SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 10 November 2021

doi: 10.2903/j.efsa.2021.6982

Safety and efficacy of a feed additive consisting of monosodium L-glutamate produced by fermentation with *Corynebacterium glutamicum* KCCM 80187 for all animal species (CJ Europe GmbH)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of monosodium L-glutamate monohydrate produced by fermentation using *Corynebacterium glutamicum* KCCM 80187 when used as a sensory additive (flavouring compound) in feed and water for drinking for all animal species. The production strain is genetically modified and viable cells of the production strain, and its DNA were not detected in the final additive. The additive does not give rise to any safety concern regarding the production strain. Monosodium L-glutamate monohydrate produced using *C. glutamicum* KCCM 80187 is considered safe for the target species, for the consumer and for the environment. Moreover, it is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser. The FEEDAP Panel expressed reservations on the use of the additive in water for drinking due to concerns on its impact on the hygienic conditions of the water. The Panel concluded that the additive is efficacious to contribute to the flavour of feed.

Keywords: sensory, flavouring compounds, monosodium glutamate, safety, efficacy

Requestor: European Commission

Question Number: EFSA-Q-2020-00015

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to acknowledge the contribution of the Working Group on Microbiology to this opinion.

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, Fašmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Edoardo Villa R, Woutersen R, Galobart J, Manini P, Pettenati E, Tarrés Call J and Anguita M, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting of monosodium L-glutamate produced by fermentation with *Corynebacterium glutamicum* KCCM 80187 for all animal species (CJ Europe GmbH). EFSA Journal 2021;19(12):6982, 12 pp. https://doi.org/10.2903/j.efsa.2021.6982

ISSN: 1831-4732

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

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1. Introduction

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

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 additive consisting of monosodium L-glutamate produced by *Corynebacterium glutamicum* KCCM 80187, when used as a feed additive for all target species (category: sensory additives; functional group: flavouring compounds).

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 17 November 2020.

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 monosodium L-glutamate produced by *Corynebacterium glutamicum* KCCM 80187, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The product under assessment, which is not authorised as a feed additive in the European Union, is based on monosodium L-glutamate and is produced by fermentation with a genetically modified strain of *C. glutamicum* (KCCM 80187).

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[3] in support of the authorisation request for the use of monosodium L-glutamate produced by *C. glutamicum* KCCM 80187 as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of monosodium L-glutamate produced by *C. glutamicum* KCCM 80187 is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents:, 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

[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 4, 65824, Schwalbach/Taunus, Germany.
[3] FEED dossier reference: FAD-2019-0055.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0090-msglutamate.pdf
[5] 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.

(EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The additive under assessment contains monosodium L-glutamate produced by *Corynebacterium glutamicum* KCCM 80187 and it is intended to be used as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production strain

The L-glutamic acid is produced by a genetically modified strain of *C. glutamicum* which is deposited in the Korean Culture Collection of Microorganisms with the accession number KCCM 80187.[6] This strain was developed from the parental strain [redacted]

The whole genome sequence (WGS) of the production strain was sequenced and the data was used for the characterisation of the strain.[7] The WGS-based data confirmed the identification of the production strain as *C. glutamicum*, based [redacted]

The applicant tested the susceptibility of the parental [redacted] and the production (KCCM 80187) strains against the list of antibiotics proposed for '*Corynebacterium* and other Gram-positive' in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a,b).[8] All the minimum inhibitory concentrations were below the cut-off values established in that guidance.

The WGS data were searched for the presence of antimicrobial resistance genes [redacted] No relevant hits were identified. The applicant also conducted a search for the presence of toxigenic and pathogenic genes in the production strain [redacted] which did not reveal genes of concern.

3.1.1.1. Description of the genetic modification

[redacted]

[6] Technical dossier/Section II/Annex Confid II.2.01.
[7] Technical dossier/Section II/Annex Confid II.2.08.
[8] Technical dossier/Section II/Annex Confid II.2.06.
[9] Technical dossier/Section II/Annex CONFID II.2.3.

[redacted]

These modifications were all confirmed with the analysis of the WGS-based data[7]: [redacted]

3.1.2. Manufacturing process

L-Glutamic acid is produced by fermentation with the production strain. [redacted]

The applicant states that no antimicrobial substances are used in the manufacturing of the additive.[10]

3.1.3. Characterisation of the additive

Monosodium L-glutamate monohydrate (MSG; International Union of Pure and Applied Chemistry (IUPAC) name: sodium (2*S*)-2-amino-4-carboxybutanoate hydrate (synonyms: L-2-aminopentandioic acid, L-glutamic acid monosodium salt monohydrate), a compound identified with the Chemical Abstracts Service (CAS) No 6106-04-3, the European Inventory of Existing Commercial chemical Substances (EINECS) No 205-538-1) is the active substance of the additive and has a molecular mass of 187.13 g/mol. The molecular formula of monosodium L-glutamate is $C_5H_8O_4NNa \cdot H_2O$ and the structural formula is given in Figure 1.

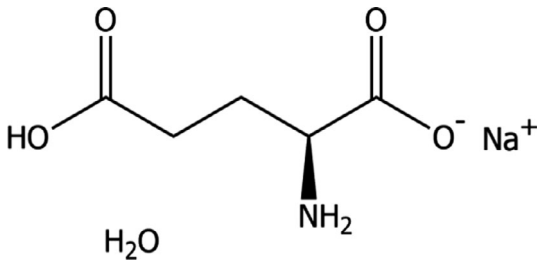

Figure 1: Structural formula of monosodium L-glutamate monohydrate

[10] Technical dossier/Section II/Annex Confid II.3.22.

The additive contains ≥ 99% monosodium L-glutamate monohydrate as is basis and ≤ 0.5% water.[11] The analysis of five batches showed an average value of monosodium L-glutamate 99.7% on 'dry matter basis' (range 99.2–100.2%) with water loss on drying of 0.12% (range 0.09–0.16%).[12] In another analytical report,[13] the applicant provided further information regarding the same batches (MSG higher than 99.5% 'as is' basis, measured by HPLC, validated method), water (0.28%), nitrogen containing components (ammonium, nitrates, nitrites and betaine, not detected, limit of detection (LOD) 0.01 mg/kg), free amino acids (not detected, LOD 0.5 mg/kg), organic acids (formic, acetic, citric, malic, succinic, lactic, not detected, LOD 1 mg/kg) and other elements (sodium 12.7%[14] 'as is' basis, and potassium, magnesium, calcium, fluoride, bromide, chloride, phosphate, sulfate were not detected, LOD 0.01 mg/kg). These data show that 0.26% of the final additive is unidentified.

The specific optical rotation was measured in three batches of the final product and the average was +25.0°,[15] which is within the range for monosodium L-glutamate monohydrate (+24.2 to +25.5, pubChem)[16] confirming the identity of the L-enantiomer and in accordance with the specifications set for monosodium glutamate (E 621) as a food additive.[17]

Three batches of the additive were analysed for other chemical impurities. Heavy metals, (lead, cadmium and mercury) and arsenic were not detected.[18] Polychlorinated dibenzodioxins (PCDDs) 0.036 WHO-TEQ ng/kg, polychlorinated dibenzofurans (PCDFs) 0.034 WHO-TEQ ng/kg, the sum of PCDD/PCDF and dioxin-like polychlorinated biphenyl (PCB) 0.136 WHO-TEQ ng/kg and indicator PCBs 0.60 μg/kg.[19] Aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, deoxynivalenol, fumonisins B1 and B2 were below the corresponding LOD.[20]

Microbial contamination showed values for yeasts and filamentous fungi < 100 CFU/g, Enterobacteriaceae and *Escherichia coli* < 10 CFU/g and absence of *Salmonella* spp. in 25 g of product.

The presence of viable cells of the production strain was assayed in three batches of the additive tested in triplicate, and using positive and negative controls.[21] [redacted] No growth was found in the samples of the additive.

The presence of recombinant DNA was analysed by PCR in three independent batches of monosodium L-glutamate tested in triplicate.[21] [redacted] DNA from the production strain was not detected.

The additive is a white powder with a solubility in water of 740 g/L. The dusting potential of the additive measured in three batches following the Stauber–Heubach method gave results ranging from 71 to 101 mg/m^3, and therefore the dusting potential is low and the potential exposure of the users by inhalation is expected to be low.[22] The particle size was measured by sieving method, particles below 100 μm amounted > 75% (w/w), particles below 44 μm amounted up to 12%.[23]

[11] Technical dossier/Section II/Annex II.1.01.
[12] Technical dossier/Section II/Annex II.1.03.
[13] Technical dossier/Section II/Annex II.1.04.
[14] This content of sodium would be in line with the stechiometry of the molecule assumed.
[15] Technical dossier/Section II/Annex II.1.02.
[16] https://pubchem.ncbi.nlm.nih.gov/compound/23689119
[17] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Text with EEA relevance). OJ L 83, 22.3.2012, p.1.
[18] Technical dossier/Section II/Annex II.1.05. LOD (in mg/kg) were 0.04 for arsenic, 0.015 for lead, 0.01 for cadmium and 0.01 for for mercury.
[19] Technical dossier/Annex II.1.05.
[20] Technical dossier/Section II/Annex II.1.05. LOD (in μg/kg) was 0.2 for aflatoxins (B1, B2, G1, G2), 0,5 for ochratoxin A, 10 for zearalenone, and 50 for deoxynivalenol.
[21] Technical dossier/Supplementary information April 2021/Annex Confid 1.
[22] Technical dossier/Section II/Annex II.1.07.
[23] Technical dossier/Section II/Annex II.1.06.

3.1.4. Stability

The applicant provided studies on the shelf-life, stability in a vitamin mineral premixture and feed for chickens for fattening, and in water for drinking.[24] In all those studies, however, the test item was monosodium glutamate produced by a different production strain (*C. glutamicum* KCCM 80188). Those studies have been assessed in the context of a previous opinion (EFSA FEEDAP Panel, 2020). Since the production process is similar and the product characteristics are nearly the same, the FEEDAP Panel considers that the results of those studies can be applicable to the product under assessment.

3.1.5. Conditions of use

Monosodium L-glutamate monohydrate is proposed to be used in feedingstuffs/complementary feedingstuffs or water for drinking in all animal species as a flavouring compound. The applicant proposes a maximum use level of 25 mg monosodium L-glutamate monohydrate/kg feed. For its use in water, the applicant recommends 12.5 mg/L water for drinking for rabbits, poultry species and pigs, for the rest of the species, the applicant recommends that the use in water should not exceed the daily amount that would be consumed via feed and established the following values (in mg/day): 50 for veal calf, 227 for cattle for fattening, 568 for dairy cow, 34 for sheep/goat, 227 for horses, 7.1 for dog and 1.7 for cats.[25]

3.2. Safety

3.2.1. Safety for the target species, consumer and environment

Safety concerns from the additive may derive either from the active substance or from the residues of the fermentation process/production strain remaining in the final product. The product under assessment is highly purified (less than 1% unidentified material is present in the additive). The production strain, KCCM 80187, belongs to a species, *C. glutamicum*, that qualifies for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007) when used for production purposes (EFSA BIOHAZ Panel, 2020). The production strain was unambiguously identified as *C. glutamicum*, was shown to be susceptible to the relevant antibiotics, the strain does not to contain antimicrobial resistance genes, the genetic modification raises no concerns, and no viable cells and DNA of the production strain were detected in the final product. It can be concluded that no safety concerns for target animals, consumers and the environment would arise from the use of *C. glutamicum* KCCM 80187 as the production strain.

The recommended levels of use of monosodium L-glutamate monohydrate in feed are well below the ones that may be present in the diets when using feedstuffs like soya bean meal, which contains 9.2% of glutamic acid (FAO, 2014).[26] Therefore, the Panel considers that no concerns for the target animals would arise for the supplementation of the diets with monosodium L-glutamate monohydrate at 25 mg/kg feed. The applicant established conditions of use in water that would mirror the intakes resulting from the supplementation in feed; however, the FEEDAP Panel has reservations on the use of the additive via water due to hygienic reasons (EFSA FEEDAP Panel, 2010).

Regarding the safety for consumers, the vast majority of monosodium L-glutamate monohydrate is metabolised in the gastrointestinal tract of the target animals and only a very small proportion enters either the systemic or the portal blood supply. It is not expected that the composition of tissues and products of animal origin will be affected by the use of monosodium L-glutamate monohydrate as a feed additive. The FEEDAP Panel also notes that glutamic acid (E 620) and its salts (E 621 to E 625) are included in the Union list of food additives as 'additives other than colours and sweeteners', 'group I (with a maximum of 10 g/kg), 'other additives that may be regulated combined', category 12.1.2 salt substitutes and category 12.2.2. seasoning and condiment.[27]

The use of monosodium L-glutamate monohydrate as a feed additive at the levels proposed is not expected to increase its concentration in the environment, and therefore, it is of no safety concern for the environment. No viable cells and no DNA from the production strain were detected in the additive.

[24] Technical dossier/Section II/Annex_II_4_01 to II_4_05.
[25] Technical dossier/Section II/Annex II.5.01 and main text of section II/Supplementary information May 2021/Annex_SIN 01.
[26] A complete feed with a 20% of soya bean meal would present 1.8 g of glutamic acid per kg feed.
[27] Commission Regulation (EU) No 1129/2011 of 11 November 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 Text with EEA relevance OJ L 295, 12.11.2011, p. 1.

Overall, the FEEDAP Panel concludes that monosodium L-glutamate monohydrate produced by *C. glutamicum* KCCM 80187 is safe under the proposed conditions of use for the target species, for the consumer and for the environment. However, the Panel has reservations on the use of the additive in water for drinking of the target animals due to concerns on its impact on the hygienic conditions of the water.

3.2.2. Safety for user

The applicant submitted an acute inhalation study,[28] *in vitro* skin[29] and eye[30] irritation studies and a skin sensitisation study.[31] In all these studies, the test item was monosodium glutamate produced by a different production strain (*C. glutamicum* KCCM 80188) and were evaluated in the context of a previous opinion (EFSA FEEDAP Panel, 2020). The Panel concluded that the additive produced with KCCM 80188 is not toxic by inhalation, it is not irritant to skin or eyes and is not a dermal sensitiser. Since the two products share a similar production process and characteristics the FEEDAP Panel considers that the results of those studies are applicable to the additive under assessment. Therefore, the additive is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser.

3.3. Efficacy

Monosodium glutamate is mentioned in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2010), by the Flavour and Extract Manufactures Association (FEMA) as a flavour enhancer, i.e. a substance with no specific taste on its own but which has an ability to enhance existing flavours. L-Glutamic acid is listed in Fenaroli's Handbook of Flavour Ingredients with the reference number 3285. Further, monosodium glutamate is authorised under Commission Regulation (EU) No 1129/2011 on food additives.

The Panel considers that the effect of monosodium L-glutamate monohydrate to increase the taste of food is well documented and therefore no further demonstration of efficacy is necessary.

4. Conclusions

The additive is produced with a genetically modified strain of *C. glutamicum,* KCCM 80187, and no viable cells of the production strain nor its DNA were detected in the final additive. The additive does not give rise to any safety concern regarding the production strain.

Monosodium L-glutamate monohydrate produced with *C. glutamicum* KCCM 80187 is considered to be safe for the target species, for the consumer and for the environment. However, the use of the additive in water for drinking raises concerns for the target species due to its likely impact on the hygienic conditions of the water.

Monosodium L-glutamate monohydrate produced with *C. glutamicum* KCCM 80187 is considered not toxic by inhalation, not irritant to skin or eyes and not a dermal sensitiser.

The FEEDAP Panel concludes that the additive is efficacious to contribute to the flavour of feed.

5. Documentation as provided to EFSA/Chronology

Date	Event
07/01/2019	Dossier received by EFSA. Monosodium L-glutamate produced by fermentation with *C. glutamicum* (KCCM 80187). Submitted by CJ Europe GmbH.
19/02/2019	Reception mandate from the European Commission
17/11/2020	Application validated by EFSA – Start of the scientific assessment
03/02/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation*
16/02/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
17/02/2021	Comments received from Member States

[28] Technical dossier/Section III/Annex III.3.04 and Annex III.3.08.
[29] Technical dossier/Section III/Annex III.3.06.
[30] Technical dossier/Section III/Annex III.3.05.
[31] Technical dossier/Section III/Annex III.3.07.

Date	Event
27/04/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
06/07/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation*
31/08/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
10/11/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviation

FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Monosodium L-glutamate produced by fermentation with *Corynebacterium glutamicum* KCCM 80187

In the current application an authorisation is sought under Article 4(1) for *monosodium L-glutamate (MSG)* produced by fermentation with *Corynebacterium glutamicum* KCCM 80187, under the category/ functional group 2(b) 'sensory additives'/'flavouring compounds', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species. According to the Applicant *MSG* has a minimum purity (mass fraction) of 99%. The *feed additive* is intended to be added directly into *feedingstuffs* (or through *premixtures*) and *water* for drinking. The Applicant proposed a recommended maximum content of *MSG* in *feedingstuffs* of 25 mg/kg.

For the quantification of *MSG* in the *feed additive* the Applicant submitted an in-house validated analytical method based on reversed phase high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). While in the frame of the validation study satisfactory performance characteristics were derived, the Applicant did not present a verification study, or any additional test performed by a second independent laboratory applying the above-mentioned method.

For the quantification of *MSG* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on ion-exchange chromatography 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 amino acids and their salts, or between different salts of the same amino acids, and it cannot differentiate between enantiomers.

The method was further ring-trial validated resulting in the EN ISO 13903:2005 method. The following performance characteristics were reported for the quantification of glutamic acid: a relative standard deviation for *repeatability* (RSDr) ranging from 0.9 to 2.7% and a relative standard deviation for *reproducibility* (RSDR) ranging from 6.2 to 9.1%. However, while a lowest limit of quantification (LOQ) of 30 mg/kg has been reported for the analysis of certain amino acids, a specific LOQ for glutamic acid has not been indicated. Therefore, the method does not ensure the accurate determination of *MSG* when added into feed at the proposed recommended maximum content (i.e. 25 mg/kg *feedingstuffs*).

Hence, the EURL recommends for official control the European Union method based on IEC-VIS for the quantification of *MSG* in *premixtures* only. The Applicant did not provide any experimental data to determine *MSG* in *water*. Nevertheless, as concluded in previous EURL reports on amino acids, the EURL recommends for official control the procedure based on IEC-VIS and described in the ring-trial validated European Union method (or in equivalent ring-trial validated methods e.g. VDLUFA Method 4.11.6.) to quantify MSG in in the feed additive and water.

The methods described above do not distinguish between the amino acids and their salts, or between different salts of the same amino acids, and they cannot differentiate between enantiomers. As a consequence, MSG is detected as glutamic acid.

In addition, the EURL recommends the "Monosodium L-glutamate monograph" of the Food Chemical Codex (FCC) for the identification of the feed additive.

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.