium and L-malic acid are natural constituents of the diet and also of the body, for which extensive data on their biological effects exist.

3.4.1. Bioavailability of calcium from DCM

Data on dissociation of DCM

According to the applicant, DCM dissociates at low pH into calcium and malic acid and any DCM not dissociated is assumed to be excreted in the faeces or metabolised by gut microflora. However, the Panel noted that the applicant did not provide any analytical data in support of these assumptions ('Documentation provided to EFSA' n. 1).

Following EFSA's request, the applicant submitted a study report designed to provide some evidence to support that DCM, when placed into acidic solution like that found in the stomach, dissociated into its individual components (calcium and malic acid) ('Documentation provided to EFSA' n. 4).

A method using a calcium ion selective electrode (ISE) was modelled to measure the concentration of calcium ions (Ca^{2+}) in an environment similar to the human stomach.

A sample of DCM having the equivalent of 1,000 ppm (1 mg/L = 1 ppm) of calcium or calcium hydroxide ($Ca(OH)_2$) was added to 1 L of water heated to 37°C. The solution was titrated in 1 mL portions using simulated gastric fluid (0.1 M HCl; pH 1.2). The concentration of Ca^{2+} ions was measured and recorded after each 1 mL added. A maximum of 50 mL simulated gastric fluid (SE) was added to the solution, and plotted using ml's of acid vs ppm of Ca^{2+} ions. In addition, a pH electrode was setup to capture the pH of the solution.

The results showed that unbound Ca^{2+} ranged from 600 to 900 ppm (60–90% dissociated) but never completely dissociated at 1,000 ppm (100%). The results also show that maximum dissociation occurred at pH 6, but never continues to increase higher as pH drops lower. According to the applicant, this suggests that the ionic strength of the solution increases at lower pH causing the calcium to bind to a surplus of anions present in solution.

On the basis of the results provided, the Panel considered that DCM does not completely dissociate into calcium and malic acid.

Human study

The bioavailability and tolerability of calcium from four calcium supplements (DCM, calcium amino acid chelate (18%), calcium amino acid chelate (26%) and calcium carbonate) were investigated in a double-blind, cross-over trial in 15 healthy volunteers ('Documentation provided to EFSA' n. 1), performed in accordance with Good Clinical Practice (GCP) in 2007 (Documentation provided to EFSA n. 1). Each subject received 900 mg of calcium from one of the four different formulations on four separate occasions. Serum samples were taken at 0, 0.5, 2, 4, 6, 9 and 12 h post-treatment. The Panel noted that with a such high level of calcium, intestinal calcium uptake may be saturated and therefore differences in uptake between different preparations may not be evident.

In comparison to baseline levels, a statistically significant increase in the concentration of serum calcium was observed beginning 4 h after supplementation with DCM, persisting for the remainder of the sampling period. A maximum calcium serum concentration (C_{max}) of 2.44 mmol/L (vs 2.30 mmol/L at baseline) was attained at 7.23 h following oral treatment with DCM. In comparison to baseline calcium levels, ingestion of calcium amino acid chelate (18%) resulted in the greatest increase in the concentration of serum calcium. Compared to supplementation with DCM or calcium amino acid chelate (26%), a significantly higher maximum serum calcium level was obtained within the shortest period of time when subjects received calcium from calcium carbonate.

The greatest area under the curve (AUC) was observed with calcium amino acid chelate (18%); however, no significant differences were observed in the AUC measurements among any of the 4 calcium preparations. Generally, all four AUC values were relatively low (i.e. in the range of 27.34–28.86 mmol·h/L) indicating that the bioavailability of calcium from all of these sources was generally poor. According to the study authors, this may have been due to the elimination of absorbed calcium during the first-pass effect. The half-life ($t_{1/2}$) for calcium from DCM was determined to be 42.28 h, which was significantly longer than the $t_{1/2}$ reported for calcium from calcium amino acid chelate (26%) and calcium carbonate. Generally, calcium from calcium carbonate appeared to be absorbed most efficiently; however, calcium from DCM exhibited the longest $t_{1/2}$.

According to the applicant, the increase in calcium serum levels following ingestion of DCM supports that the compound dissociates under physiological conditions, allowing for the subsequent absorption of calcium, as well as malic acid. In the same manner as other similar salts, the undissolved portion of DCM is expected to pass through the gastrointestinal tract, and will be excreted in the faeces.

***In Vitro* Study – Absorption of calcium from DCM**

The applicant provided results on an *in vitro* assay carried out to determine the solubility of DCM under different pH conditions ('Documentation provided to EFSA' n. 1). A quantity of 5 g of DCM were dissolved in 40 mL of water and the pH adjusted to different levels ranging from pH 2 to 7 by addition of 6 M hydrochloric acid. The solubility of DCM was shown to be pH-dependent, such that at a pH of 7, 5.2% of DCM was dissolved, whereas at a pH of 2, solubility increased more than twofold to 12.6%. At pH 4, the solubility of DCM was calculated to be 5.9%. According to the applicant, on the basis of these results, DCM is expected to dissolve under the acidic conditions encountered in the stomach upon ingestion. However, according to the applicant, calcium absorption does not occur in the stomach, but instead calcium is absorbed predominantly from the small intestine. As free calcium ions enter the small intestine, where the pH is approximately 6.5, the dissociated ions may at least in part re-precipitate (Heaney et al., 1990). Moreover, according to the applicant, absorption of the ionised calcium will also depend on other factors such as concomitant intake of foods that may increase or decrease its absorption.

3.4.2. Acute toxicity

DCM from a single lot has been evaluated in an unpublished single-dose oral toxicity study conducted in accordance with Good Laboratory Practice (GLP) in three healthy female Wistar albino rats (Documentation provided to EFSA n. 1). No mortality was recorded at 5,000 mg/kg body weight.

4. Discussion

The current assessment by the Panel is based on the information submitted in the application dossier (Documentation provided to EFSA n. 1) and the additional information provided by the applicant in response to EFSA's requests (Documentation provided to EFSA n. 4, 5).

According to the applicant, DCM is a salt made of two calcium ions and one malic acid and it is different from calcium malate (CAS 17482-42-7). Calcium malate is included in Annex II of Directive 2002/46/EC on vitamin and mineral substances which may be used in the manufacture of food supplements following a scientific opinion by EFSA (EFSA AFC Panel, 2006).

The Panel noted that the manufacturing process described for DCM does not demonstrate that the synthesis of DCM is different from that of calcium malate.

The Panel has requested scientific data to demonstrate that the substance under evaluation is different from 'calcium malate', and the applicant was not able to provide any scientific evidence. In the absence of these data, the Panel is unable to conclude that the substance presented as DCM would be different from calcium malate already authorised as a nutrient source.

The applicant has proposed chemical and microbiological specifications for DCM and provided results from five non-consecutive batches. The Panel noted that some parameters in the proposed specifications were not commensurate with the structure proposed.

The Panel noted that in the stability report provided by the applicant, a significant reduction in the calcium content occurred after storage at room temperature for 3 years. The Panel noted that no explanation for that decrease was provided by the applicant.

On the basis of the information provided by the applicant, the Panel was unable to calculate the typical and maximum daily calcium intake resulting from the proposed uses of DCM as a source of calcium intended to be added as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and FSMP.

The Panel noted that the toxicological data set was limited to an acute oral toxicity study, an *in vitro* study and a double-blind, cross-over trial performed in healthy human volunteers with limited safety parameters.

According to the applicant, DCM dissociates into its main components (calcium and malic acid); however, on the basis of the data available, the Panel concluded that DCM does not completely dissociate into calcium and malic acid, consequently, Tier 1 toxicity studies would be needed to evaluate the safety of DCM.

On the basis of the data submitted by the applicant, the Panel considered that calcium is bioavailable from the source.

5. Conclusions

On the basis of the available data, the Panel concluded that there was insufficient scientific evidence of a difference between the proposed novel food ingredient named as DCM and calcium malate already authorised as a source of calcium included in Annex II to Directive 2002/46/EC. Accordingly, the Panel was unable to assess the safety of DCM as a novel food ingredient.

The Panel concluded that based on the data provided DCM does not completely dissociate into calcium and malic acid.

The Panel concluded that when DCM dissociates, calcium would be available following ingestion of DCM and the bioavailability would appear similar to values reported for other sources of calcium already permitted.

Furthermore, the Panel concluded that on the basis of the information available it was not possible to calculate the exposure to DCM as a source of calcium to foods for the general population, food supplements, total diet replacement for weight control and FSMP.

Documentation provided to EFSA

1) Dossier 'Application for the approval of dicalcium malate as a source of calcium for use in the manufacture of PARNUTS products, food supplements and fortified foods'. March 2016. Submitted by Albion Laboratories, Inc.
2) Initial assessment of di-calcium malate (DCM) as a novel food ingredient. Food Safety Authority Ireland (FSAI). March 2016.
3) Member States comments and objections. March 2016.
4) Additional information. January 2018. Submitted by Albion Laboratories, Inc. in response to a request from EFSA.
5) Additional data provided as follow-up from technical hearing at ANS SWG on applications on 29 January 2018 as a web/teleconference. March 2018.

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Abbreviations

ADI	acceptable daily intake
AFC	EFSA 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
AUC	area under the curve
BAM	bacteriological analytical manual
CAS	Chemical Abstracts Service
CFU	colony forming units
C_{max}	maximum concentration
DCM	di-calcium malate
FCC	Food Chemical Codex
FSAI	Food Safety Authority of Ireland
FSMP	Food for special medical purposes
FTIR	Fourier-transforming infrared
GCP	Good clinical practices
GLP	Good laboratory practices
GMP	Good manufacturing practices
HACCP	Hazard Analysis Critical Control Point
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectroscopy

ISE	ion selective electrode
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MS	mass spectroscopy
NLT	Not less than
NMT	Not more than
PARNUTS	Foods for Particular Nutritional Uses
SCF	Scientific Committee on Food
$t_{1/2}$	half-life