SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 12 November 2019

doi: 10.2903/j.efsa.2019.5917

Safety and efficacy of L-methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* KCCM 80096 for all animal species

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Abstract

The European Commission asked EFSA for an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of L-methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* KCCM 80096 for all animal species. The two producing microorganisms were obtained by genetic modification. L-Methionine is intended to be used in feed or water for drinking for all animal species and categories. Neither viable cells of the production strains, nor their recombinant DNA were detected in the final product. The additive does not pose any safety concern associated with the genetic modification of the production strains. L-methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096. The additive is considered safe for the target species, for the consumer and for the environment. L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is considered not toxic by inhalation, non-irritant to skin or eyes and not a dermal sensitiser. Regarding the use in water, the FEEDAP Panel reiterates its concerns over the safety of L-methionine for target species when administered via water for drinking owing to the risk of nutritional imbalances and hygienic reasons. L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is considered as an efficacious source of the essential amino acid L-methionine for non-ruminant animal species. For the supplemental L-methionine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

Keywords: nutritional additives, amino acid, L-methionine, safety, efficacy, *C. glutamicum*, *E. coli*

Requestor: European Commission

Question number: EFSA-Q-2018-01017

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Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Montserrat Anguita, Elisa Pettenati and Jordi Tarrés-Call.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Brozzi R, Galobart J, Gregoretti L, Innocenti ML, Sofianidis K, Vettori MV and López-Gálvez G, 2019. Scientific Opinion on the safety and efficacy of L-methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* KCCM 80096 for all animal species. EFSA Journal 2019;17(12):5917, 15 pp. https://doi.org/10.2903/j.efsa.2019.5917

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from CJ Europe GmbH[2] for authorisation of the product L-Methionine (feed grade) produced by fermentation with *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids and their salts and analogues).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 7 February 2019.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product L-Methionine produced by fermentation with genetically modified *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 for all animal species when used under the proposed conditions of use (see Section 3.1.7).

1.2. Additional information

L-Methionine produced by fermentation with *Escherichia coli* (KCCM 11252P and KCCM 11340P) is authorised as a nutritional feed additive by Commission Implementing Regulation (EU) No 852/2014[3]; this regulation followed an EFSA Scientific Opinion on the safety and efficacy of L-methionine produced by *E. coli* (KCCM 11252P) and *E. coli* (KCCM 11340P) for all animal species (EFSA FEEDAP Panel, 2013). The product under assessment, L-Methionine produced by fermentation with genetically modified *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, has not been previously authorised as a feed additive in the European Union (EU).

L-Methionine is currently authorised as a flavouring substance in feed (FLAVIS number: No 17.027).[4]

L-Methionine may be used as a nutritional substance (list of amino acids and other nitrogen compounds) in the manufacture of infant formulae and follow-on formulae.[5] Methionine (DL-Methionine) is registered as an ingredient for use in cosmetics as antistatic and for skin conditioning.[6]

A medicine for human use containing L-Methionine is registered in the Community Register of Orphan medicinal product under number EU/3/18/2076.[7]

The European Pharmacopoeia has a dedicated monograph to L-methionine (Ph. Eur., 2019).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] CJ Europe GmbH. Ober der Roeth 04, 65824 Schwalbach am Taunus, Germany.
[3] Commission Implementing Regulation (EU) No 852/2014 of 5 August 2014 concerning the authorisation of L-methionine as a feed additive for all animal species. OJ L 233, 8.8.2014, p. 22.
[4] Commission Implementing Regulation (EU) 2018/249 of 15 February 2018 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D,L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs. OJ L 53, 23.2.2018, p. 134.
[5] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1.
[6] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p. 1.
[7] Commission Implementing Decision of 26.10.2018 relating to the designation of 'Glycine, L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, L-histidine, L-lysine monohydrate, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, taurine' as an orphan medicinal product under Regulation (EC) No 141/2000 of the European Parliament and of the Council.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of L-Methionine produced by fermentation with *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 as a nutritional additive in feed or water for drinking for all animal species.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of L-Methionine in animal feed. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-Methionine produced by fermentation with *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

3. Assessment

The product subject of this application is L-methionine produced by fermentation with genetically modified strains of *C. glutamicum* and *E. coli*. The applicant is seeking the authorisation of this new source L-methionine, intended to be used for all animal species in feed and water for drinking as a nutritional additive (functional group: amino acids, their salts and analogues).

The additive under assessment, L-Methionine, corresponds with the active substance.

Methionine is an indispensable amino acid for all animal species. Methionine is clearly recognised as the first limiting amino acid in poultry, probably also the first for high-yielding cows and the third one in pigs fed conventional diets. Therefore, additives containing L-methionine or the hydroxy-analogue of methionine as the active substance are frequently used in the feed industry to adjust the dietary methionine to the requirements of target animals in order to achieve more efficient conversion of feed and reduce nitrogen emissions.

3.1. Characterisation

3.1.1. Manufacturing process

L-Methionine is produced via two independent fermentation processes by means of *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096. [redacted]

[8] FEED dossier reference: FAD-2018-0085.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0085-methionine.pdf

[10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[redacted]

3.1.2. Characterisation of the production microorganisms

3.1.2.1. Information related to the genetically modified microorganisms

[redacted]

The strain KCCM 80184 was identified as *C. glutamicum* by sequence comparison of the gene encoding [redacted]

The strain KCCM 80096 was identified as *E. coli* by sequence comparison of the gene encoding [redacted]

a) Characteristics of the recipient or parental microorganisms

[redacted]

b) Characteristics of the donor organisms

[redacted]

c) Description of the genetic modification process

[redacted]

[redacted]

[redacted]

3.1.3. Characterisation of the additive

L-Methionine has the International Union of Pure and Applied Chemistry (IUPAC) name 'L-2-amino-4-(methylthio)butanoic acid' and its CAS number is 63-68-3. Its molecular weight is 149.2 g/mol, the molecular formula is $C_5H_{11}NO_2S$ and its molecular structure is given in Figure 1.

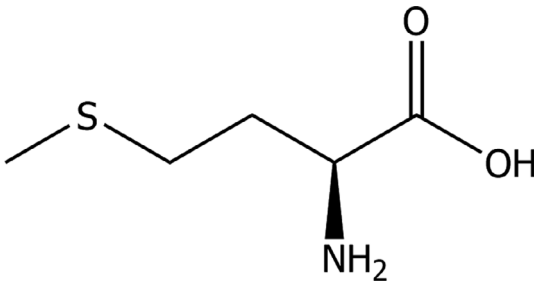

Figure 1: Molecular structure of L-methionine

The applicant declared that the product contains by specification ≥ 98.5% L-methionine *as is* (≥ 99% on dry matter), ≤ 0.5% water and ≤ 0.1 ash.[17] The analysis of five batches of L-methionine *as is* confirmed the specification: L-methionine and water were found in the ranges of 99.24–99.39% and 0.11–0.18%, respectively. The applicant provided also an analysis of other free amino acids (i.e. valine, leucine and phenylalanine) with a total content of 0.38–0.44%.[18]

Heavy metals (lead Pb, mercury Hg, cadmium Cd) and arsenic (As) were analysed in three batches of the additive[19]; the results showed that those analytes could not be detected.[20] The determination of bacterial counts (by total plate count), coliform bacteria, *E. coli*, *Salmonella* and yeasts and moulds in three batches of the additive showed compliance with the specifications.[19,21] Aflatoxins (B1, B2, G1, G2), ochratoxin A, deoxynivalenol, zearalenone and fumonisins (B1 and B2) analysed in three batches of the additive were not detected.[19,22] Three batches of L-methionine were analysed for dioxins and furans, dioxin-like PCBs and pesticides[23]; the results showed that those impurities were not detected in the additive.[24] The concentrations of the impurities reported did not raise concern.

Analytical data on the content of lipopolysaccharide (LPS)[25] in three batches of the additive showed a range of 320–380 IU/g.[26]

The absence of viable cells of the production strains in three samples of L-methionine was tested in triplicate by plating a solution of 1 g of the sample on a non-selective, rich agar medium and cultivating at 30°C for 32 h. Positive controls of *C. glutamicum* and *E. coli* were included.[27]

[17] Technical dossier/Section II/Annex.II.1.01.
[18] Technical dossier/Section II/Annex.II.1.02.
[19] Technical dossier/Section II/Annex.II.1.03.
[20] The following limits of detection were reported in the certificates of analysis: Pb, Cd and As, 1 ppb; Hg, 5 ppb.
[21] Total plate count: 1000 max; yeasts & moulds: 50 max; coliform bacteria, *E. coli* and *Salmonella*: negative.
[22] The following limits of detection were reported in the certificates of analysis: Aflatoxins and ochratoxin, 0.1 ppb; zearalenone, 1.5 ppb; deoxyvalenol, 0.5 ppb; fumonisins, 5 ppb.
[23] Technical dossier/Section II/Annexes II.1.03 and II.104.
[24] The following limits of detection were reported in the certificates of analysis: Dioxins and furans, 0.05 ppb; dioxin-like PCBs, 0.13 ppb; pesticides, 0.5–0.8 ppb.
[25] Lipopolysaccharides (LPS) represent the purified endotoxin material. Endotoxins are components of the external membrane of most Gram-negative bacteria. From HCN (2010). Available online: https://www.healthcouncil.nl/binaries/healthcouncil/documents/advisory-reports/2010/07/15/endotoxins-health-based-recommended-occupational-exposure-limit/advisory-report-endotoxins-health-based-recommended-occupational-exposure-limit.pdf
[26] Technical dossier/Supplementary information June 19/Annex_SIN_01.
[27] Technical dossier/Supplementary information June 19/Annexes 03_SIN_CJ_L-MET_190611.pdf and CONFID_SIN_02.

No recombinant DNA of the production strains was detected in three batches of the final additive.[28] Batches were analysed in triplicate (using 5 g of sample) by polymerase chain reaction (PCR) [redacted]. Batches were analysed in triplicate; 5 g of sample were used. The limit of detection of the two genes was 5 ng of DNA per g of product for both strains.

3.1.4. Physical properties of the additive

The product is a white crystalline powder. Its solubility in water is 5.66 g/100 g water at 25°C. The bulk density of the additive is 550–700 kg/m^3 and its melting point of 284°C.[17] The optical rotation examined in three batches of the additive was +23.3° to +23.7, thus meeting the specification (+21.1° ~ +25.1° at 25°C),[19,29] and confirming the presence of the L-enantiomer of methionine.

The analytical data of dusting potential from three batches of the additive were provided.[30] The results showed an average dusting potential of 0.39 g/m^3 (range: 0.35–0.42).

Particle size distribution of three batches of the additive analysed by sieving was provided.[31] The results showed on average 10.7% (range 8.0–12.9) of particles below 75 μm, from which only a small fraction of particles (0.51%, range 0.45–0.64%) were below 43 μm.

3.1.5. Stability and homogeneity

The data provided on shelf life, stability in premixtures and in feedingstuffs and on the capacity of the additive to distribute homogeneously in feed were not produced with the product under assessment. Those data were obtained from the testing of another L-methionine produced from *E. coli* KCCM 1152P and 11340P and have been evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2013). Since the physical characteristics, the purity and the production process of the two L-methionine products – produced by different microorganisms – are similar, the FEEDAP Panel considers the previous data as representative for the product under assessment.

3.1.6. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities in feed are expected with other authorised additives, medicinal products or feed materials.

3.1.7. Conditions of use

The additive L-methionine is proposed to be used in feed in order to achieve the adequate amino acid profile and meet the requirements on L-methionine for all animal species. It can be added directly to the feed or via premixture. The use of L-methionine in water for drinking is also proposed. No inclusion levels are recommended as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid content of the unsupplemented diet.

3.2. Safety

3.2.1. Safety of the genetic modification

The recipient organism *C. glutamicum* is considered to be safe. *C. glutamicum* qualifies for the qualified presumption of safety (QPS) approach to safety assessment when used for production purposes (EFSA BIOHAZ Panel, 2019). [redacted]

The recipient organism *E. coli* K-12 is considered to be safe. [redacted]

[28] Technical dossier/Supplementary information October 19/Annex_CONFID_SIN_01_The absence of recombinant DNA.pdf.
[29] The FEEDAP Panel notes that the specifications for optical rotation proposed by the applicant do not meet the specifications for L-methionine established in the European Pharmacopoeia which are +22.5° to +24.0°.
[30] Technical dossier/Supplementary information September 2019/Annex_SIN_01.
[31] Technical dossier/Section II/Annex.II.1.05.

The product L-methionine, produced by fermentation with *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, is considered to be safe with regard to the genetic modification of the production strains.

3.2.2. Safety for the target species, consumer and environment

Safety concerns from the additive may derive either from the amino acid or from the residues of the fermentation process/production strain remaining in the final product. The L-methionine under assessment is highly purified (> 98.5% methionine and < 1% unidentified substances). Neither the production strains, *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, nor their recombinant DNA were detected in the final product; the safety assessment of the production strains, *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, did not raise elements of concern.

The requirements L-methionine of different animal species (non-ruminants and ruminants) and categories and the tolerance to L-methionine excess in the diet have been described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013).

The FEEDAP Panel considers that safety concerns for target species are highly unlikely to arise from the L-methionine under application. The safety of L-methionine for the target animals has been assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The Panel based its assessment on the previously established safety of DL-methionine (EFSA FEEDAP Panel, 2012b) and on the specific metabolism of L-methionine and concluded that no safety concerns were expected. Regarding the use in water, the FEEDAP Panel reiterates its concerns over the safety of L-methionine for target species when administered via water for drinking owing to the risk of nutritional imbalances and hygienic reasons (EFSA FEEDAP Panel, 2010). The level of endotoxins (LPS) in the product (320–380 IU/g) is one order of magnitude lower than that commonly observed in feedingstuffs (1,000 IU/mg; Cort et al., 1990) and is therefore of no concern for the target species.

The absorption, distribution, metabolism and excretion of methionine have been extensively described in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2012b). The amino acid L-methionine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any potential excess will be catabolised and excreted as urea/uric acid, sulfate and carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-methionine in animal nutrition.

The product does not pose any environmental concern associated with the production strains. The amino acid L-methionine is a physiological and natural component in plants and animals. The use of L-methionine in animal nutrition would not expectedly lead to any localised increase in its concentration in the environment.

The FEEDAP Panel concludes that L-methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is safe for the target species, for the consumer and for the environment.

3.2.3. Safety for the user

No studies to support the safety for the user were performed with the product under assessment. The applicant provided an acute inhalation toxicity study,[32] a skin irritation study,[33] an eye irritation study[34] and a dermal sensitisation study[35] performed with L-methionine produced by *E. coli* KCCM 11252P and *E. coli* KCCM 11340P; those studies had been evaluated previously by the FEEDAP Panel (EFSA FEEDAP Panel, 2013). As the two L-methionine characteristics and the production process are very similar, the production strains of the L-methionine under assessment are *C. glutamicum* and an *E. coli* K-12 derivative for which the genetic modifications raise no safety concerns, the FEEDAP Panel considers that the results of these studies submitted to support the safety for the users can be applied to the product under assessment.

3.2.3.1. Effects on the respiratory system

From the results of a previously assessed acute inhalation study (EFSA FEEDAP Panel, 2013), the additive can be considered not toxic by inhalation.

[32] Technical dossier/Section III/Annex.III.3.02.
[33] Technical dossier/Section III/Annex.III.3.04.
[34] Technical dossier/Section III/Annex.III.3.03.
[35] Technical dossier/Section III/Annex.III.3.05.

The dusting potential of the additive was up to 0.42 g/m^3 (see Section 3.2.3) and the content of LPS was up to 0.38 IU/mg. The effects of endotoxin (LPS) inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015). The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012a) is described in the Appendix A. The health based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (HCN, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure could be up to 89 IU per 8-h working day, indicating that there is no risk by inhalation exposure to endotoxins for persons handling the additive.

The FEEDAP Panel considers therefore that the additive is not toxic by inhalation.

3.2.3.2. Effects on the skin and eyes

From the results of previously assessed studies (EFSA FEEDAP Panel, 2013), the additive can be considered not irritant to skin and eyes, and not a dermal sensitiser.

3.2.3.3. Conclusions on safety for the user

L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli K12* KCCM 80096 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser.

3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of the amino acid L-methionine is well established in the scientific literature. The additive L-methionine is regarded as an effective source of methionine for non-ruminant animal species. For the supplemental L-methionine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen (Chalupa, 1976; Broderick and Balthrop, 1979).

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[36] and Good Manufacturing Practice.

4. Conclusions

Neither the production strains, *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096, nor their recombinant DNA were detected in the final product. The additive does not pose any safety concern associated with the genetic modification of the production strains.

L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is considered safe for the target species, for the consumer and for the environment. L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli K12* KCCM 80096 is considered not toxic by inhalation, non-irritant to skin or eyes and not a dermal sensitiser.

Regarding the use in water, the FEEDAP Panel reiterates its concerns over the safety of L-methionine for target species when administered via water for drinking owing to the risk of nutritional imbalances and hygienic reasons.

L-Methionine produced by *C. glutamicum* KCCM 80184 and *E. coli* KCCM 80096 is considered as an efficacious source of the essential amino acid L-methionine for non-ruminant animal species. For the supplemental L-methionine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

[36] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Documentation provided to EFSA/Chronology

Date	Event
28/11/2018	Dossier received by EFSA. L-Methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* KCCM 80096 for all animal species. Submitted by CJ Europe GmbH
17/12/2018	Reception mandate from the European Commission
07/02/2019	Application validated by EFSA – Start of the scientific assessment
25/03/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issue: characterisation*
07/05/2019	Comments received from Member States
07/05/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
13/06/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
03/07/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issue: characterisation*
05/09/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
08/10/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issue: characterisation*
14/10/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
12/11/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ARDB	Antibiotic Resistance Genes database
As	Arsenic
BIOHAZ	EFSA Panel on Biological Hazards
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
IEC-VIS/FLD	ion-exchange chromatography coupled to visible or fluorescence detection
IUPAC	International Union of Pure and Applied Chemistry
KCCM	Korean Culture Collection of Microorganisms
LPS	lipopolysaccharide
MIC	minimum inhibitory concentration
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
WGS	whole genome sequencing

Appendix A – Safety for the user

The effects of the endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

Calculation of maximum acceptable levels of exposure from feed additives

For additives added in premixtures, the likely exposure time according to the relevant EFSA guidance assumes a maximum of 40 periods of exposure per day, each comprising 20 s, equal to = 800 s/day (EFSA FEEDAP Panel, 2012a,b). With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s (0.444 h/day). Again, assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m^3/day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900/0.556 = 1,619 IU/m^3.

Calculation of endotoxin content of dust

Two key measurements are required to evaluate the potential respiratory hazard associated with endotoxin content of the product (the dusting potential of the product, expressed in g/m^3; the endotoxin activity of the dust, determined by the *Limulus* amoebocyte lysate assay (expressed in IU/g). If data for the dust are not available, the content of endotoxins of the product can be used instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by the simple multiplication a × b. This resulting value is further used for calculation of potential inhalatory exposure by users to endotoxin from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012a,b).

Table A.1: Estimation of user expossure to endotoxins from the additive L-methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* K12 KCCM 80096 for all animal species, including consideration of using filter half mask (FF P2 or FF P3)[37] as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	380	Technical dossier
	b	Dusting potential (g/m^3)	0.42	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	159.6	
	d	No of premixture batches made/working day	40	EFSA FEEDAP Panel (2012a,b)
	e	Time of exposure (s)/production of one batch	20	EFSA FEEDAP Panel (2012a,b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA FEEDAP Panel (2012a,b)
f × *g*	*h*	Refined total duration of daily exposure (s)	1600	
h/3 600	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3)/eight-hour working day	10	EFSA FEEDAP Panel (2012a,b)
j/8 × *i*	*k*	Inhaled air during exposure (m^3)	0.56	
c × *k*	*l*	**Endotoxin inhaled (IU) during exposure/eight-hour working day**	**88.7**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3)/eight-hour working day	90	Health Council of the Netherlands (2010)
m × *j*	*n*	**Health-based recommended exposure limit of total endotoxin exposure (IU)/eight-hour working day**	**900**	

[37] Filtering face piece or filtering half mask according to European standard EN 149. They are graded from 1 to 3 depending on their filtering capacity.

Calculation	Identifier	Description	Amount	Source
//10		Endotoxins inhaled (IU)/eight-hour working day reduced by filter half mask FF P2 (reduction factor 10)	8.9	
//20		Endotoxins inhaled (IU)/eight-hour working day reduced by filter half mask FF P3 (reduction factor 20)	4.4	

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-methionine produced by fermentation with *Corynebacterium glutamicum* KCCM 80184 and *Escherichia coli* K12 KCCM 80096

In the current application authorisation is sought under Article 4(1) for *L-methionine produced by fermentation with Corynebacterium glutamicum KCCM 80184 and Escherichia coli K12 KCCM 80096*, under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species.

According to the Applicant, *L-methionine* has a minimum purity (mass fraction) of 98.5%. The *feed additive* is intended to be added directly into *feedingstuffs* (or through *premixtures*) and *water* for drinking. However, the Applicant did not propose any minimum or maximum content of *L-methionine* in *feedingstuffs*.

For the quantification of *L-methionine* in the *feed additive* the Applicant submitted the method based on titration described in the corresponding European Pharmacopoeia monograph. For the quantification of *methionine* in the *feed additive* the EURL identified instead the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). This standard method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. It applies for products containing more than 10% of amino acid. The following performance characteristics were reported: a relative standard deviation for repeatability (RSDr) ranging from 0.5 to 1.6% and a relative standard deviation for reproducibility (RSDR) ranging from 1.5 to 2.6%. In addition, the EURL identified the "L-methionine monograph" of the Food Chemical Codex (FCC) for the identification of *L-methionine* in the *feed additive*.

For the quantification of *L-methionine* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on IEC coupled to photometric detection (IEC-VIS). This method, designed only for the analysis of amino acids in *premixtures* and *feedingstuffs*, does not distinguish between the salts and the amino acid enantiomers. The Community method was ring-trial validated using four different matrices. This method was further ring-trial validated by twenty-three laboratories, resulting in the EN ISO 13903:2005 method. The following performance characteristics were reported for the quantification of total *methionine*: RSDr ranging from 1.1 to 5.6% and RSDR ranging from 6.9 to 13%.

The Applicant did not provide experimental data to determine *L-methionine* in *water*. Nevertheless, for the quantification of *methionine* in *water*, as concluded in the previous EURL reports and further specified in the corresponding legislation, the EURL recommended the Community method for official control.

In the frame of this authorisation the EURL recommends for official control (i) the "L-methionine monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-methionine* in the *feed additive*; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) to quantify free *methionine* in the *feed additive* and *premixtures* (containing more than 10% *methionine*); and (iii) the Community method based on IEC-VIS for the quantification of *methionine* in *premixtures*, *feedingstuffs* and *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005), as last amended by Regulation (EU) 2015/1761) is not considered necessary.