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Safety and efficacy of a feed additive consisting of L-lysine monohydrochloride and L-lysine sulfate produced by *Corynebacterium glutamicum* CGMCC 14498 for all animal species (Kempex Holland BV)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of L-lysine monohydrochloride (L-lysine HCl) and L-lysine sulfate produced by *Corynebacterium glutamicum* (*C. glutamicum*) CGMCC 14498 as a nutritional feed additive for all animal species. The active substance is L-lysine and it is produced in two different forms (monohydrochloride or sulfate). The production strain *C. glutamicum* CGMCC 14498 and its recombinant DNA were not detected in the final products. The products L-lysine HCl and L-lysine sulfate do not pose any safety concern associated with the production strain. L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are considered safe for the target species. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account. L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are safe for the consumer and the environment. In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-lysine HCl produced by the strain *C. glutamicum* CGMCC 14498 to be toxic by inhalation, and on the potential of L-lysine HCl and L-lysine sulfate produced by the above-mentioned strain to be irritant to skin or eyes, or on their potential to be dermal sensitisers. L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are considered efficacious sources of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as in non-ruminant species, this would require protection against degradation in the rumen.

Keywords: nutritional additive, amino acid, lysine monohydrochloride, lysine sulfate, safety, efficacy, CGMCC 14498

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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 Kempex Holland B.V.[2] for authorisation of the products L-lysine monohydrochloride and L-lysine sulfate produced using *Corynebacterium glutamicum* CGMCC 14498 when used as a feed additive for all animal species (category: nutritional additive; functional group: amino acids, 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 8 December 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 products L-lysine monohydrochloride and L-lysine sulfate produced using *Corynebacterium glutamicum* CGMCC 14498, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

L-Lysine produced using different microbial strains is currently authorised for its use in all animal species as a nutritional additive.[3]

L-Lysine is authorised for use in food,[4] and as a veterinary medicinal product.[5,6]

L-Lysine hydrochloride is described in a monograph of the European Pharmacopoeia (European Pharmacopeia, 2019) monograph 01/2008:0930.

The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has published several opinions on the safety and efficacy of L-lysine and/or its salts produced by fermentation using different strains of *C. glutamicum* for all animal species (EFSA, 2007a; EFSA FEEDAP Panel, 2015b, 2016b, 2019a-e, 2020a-e, 2021a-e one opinion on the safety and efficacy of concentrated liquid L-lysine (base) and L-lysine HCl produced by fermentation with *Corynebacterium casei* KCCM 80190 for all animal species (EFSA FEEDAP Panel, 2020d); and others on the safety and efficacy of L-lysine and/or its salts produced by fermentation using different strains of *Escherichia coli* (EFSA FEEDAP Panel, 2013, 2014, 2015a-c, 2016a, 2017a).

[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] Kempex Holland BV. (Zeelandsedijk 15, 5408SL, Volkel, The Netherlands).
[3] European Union Register of feed additives, available online: https://ec.europa.eu/food/system/files/2021-09/animal-feed_additives_eu-register_1831-03.pdf
[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, OJ L 181, 29.6.2013, p. 35.
[5] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.
[6] Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OL L 152, 16.6.2009, p. 11.

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[7] in support of the authorisation request for the use of L-lysine monohydrochloride (HCl) and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 as an additive in feed and water for drinking.

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, 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-lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), 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, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), 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, 2019f).

3. Assessment

The current application is for the authorisation of L-lysine monohydrochloride (HCl) (minimum 78.8% L-lysine on dry matter basis) and L-lysine sulfate (minimum 55% L-lysine on dry matter basis) produced by fermentation by a genetically modified strain of *C. glutamicum* (CGMCC 14498). These products are intended to be used in feed and water for drinking for all animal species as nutritional additives (functional group: amino acids, their salts and analogues). The active substance of both forms of the additive is L-lysine.

3.1. Characterisation

3.1.1. Characterisation of the production organism

L-Lysine is produced by a genetically modified strain of *C. glutamicum* which has been deposited in the China General Microbiological Culture Collection Center (CGMCC) with deposition number CGMCC 14498.[10]

A bioinformatic analysis based on whole genome sequence (WGS) confirmed the taxonomic identification of the production strain as *C. glutamicum*.[11] [redacted][11]

The susceptibility of the production strain to the antimicrobials listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP

[7] FEED dossier reference: FAD-2020-0067.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep_fad-2020-0067-lyshcl-lys-sulfate.pdf
[9] 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.
[10] Technical dossier/Section II/Annex 2.2.1.2b CONFID.
[11] Technical dossier/Section II/Annex 2.2.1.2f CONFID and Supplementary information August 2021/Annex 12.

Panel, 2018a) for '*Corynebacterium* and other Gram +' was assessed by broth microdilution method.[12] All the minimum inhibitory concentration (MIC) values were lower than the corresponding cut-off values.

The whole genome sequence (WGS) of the production strain was interrogated for the presence of acquired antimicrobial resistance (AMR) genes. The applicant has provided two data sets: in the first one, the interrogation was performed using [redacted][13] No genes of concern were identified. Similarly, in the second data set, the WGS of the production strain was interrogated [redacted] The search confirmed that the strain does not harbour any AMR gene of concern.

3.1.1.1. Information related to the genetically modified microorganism

Description of the genetic modification

[redacted][15]

3.1.2. Manufacturing process

L-Lysine is produced by fermentation using *C. glutamicum* CGMCC 14498. [redacted]

[12] Technical dossier/Section II/Annex 2.2.2.2 MIC sensitivity.
[13] Technical dossier/Section II/Annex 2.2.1.2f CONFID.
[14] Technical dossier/Supplementary information August 2021/Annex 12.
[15] Technical dossier/Supplementary information August 2021/Annex 10, 12 and 13.

[redacted][16] [redacted][17] Remnants of the antifoamer used were not detected in three batches of L-lysine sulfate.[18]

The applicant stated that no antibiotics are used during the production process.[19]

The presence of viable cells of the production strain in the final additive was investigated in three batches of L-lysine HCl and three batches of L-lysine sulfate.[20] [redacted] No viable cells of the production strain were found in three independent batches of the final product L-lysine HCL and L-lysine sulfate.

The presence of recombinant DNA from the production strain was tested in three batches of L-lysine HCL and in three batches of L-lysine sulfate, each tested in triplicate.[21] [redacted] No DNA of the production strain was detected.

3.1.3. Characterisation of L-lysine monohydrochloride

l-Lysine HCl (IUPAC name: (2*S*)-2,6-diaminohexanoic acid monohydrochloride, synonym L-lysine hydrochloride, a compound identified with the CAS No 657-27-2 and the EINECS No 211-519-9), has a molecular weight of 182.65 g/mol. The theoretical content of lysine in lysine monohydrochloride is 80%. The molecular formula is $C_6H_{15}ClN_2O_2$ and the molecular structure is given in Figure 1.

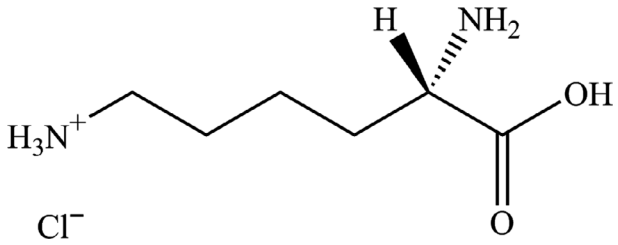

Figure 1: Molecular structure of L-lysine HCl

The product is specified to contain ≥ 98.5% L-lysine HCl (representing ≥ 78.8% L-lysine on a dry matter [DM] basis), and ≤ 1% moisture.

The average lysine content analysed in five batches was 79.0% (range 78.0–80.4%) on DM basis.[22] Three of the five batches analysed had a lysine content on DM basis below the specification level. The content of chloride was not analysed. It was calculated (stoichiometry) to correspond to 19.8% on DM basis. Loss on drying was on average 2.3% (range 1.2–3.4%), and all five batches were above specification. On a DM basis, the sum of quantified material including calculated chloride was on average 98.8%.

[16] Technical dossier/Section II/Annex 2.3.1i CONFID.
[17] Technical dossier/Section II/Annex 2.3.1j CONFID.
[18] Technical dossier/Supplementary information of August 2021/Annexes 6.4, 7.5, 8.6 and 9.7. [redacted]
[19] Technical dossier/Section II/Annex 2.3.1h.
[20] Technical dossier/Section II/Annex 2.1.4i and Supplementary information August 2021/Annex 14.
[21] Technical dossier/Section II/Annex 2.1.4j and Annex 2.1.4k.
[22] Technical dossier/ Section II/Annex 2.1.3a, and supplementary information of August 2021, Annex 1. Analytical method stated to be in accordance with Commission Regulation 152/2009 App III (Annex 2).

The specific optical rotation was measured in six batches and ranged from +19.12° to +20.32°.[23] This is within the acceptable criteria provided by the applicant (+18.0 to +21.5°) but outside the range specified in the European Pharmacopoeia for L-lysine HCl (+21.0 to +22.5°). The conditions of the test (e.g. amount of lysine HCl, molarity of the hydrochloric acid used it the test) were not provided.

Three batches of L-lysine HCl were analysed for undesirable substances. As regards heavy metals, lead and cadmium were found below the LOD,[24] and mercury ranged from < 0.002 mg/kg (LOD) to 0.016 mg/kg. Arsenic ranged from < 0.01 (LOD) to 0.17 mg/kg. In relation to the mycotoxin concentrations, aflatoxins (unspecified), ochratoxin A, zearalenone, fumonisins (B1, B2 and B3) and deoxynivalenol were below the LOD while citrinin ranged from < 15 (LOD) to 36 μg/kg.[25]

Dioxins (polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo(p)dioxins (PCDDs)) and dioxin-like polychlorinated biphenyls (DL-PCBs) were measured in three batches of the final product and were below the corresponding limits of quantification (LOQs). The levels of PCDD/F and the sum of PCDD/F and DL-PCB (upper limit) were calculated to be 0.14–0.30 ng WHO-TEQ/kg and 0.27–0.43 ng WHO-TEQ/kg, respectively, depending on the batch considered.[26]

The analysis of the microbial contamination of L-lysine HCl showed that *Salmonella* spp. was not detected in 25 g samples; and *Escherichia coli*, Enterobacteriaceae, yeasts and filamentous fungi could not be detected in 1-g samples.[28]

3.1.3.1. Physico-chemical characteristics

L-Lysine HCl is a white or light brown microgranular product with an approximate density of 0.4 to 0.6 kg/L, and a water solubility of about 600 g/L at 20°C.[27]

The dusting potential analysed by Stauber–Heubach method in three batches ranged from 7 to 10 g/m^3.[29] Concerning the particle size distribution, three batches were analysed by laser diffraction. The fraction of particles having a diameter smaller than 18, 50 and 100 μm ranged 1–2%, 2–3% and 3–5%, respectively.[30]

3.1.3.2. Stability and homogeneity of L-lysine HCl

The shelf life of L-lysine HCl was studied in three batches, kept in sealed bags protected from light and air exchange, either at room temperature for 12 months or at 40°C for 6 months. No losses were observed.[31]

The stability of the additive when supplemented with 7.7% (three batches) in a vitamin/mineral premixture containing 0.24% lysine and 46,000 mg choline/kg was studied and stored in sealed bags at ambient temperature for 6 months.[32] Losses observed ranged from 0% to 4% depending on the batch considered.

The stability of the additive (three batches) in feedingstuffs was tested in meal and pelleted compound feed for pigs for fattening (basal diet containing barley and wheat and 0.78% background lysine) when supplemented at 0.5% (corresponding to 0.39% lysine).[33] The pelleting process was performed at 70 °C. The samples were kept at room temperature in individual bags for 3 months. Total (free plus protein-bound) lysine was analysed. No losses were observed. The effect of pelleting at 70°C on the stability of the additive produced a loss of 1%.

The stability of the additive (three batches) was studied in water at a concentration of 0.5% at room temperature for 24 h.[34] No losses were detected.

The capacity of one batch of L-lysine HCl to homogeneously distribute in the pelleted feed described above was studied in 10 subsamples. Total lysine was measured in each subsample and the

[23] Technical dossier/Section II/Annex 2.1.5f, and supplementary information of August 2021/Annex 15 and Annex 4.2 method.
[24] Technical dossier/Section II/Annex 2.1.3a. LOD in mg/kg were 0.002 for cadmium and 0.01 for lead.
[25] Technical dossier/Section II/Annex 2.1.3a. LOD in μg/kg were 1.7 for aflatoxins, 5 for ochratoxin A, 17 for zearalenone, 25 for fumonisins and 134 for deoxynivalenol.
[26] Technical dossier/Section II/Annexes 2.1.4.b1, c1 and d1.
[27] Technical dossier/Supplementary information August 2021/FAD-2020-0067 Sin 050321 Answers pdf.
[28] Technical dossier/Section II/Annexes 2.1.3a and 2.1.4a.
[29] Technical dossier/Section II/Annexes 2.1.5d1, d2 and d3.
[30] Technical dossier/Section II/Annexes 2.1.4b2, c2 and d2.
[31] Technical dossier/Section II/Anne 2.1.3a.
[32] Technical dossier/Section II/Annexes 2.4.1a and 1b; and supplementary information August 2021/ FAD-2020-0067 Sin 050321 Answers pdf.
[33] Technical dossier/Section II/Annexes 2.4.1b and 1d; and supplementary information August 2021/ FAD-2020-0067 Sin 050321 Answers pdf.
[34] Technical dossier/Section II/Annex 2.1.3a.

background lysine concentration of the basal diet at the beginning of the study was subtracted from each subsample. The coefficient of variation (CV) was 1.5% when total lysine was considered and 5% when the background lysine in feed was subtracted from each measurement of total lysine concentration.[35]

3.1.4. Characterisation of L-lysine sulfate

L-Lysine sulfate (CAS No 60343-69-3) has a molecular weight of 390.38 g/mol. The molecular formula is $[C_6H_{14}N_2O_2]_2\ SO_4$ and the molecular structure is given in Figure 2. The theoretical content of lysine in the lysine sulfate is 75%.

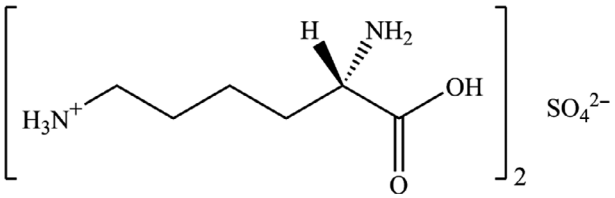

Figure 2: Molecular structure of L-lysine sulfate

The additive L-lysine sulfate contains by specification ≥ 55% of lysine on DM basis, ≤ 3% loss on drying and ≤ 4% residues on ignition. Additional specifications were ≥ 73% L-lysine sulfate and ≥ 10% other amino acids.

The compositional data of five batches showed an average lysine concentration of 57.7% (range 56.7–58.6%) on DM basis.[36] Loss on drying was on average 2.4% (range 2.2–2.6%). The following parameters were analysed in only three batches: residues on ignition ranged from 1.9% to 2.8% on DM basis; sulfate ranged from 21.4% to 22.9%; and other amino acids ranged from 8.3% to 9.1%.

3.1.4.1. Impurities

Three batches of L-lysine sulfate were analysed for undesirable substances. Regarding the levels of heavy metals, cadmium ranged 0.0044–0.0052 mg/kg, lead ranged 0.058–0.23 mg/kg and mercury 0.003–0.056 mg/kg. Arsenic ranged 0.05–0.11 mg/kg.

Regarding the mycotoxin content, analytical data of the same batches showed levels of aflatoxins (not specified) ranging 0.7–2.3 μg/kg, ochratoxin A ranging from < 5 (LOD)–7.3 μg/kg, zearalenone ranging 42.5–127.5 μg/kg, fumonisins (B1 + B2 + B3) ranging 35–90 μg/kg, deoxynivalenol ranging 710–938 μg/kg and citrinin ranged from < 15 (LOD)–6 μg/kg.

Dioxins (PCDFs, PCDD) and DL-PCBs were measured in three batches of the final product and were below the corresponding LOQs. The levels of PCDD/F and the sum of PCDD/F and DL-PCB (upper limit) were calculated to be 0.14–0.95 ng WHO-TEQ/kg and 0.27–1.08 ng WHO-TEQ/kg, respectively depending on the batch considered.[38]

The analysis of the microbial contamination of L-lysine sulfate showed absence of *Salmonella* spp. in 25 g samples; and *E. coli*, Enterobacteriaceae, yeasts and filamentous fungi could not be detected in 1-g samples.[37]

3.1.4.2. Physico-chemical characteristics

L-Lysine sulfate is an odourless light brown granular product, with a density of 500–700 kg/m^3, and a solubility in water stated to be of 200 g/100 mL at 20°C.[39]

The dusting potential analysed by the Stauber–Heubach method in three batches ranged from 0.3 to 0.4 g/m^3.[40] Concerning the particle size distribution, three batches were analysed by laser diffraction. No particles having a diameter < 100 μm were detected.[41]

[35] Technical dossier/Section II/Annex 2.4.1b.
[36] Technical dossier/Supplementary information August 2021/FAD-2020-0067 Sin 050321 Answers pdf. The analytical method is stated to be in accordance with is in accordance with Commission Regulation 152/2009 App. III (Annex 2).
[37] Technical dossier/Section II/Annex 2.1.4a.
[38] Technical dossier/Section II/Annexes 2.1.4e, 4f and 4g.
[39] Technical dossier/Supplementary information August 2021/FAD-2020-0067 Sin 050321 Answers.
[40] Technical dossier/Section II/Annexes 2.1.5d1 to d3.
[41] Technical dossier/Section II/Annexes 2.1.5.a2, 2.1.5.b2, and 2.1.5.c2.

3.1.4.3. Stability and homogeneity

The shelf life of the additive (three batches) was studied when stored in closed bags and protected from light, either at room temperature for 12 months or at 40°C for 6 months. No losses were observed.

The stability of the additive (three batches) in a vitamin/mineral premixture containing 0.24% of lysine and choline (16,000 mg/kg) was studied when added at 10% (corresponding to 5.6% lysine) and stored in sealed plastic bags at ambient temperature for 6 months. No losses were observed in one batch whereas the other two had losses of 15% and 27%.[43]

The stability of the additive (three batches) in feedingstuffs was tested in mash and pelleted compound feed for pigs for fattening (basal diet containing barley and wheat, and with 0.81% background lysine) with the addition of 0.5% lysine sulfate (corresponding to 0.28% lysine).[44] The samples were kept at room temperature in individual sealed bags for 3 months. Total lysine (free plus protein-bound) was analysed. Losses ranged from 1% to 12% in the meal feed and from 0% to 2% in the pelleted feed, depending on the batch considered. The effect of pelleting at 70°C on the stability of the additive (comparing average concentrations between meal and pellet feed analysed at T0) produced a loss of 1%.

The stability of the additive (three batches) was studied in water at a concentration of 0.5% (corresponding to 0.28% lysine) when stored at room temperature for 24 h.[42] No losses were detected. It is noted that the duration of the stability study is half that recommended in the Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b).

The capacity of L-lysine sulfate (one batch) to distribute homogeneously in the pelleted feed mentioned above was studied in 10 subsamples. Total lysine was measured in each subsample and the background lysine concentration of the basal diet at the beginning of the study was subtracted from the concentration of each subsample. The CV was 2% when total lysine was considered and 8% when the background lysine in feed was subtracted from each measurement of total lysine concentration.[45]

3.1.5. Conditions of use

Both forms of the additive are intended to be used in feed for all animal species and can be added directly in compound feed or be administered via complementary feed or premixtures. No proposed inclusion levels are provided, as the optimal daily allowance in quantitative terms depends on the species, the physiological state of the animal, the performance level and the environmental conditions, the water intake and, in particular, the amino acid composition of the unsupplemented diet.

The applicant states that both forms of the additive can be used in water for drinking. No inclusion levels in water were proposed by the applicant.

3.2. Safety

3.2.1. Safety of the production microorganism

The production organism *C. glutamicum* CGMCC 14498 was developed to increase the production of L-lysine. The production strain belongs to a species, *C. glutamicum*, that is eligible for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007b) when used for production purposes (EFSA BIOHAZ Panel, 2020). The production strain has been properly identified at species level, it does not carry acquired antimicrobial resistance genes, and the genes inserted during the genetic modification do not raise safety concerns. The production strain and its DNA were not detected in the additives. Therefore, the additives do not pose any safety concern as regards the genetic modification of the production strain.

[42] Technical dossier/Section II/Annex 2.1.3b.
[43] Technical dossier/Section II/Annexes 2.4.1a and 2.4.1c; and supplementary information August 2021/Annex FAD-2020-0067 SIN 050221 Answers.
[44] Technical dossier/Section II/Annexes 2.4.1b and 2.4.1d for feed composition; and supplementary information August 2021/ Annex FAD-2020-0067 SIN 050221 Answers.
[45] Technical dossier/Section II/Annex 2.4.1c.

3.2.2. Safety of L-lysine HCl and L-lysine sulfate for the target species, consumer and the environment

The L-lysine requirements of different non-ruminant species and animal categories, the absorption and metabolic fate of L-lysine, the tolerance to L-lysine excess and the lysine to arginine antagonism have been described in detail in previous opinions (EFSA FEEDAP Panel, 2013, 2014). No safety concerns for ruminants would arise from ruminal lysine metabolism (EFSA FEEDAP Panel, 2013, 2014). The use of the amino acid *per se* will not raise safety concerns for the target animals, provided that it is supplemented in appropriate amounts to satisfy the nutritional requirements of the animals in L-lysine-deficient diets. However, due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the use of the amino acid via water for drinking.

There is a high inherent content of sulfate in L-lysine sulfate which could be a safety concern for the target species, depending on the supplementation level and the tolerance of the target species. The EFSA FEEDAP Panel (2019a) has already concluded that the formulation of the complete feed should carefully take into account the maximum tolerable level of total sulfur (S), as established by NRC (2005) and set in ruminant diets at 3 g S/kg DM (diet rich in concentrate) and at 5 g S/kg DM (diet rich in roughage) and in non-ruminant diets at 4 g S/kg DM. Also, the contribution of S/sulfate present in water for drinking to the total S intake should be considered, especially when the content is high. Consequently, no negative effects are expected at normal use levels for the target species provided that the total S intake complies with the recommendations of established scientific bodies.

The absorption, distribution, metabolism and excretion of L-lysine were described in a previous scientific opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). Potential concerns for consumers might arise from the fermentation process. However, the production strain is considered safe (see Section 3.2.1). The use of the amino acid L-lysine itself in animal nutrition is considered safe for consumers.

The amino acid L-lysine is a physiological and natural component of animals and plants. When supplemented to feed it 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 and carbon dioxide. The use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The use of L-lysine in animal nutrition would not lead to localised increase in the concentration of L-lysine or its metabolites in the environment.

The FEEDAP Panel considers that L-lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are safe for the target species, consumer and the environment.

3.2.3. Safety of L-lysine HCl and L-lysine sulfate for the user

No specific studies to support the assessment of the safety for the user were submitted.

The dusting potential of L-lysine HCl is up to 10 g/m^3 and the product has up to 5% of particles with a diameter < 100 μm (see Section 3.1.3.1), indicating that users can be likely exposed to dust from the additive.

The physical properties of L-lysine sulfate showed that there were no particles having a diameter < 100 μm and the dusting potential ranged from 0.3 to 0.4 g/m^3 (see Section 3.1.4.2). Thus, exposure of users by inhalation is unlikely.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-lysine HCl produced by the strain *C. glutamicum* CGMCC 14498 to be toxic by inhalation; and on the potential of L-lysine HCl and L-lysine sulfate produced by the abovementioned strain to be irritant to skin or eyes, or on their potential to be 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-lysine is well established in the scientific literature. The efficacy of L-lysine for both non-ruminant and ruminant species was described in two previous EFSA opinions (EFSA FEEDAP Panel, 2013, 2014). In general, the products L-lysine HCl and L-lysine sulfate are considered as efficacious sources of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as in non-ruminant species, this would require protection against degradation in the rumen.

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[46] and Good Manufacturing Practice.

4. Conclusions

The production strain *C. glutamicum* CGMCC 14498 and its recombinant DNA were not detected in the final products. The products L-lysine HCl and L-lysine sulfate do not pose any safety concern associated with the production strain.

L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are considered safe for the target species. When using L-lysine sulfate, the background sulfur/sulfate content in the compound feed should be taken into account. L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are safe for the consumer and for the environment.

In the absence of data, the FEEDAP Panel cannot conclude on the potential of L-lysine HCl produced by the strain *C. glutamicum* CGMCC 14498 to be toxic by inhalation; and on the potential of L-lysine HCl and L-lysine sulfate produced by the above-mentioned strain to be irritant to skin or eyes, or on their potential to be a dermal sensitiser.

L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498 are considered as efficacious sources of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as in non-ruminant species, this would require protection against degradation in the rumen.

5. Documentation as provided to EFSA/Chronology

Date	Event
16/09/2020	Dossier received by EFSA. L-lysine monohydrochloride and L-lysine sulfate produced by *C. glutamicum* CGMCC 14498. Submitted by Kyowa Hakko Europe GmbH.
28/09/2020	Reception mandate from the European Commission
8/12/2020	Application validated by EFSA – Start of the scientific assessment
05/03/2021	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 of the production strain, characterization of the additive, conditions of use, safety for the user*
09/03/2021	Comments received from Member States
09/03/2021	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
30/08/2021	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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Abbreviations

AMR	antimicrobial resistance
CAS	Chemical Abstracts Service
CGMCC	China General Microbiological Culture Collection Centre
CFU	colony forming unit
CV	coefficient of variation

DM	dry matter
EINECS	European Inventory of Existing Commercial chemical Substances
EURL	European Union Reference Laboratory
FCC	Food chemical codex
FEEDAP	Panel on additives and products or substances used in animal feed
GLP	Good Laboratory Practice
IEC-VIS/FLD	Ion exchange chromatography coupled to visible or fluorescence detection
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MCE	mixed cellulose esters
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzodioxins/dibenzofurans
PCR	polymerase chain reaction
PVDF	polyvinylidene fluoride
QPS	Qualified presumption of safety
TEQ	Toxic equivalents
VDLUFA	Association of German agricultural analytic and research institutes
WGS	whole genome sequence
WHO	World Health Organization

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-lysine monohydrochloride and L-lysine sulfate from *Corynebacterium glutamicum* CGMCC 14498

In the current application authorisation is sought under Article 4(1) for L-lysine monohydrochloride and L-lysine sulfate produced by fermentation with *Corynebacterium glutamicum* CGMCC 14498, 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-lysine monohydrochloride has a minimum purity (mass fraction on dry matter basis) of 98.5% and 78.8% with respect to the L-lysine content, respectively, while L-lysine sulfate has a minimum purity of 73.0% and 55.0% with respect to the L-lysine content, respectively.

The two forms of the feed additive are intended to be added directly into feedingstuffs or through complementary feed, premixtures and water. However, the Applicant did not propose any minimum or maximum content of L-lysine in feedingstuffs.

For the quantification of lysine in the feed additive the Applicant proposed 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 cannot differentiate between enantiomers. It applies for products containing more than 10% of amino acid. The following performance characteristics are reported: a relative standard deviation for repeatability (RSDr) ranging from 0.7% to 1.7% and a relative standard deviation for reproducibility (RSDR) ranging from 1.5% to 2.5%. In addition, the EURL identified the "L-lysine monohydrochloride monograph" of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive and the generic European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of sulfate ions in feed additive.

For the quantification of lysine in premixtures, feedingstuffs and water the Applicant suggested using the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled with 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 following performance characteristics were reported for the quantification of total lysine: RSDr ranging from 2.1% to 2.8 % and RSDR ranging from 3.0% to 6.7%. Furthermore, as concluded in previous amino acids reports, the EURL also considers the IEC-VIS/FLD procedure described above as fit-for-purpose for the determination of lysine in water.

Based on the performance characteristics available, the EURL recommends for official control (i) the "L-lysine monohydrochloride monograph" of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive; (ii) the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of sulfate ions in feed additive; (iii) the ring-trial validated method EN ISO 17180:2013 based on IEC-VIS/FLD to quantify free lysine in the feed additive and premixtures (containing more than 10 % lysine); (iv) the European Union method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; and (v) the ion-exchange chromatography methods coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) or coupled with post-column derivatisation and photometric detection (IEC-VIS) for the quantification of lysine in 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.