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Safety and efficacy of the feed additives concentrated liquid L-lysine (base) and L-lysine monohydrochloride produced by *Corynebacterium glutamicum* KCCM 80183 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 concentrated liquid L-lysine (base) and L-lysine monohydrochloride (HCl) produced using *Corynebacterium glutamicum* KCCM 80183 when used as nutritional additives in feed and water for drinking for all animal species. The active substance is L-lysine. Concentrated liquid L-lysine (base) and L-Lysine HCl produced by the strain *C. glutamicum* KCCM 80183 do not pose any safety concern as regards the genetic modification of the production strain. The use of the additives under assessment in supplementing feed to compensate for L-lysine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel has concerns about the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed. The use of both forms of L-lysine produced by fermentation using *C. glutamicum* KCCM 80183 in animal nutrition is considered safe for the consumers and for the environment. Concentrated liquid L-lysine (base) produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, not irritant to skin and eyes and it is not a skin sensitiser. L-Lysine HCl produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, it is not irritant to skin but mildly irritant to eyes and it is not a skin sensitiser. The additives under assessment 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, it would require protection against degradation in the rumen.

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1. Introduction

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

Regulation (EC) No 1831/2003¹ 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 GmbH² for authorisation of the products L-lysine monohydrochloride (HCl), concentrated liquid L-lysine (base), and L-lysine sulfate when used as feed additives for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues). During the assessment, the applicant withdrew the application for L-lysine sulfate.³

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 23 July 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 products L-lysine HCl and concentrated liquid L-lysine (base), produced by fermentation using *Corynebacterium glutamicum* KCCM 80183 when used as additive in feed and water for drinking 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.⁴ Nevertheless, L-lysine HCl and concentrated liquid L-lysine (base) produced by fermentation using *C. glutamicum* KCCM 80183 are not currently authorised in the European Union (EU).

L-Lysine is authorised for use in food,⁵ cosmetics⁶ and as a veterinary medicinal product.^{7,8}

L-Lysine hydrochloride is described in a monograph of the European Pharmacopoeia (PhEur, 2019).

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,b,c,d,e, 2020a,b,c); 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,b,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 CJ Europe GmbH. Unterschweinstiege 2 - 14, 60549 Frankfurt am Main, Germany.

³ Letter of CJ Europe GmbH of 29 October 2019 to the European Commission (EC). Acceptance letter by the EC on 6 December 2019.

⁴ Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition. OJ L 239, 30.8.1988, pp. 36–39.

⁵ 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.

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, pp. 1–528.

⁷ 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.

⁸ 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 dossier⁹ in support of the authorisation request for the use of concentrated liquid L-lysine (base) and L-lysine HCl as additives 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 and experts' knowledge, 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 the concentrated liquid L-lysine (base) and L-lysine HCl, produced using *C. glutamicum* KCCM 80183 in animal feed. The Executive Summary of the EURL report can be found in Annex A.¹⁰

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of concentrated liquid L-lysine (base) and L-lysine HCl is in line with the principles laid down in Regulation (EC) No 429/2008¹¹ 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, 2018), 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) and Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019f).

3. Assessment

The product subject of this application is L-lysine in the forms of concentrated liquid L-lysine (base) and L-lysine HCl produced by fermentation with a genetically modified strain of *C. glutamicum*. The applicant is requesting the authorisation of these products as nutritional additives, under the functional group 'amino acids, their salts and analogues'. The products under application are intended to be used in feed and water for drinking for all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *C. glutamicum* which is deposited in the Korean Culture Collection of Microorganisms with accession number KCCM 80183.¹²

The production strain was identified as *C. glutamicum* by [REDACTED]

[REDACTED] 13
confirming the identification of the production strain as *C. glutamicum*.¹⁴

The susceptibility of the production strain to relevant antibiotics was tested against the list of antimicrobials described for '*Corynebacterium* and other Gram+' in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP

⁹ FEED dossier reference: FAD-2019-0016.

¹⁰ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-00160028-lysinehcl.pdf

¹¹ 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.

¹² Technical dossier/Section II/Annex Conf II.2.1.

¹³ Technical dossier/Section II/Annex Conf II.2.2.

¹⁴ Technical dossier/Supplementary information December 2020/Annex CONFID/Annex_SIN_CONFID_01 CJ L-Lys WGS Report.

Panel, 2018). All measured minimum inhibitory concentration (MIC) values were lower than the cut-off values specified in the guidance.¹⁵

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) [REDACTED]

[REDACTED] No hits of concern were identified.¹⁴

The WGS of *C. glutamicum* KCCM 80183 was also interrogated for the presence of toxin and virulence factor genes [REDACTED]

[REDACTED]¹⁴ No hits of concern were identified.

Information relating to the genetically modified microorganism

3.1.1.1. Characterisation of the recipient or parental microorganism

[REDACTED]

3.1.1.2. Characterisation of the donor organisms

[REDACTED]

3.1.1.3. Description of the genetic modification

[REDACTED]¹⁷

[REDACTED]

[REDACTED]¹⁴

3.1.2. Manufacturing process

Concentrated liquid L-lysine (base) and L-lysine HCl are produced by fermentation using *C. glutamicum* KCCM 80183 as production microorganism. [REDACTED]

¹⁵ Technical dossier/Section II/Annex Conf II.2.4.

¹⁶ Technical dossier/Section II/Annex Conf II.2.03.

¹⁷ Technical dossier/Supplementary information December 2020/03_SIN_CJ_L-LYS_80183_201214.pdf and Annex CONFID/Annex_SIN_CONFID_01 CJ L-Lys WGS Report.

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The presence of viable cells of the production strain in the final products was analysed in three batches of L-lysine HCl and concentrated liquid L-lysine (base), respectively.²⁰

No colonies were detected.

The presence of DNA of the production strain in the final products was analysed in three batches L-lysine HCl and concentrated liquid L-lysine (base), respectively.²¹

No DNA from the production strain was detected in the samples.

3.1.3. Characterisation of concentrated liquid L-lysine (base)

L-Lysine (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2,6-diaminohexanoic acid; synonym: (S)-2,6-diaminocaproic acid), a compound identified with the Chemical Abstracts Service (CAS) No 56-87-1 and the European Inventory of Existing Commercial chemical Substances (EINECS) No 201-300-6, has a molecular weight of 146.2 g/mol. The molecular formula is C₆H₁₄N₂O₂ and the molecular structure is given in Figure 1.

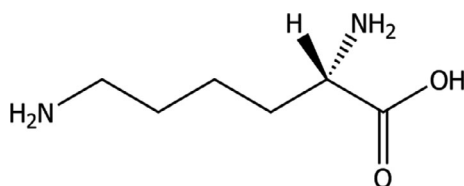


Figure 1: Molecular structure of L-lysine

The product is specified to contain $\geq 50\%$ lysine on 'as is' basis and $\leq 50\%$ water.

Compliance with the specification was shown in five batches in which L-lysine was on average 50.8% on 'as is' basis (range 50.3–51.0%). Water content was 47.9% (range 46.0–49.0%).²² Free amino acids other than lysine (glutamic acid, alanine, isoleucine and arginine) were on average 0.14%, ammonium 0.03%, sodium 0.06%, potassium 0.14%, chloride 0.9% and sulfate 0.15%. Ash was on average 0.33% (range 0.29–0.35%).²³ The amount of unidentified material on 'as is' basis was < 1%.

Three batches were analysed for undesirable substances. Levels of heavy metals (cadmium, lead, mercury) and arsenic were below the respective LOD.²⁴ Mycotoxins (aflatoxins B1, B2, G1, G2; ochratoxin A, zearalenone, deoxynivalenol (DON) and fumonisins B1, B2) were found below the respective LODs.²⁵ Pesticides (358 species) were analysed in three batches of concentrated liquid L-lysine (base) and found below the LOD.²⁶ Microbiological contaminants as total plate counts, yeasts

¹⁸ Technical dossier/Section II.3 and supplementary information December 2020/03 SIN CJ L-Lys 80183, Section 4.

¹⁹ Technical dossier/Section II/Annex Conf II.3.19.

²⁰ Technical dossier/Supplementary information December 2020/Annex CONFID/Annex_SIN_CONFID_03.

²¹ Technical dossier/Supplementary information December 2020/Annex CONFID/Annex_SIN_CONFID_04.

²² Technical dossier/Section II/Annex II.1.6. Lysine was analysed by AOAC 999.13 method.

²³ Technical dossier/Section II/Annex II.1.9 and Supplementary information December 2020/Annex Sin_01.

²⁴ Technical dossier/Section II/Annex II.1.13. LOD in $\mu\text{g}/\text{kg}$ were 1 for lead, cadmium and arsenic; 5 for mercury; for nitrofurans ranged from 0.09 to 0.15 depending on the compound considered; for nitrofurans metabolites ranged from 0.11 to 0.16 depending on the compound considered.

²⁵ Technical dossier/Section II/Annex II.1.13. LOD in $\mu\text{g}/\text{kg}$ was 0.1 for aflatoxins (B1, B2, G1, G2) and ochratoxin A; 0.5 for DON; 1.5 for zearalenone and 5 for fumonisins (B1, B2).

²⁶ Technical dossier/Section II/Annex II.1.11. LOD ranged from 0.5 to 8 $\mu\text{g}/\text{kg}$ depending on the pesticide considered.

and filamentous fungi were below the LOD; *Salmonella* spp. was absent in 25-mL samples; and *Escherichia coli* and coliforms were not detected.²⁷

The above-mentioned impurities/contaminants do not represent a safety concern.

Concentrated liquid L-lysine base is a dark brown liquid, readily soluble in water. It has a density of 1.12–1.17 g/mL,²⁸ a surface tension (three batches) ranging from 43 to 46 mN/m and a viscosity ranging from 82 to 86 cp at 25°C.²⁹ Its pH (three batches) ranged from 10.1 to 10.3.³⁰

3.1.4.1. Stability and homogeneity

The applicant provided studies on the shelf-life, stability in water for drinking, stability in premixtures and in mash feed for chicken for fattening as well as on the capacity of concentrated liquid L-lysine (base) to distribute homogeneously in premixtures, mash and pelleted feed of chicken for fattening.³¹ In all those studies, however, the test item was concentrated liquid L-lysine (base) produced by a different species (*C. glutamicum* KCCM 10227). Those studies had been assessed in a previous opinion (EFSA FEEDAP Panel, 2019d). As the production process is the same and the product characteristics are very similar, the FEEDAP Panel considers that the results of those studies can be applicable to the product under assessment.

3.1.4. Characterisation of the L-lysine HCl

L-Lysine HCl (IUPAC name: (2S)-2,6-diaminohexanoic acid monohydrochloride; synonym: L-(+)-2,6-diamino-*N*-caproic acid monohydrochloride, 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 HCl is 80%. The molecular formula is C₆H₁₅ClN₂O₂ and the molecular structure is given in Figure 2.

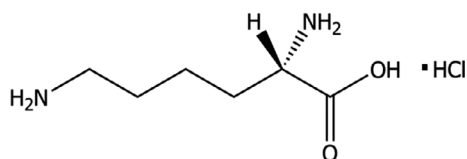


Figure 2: Molecular structure of L-lysine HCl

The product is specified to contain $\geq 78\%$ L-lysine on a dry matter (DM) basis, $\leq 1\%$ water and $\leq 0.3\%$ ash.

The applicant provided data of five batches of the additive. The average content of lysine was 79.7% (all five batches) on DM and loss on drying was on average 0.2% (range 0.1–0.4%).³² Ash was on average 0.05% (range 0.03–0.06%); chloride average was 19.1% (range 19.1–19.2%); sulphate was 0.02%; sodium, potassium and ammonium 0.01% each. The amount of identified material on DM basis was on average 99.0%.³³

The specific optical rotation was measured in three batches and ranged from +21.4 to +21.8°. This is within the range of the reference values established in the European Pharmacopoeia (range between +21.0 and +22.5°) and confirms the L enantiomer of lysine in the additive.³⁴

²⁷ Technical dossier/Section II/Annex II.1.13. Supplementary information December 2020/03_Sin_CJ_Lys_80183. LOD was 10 CFU/mL for total plate count and for yeast and filamentous fungi. For coliforms and *E. coli* the LOD was 1 CFU/mL and for *Salmonella* spp. the LOD was 1 CFU/25mL.

²⁸ Technical dossier/Section II.3.2.

²⁹ Technical dossier/Section II/Annex II.1.21 and 22.

³⁰ Technical dossier/Supplementary information December 2020/Annex SIN 04.

³¹ Technical dossier/Section II/Annexes II.4.2, II.4.5, II.4.8, II.4.11, II.4.14, II.4.16 and II.4.19.

³² Technical dossier/Section II/Annex II.1.5 and supplementary information December 2020/Annex Sin 5. Lysine analysed by AOAC 999.13 method.

³³ Technical dossier/Section II/Annex II.1.8 and Supplementary information December 2020/Annex Sin_05.

³⁴ Technical dossier/Section II/Annex II.1.4.

Three batches were analysed for undesirable substances. Levels of heavy metals (cadmium, lead, mercury) and arsenic were below the respective LODs.³⁵ Mycotoxins (aflatoxins B1, B2, G1, G2; ochratoxin A, zearalenone, DON and fumonisins B1, B2) were below the respective limits of detection.³⁶ Pesticides (358 species) were analysed in three batches of L-lysine HCl and found below the LOD.³⁷ Microbiological contaminants as total plate counts and moulds were below the LOD; coliforms, *Salmonella* spp. was absent in 25 g samples; and *Escherichia coli* was not detected in 1 g sample.³⁸

The above-mentioned impurities/contaminants did not represent a safety concern.

L-lysine HCl is a free-flowing pale brownish crystalline powder having a bulk density of 670–750 kg/m³ a solubility in water of 642 g/L at 30°C.³⁹ Its dusting potential (Stauber–Heubach method, six batches analysed) ranged from 0.331 to 0.688 g/m³.⁴⁰

3.1.4.1. Stability and homogeneity

The applicant provided studies on the shelf-life, stability in water for drinking, stability in premixtures and in mash feed for chicken for fattening; as well as on the capacity of concentrated L-lysine HCl to distribute homogeneously in premixtures, mash and pelleted feed of chicken for fattening.⁴¹ In all those studies, however, the test item was L-lysine HCl produced by a different species (*C. glutamicum* KCCM 10227). Those studies had been assessed in a previous opinion (EFSA FEEDAP Panel, 2019d). As the production process is the same and the product characteristics are very similar, the FEEDAP Panel considers that the results of those studies can be applicable to the product under assessment.

3.1.5. Conditions of use

L-Lysine in the form of concentrated liquid L-lysine (base) or L-lysine HCl, is proposed to be used in feeds in order to achieve the adequate amino acid profile and to meet the requirements on L-Lysine for all animal species. It can be added directly to the feedingstuffs, complementary feed or via premixture. Both forms of the additive are also intended for use in water for drinking.⁴² No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, the water intake as well as the amino acid content of the unsupplemented diet.

3.2. Safety

3.2.1. Safety of the production organism

The production strain belongs to a species, *C. glutamicum*, that is suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007a,b) when used for production purposes (EFSA BIOHAZ Panel, 2020). The production organism *C. glutamicum* KCCM 80183 was developed to increase the production of L-lysine. The genes inserted during the genetic modification do not raise safety concerns and the production strain does not carry acquired antimicrobial resistance genes. 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.

3.2.2. Safety of concentrated liquid L-lysine (base) and L-lysine HCl for the target species, consumer and the environment

The applicant provided two acute oral toxicity studies in rat testing L-lysine HCl and concentrated liquid L-lysine (base) produced by a different strain (*C. glutamicum* KCCM 10227) that were considered not relevant because a single dose was tested.⁴³

³⁵ Technical dossier/Section II/Annex II.1.11. LOD in µg/kg were < 1 for lead, cadmium and arsenic; < 5 for mercury; for nitrofurans ranged from 0.09 to 0.15 depending on the compound considered; for nitrofurans metabolites ranged from 0.11 to 0.16 depending on the compound considered.

³⁶ Technical dossier/Section II/Annex II.1.11. LOD in µg/kg was < 0.1 for aflatoxins (B1, B2, G1, G2) and ochratoxin A; < 0.5 for DON; < 1.5 for zearalenone and < 5 for fumonisins (B1, B2).

³⁷ Technical dossier/Section II/Annex II.1.14. LOD ranged from 0.5 to 8 µg/kg depending on the pesticide considered.

³⁸ Technical dossier/Section II/Annex II.1.11 and supplementary information December 2020/03 SIN CJ L-lys 80183, Section 3.2. LOD was < 1 × 10³ CFU/g for total plate count and < 50 CFU/g for yeast and filamentous fungi.

³⁹ Technical dossier/Section II/Annex II.3.1.

⁴⁰ Technical dossier/Section II/Annex II.1.19.

⁴¹ Technical dossier/Section II/Annexes II.4.1, II.4.4, II.4.7, II.4.10, II.4.13, II.4.15 and II.4.18.

⁴² Technical dossier/Supplementary information December 2020/Annex 03_SIN_CJ_80183 point 5.

⁴³ Technical dossier/Section III/Annexes III.3 and III.4.

L-Lysine requirements of different non-ruminant species and animal categories, absorption and metabolic fate of L-lysine, tolerance to L-lysine excess and the lysine to arginine antagonism have been described in detail in previous opinions (EFSA FEEDAP Panel, 2013, 2014, 2015a,b,c, 2016a, 2017a). No safety concerns for ruminants would arise from ruminal lysine metabolism.

Both forms of the additive are highly purified. The use of the amino acid L-lysine 'per se' will not raise safety concerns for the target animals provided that it is supplemented in appropriate amounts to the diets. Concerns from the use of the additive may arise from residues of the fermentation process/production strain remaining in the final product. The production strain (KCCM 80183) belongs to species *C. glutamicum* that is considered suitable for the QPS approach to safety assessment when used for production purposes. Viable cells of the production strain and/or its DNA were not detected in the final products. Consequently, no safety concerns for target animals, consumers of products derived from animals fed the additive and the environment would arise from the fermentation residues that may be present in the final additives.

The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed (EFSA FEEDAP Panel, 2010).

The amino acid L-lysine, supplemented to feed, will be incorporated into proteins of tissues and/or products of animal origin and any of their potential excess will be metabolised and excreted as urea/uric acid and carbon dioxide. Therefore, the composition of tissues and products of animal origin will not be affected by the use of L-lysine in animal nutrition.

The additives under assessment do not give rise to any concern for the environment associated with the production strain. The amino acid L-lysine is a physiological and natural component of the proteins of living organisms. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. 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 these additives in animal nutrition would not lead to any localised increase in the concentration of L-lysine in the environment and does not represent a risk to the environment.

3.2.2.1. Conclusions on the safety for the target species, consumer and the environment

The use of concentrated liquid L-lysine (base) or L-lysine HCl produced by *C. glutamicum* KCCM 80183 in supplementing feed to compensate for lysine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed.

The use of both forms of L-lysine produced with *C. glutamicum* KCCM 80183 in animal nutrition is considered safe for the consumer and for the environment.

3.2.3. Safety for the user

The acute inhalation toxicity studies, *in vitro* skin irritation studies, *in vitro* eye irritation studies, and skin sensitisation studies submitted were conducted using as a test item the concentrated liquid L-lysine (base) or L-lysine HCl of a different production strain (*C. glutamicum* KCCM 10227).⁴⁴ These studies had been evaluated in a previous opinion (EFSA FEEDAP Panel, 2019d). As the characteristics of the products L-lysine HCl and concentrated liquid L-lysine (base) of the two different production strains are very similar, the production process is the same and the production strains qualify for the QPS assessment, the FEEDAP Panel considers that the results of these studies are applicable to the L-lysine HCl and concentrated liquid L-lysine (base) under assessment.

Concentrated liquid L-lysine (base) produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, not irritant to skin and eyes and it is not a skin sensitiser.

L-Lysine HCl produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, it is not irritant to skin but mildly irritant to eyes and it is not a skin 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 opinions

⁴⁴ Technical dossier/Section III/Annexes III.3.6, 7, 9, 10, 12, 13, 15 and 16.

(EFSA FEEDAP Panel, 2013, 2014, 2015a,b,c, 2016a, 2017a). In general, the products concentrated liquid L-lysine (base) and L-lysine HCl 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, it 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 Regulation⁴⁵ and Good Manufacturing Practice.

4. Conclusions

Concentrated liquid L-lysine (base) and L-lysine HCl produced by the strain *C. glutamicum* KCCM 80183 do not pose any safety concern as regards the genetic modification of the production strain.

The use of concentrated liquid L-lysine (base) and L-Lysine HCl produced by the strain *C. glutamicum* KCCM 80183 in supplementing feed to compensate for L-lysine deficiency in feedingstuffs is safe for the target species. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking.

The use of both forms of L-lysine produced by fermentation using *C. glutamicum* KCCM 80183 in animal nutrition is considered safe for the consumers and for the environment.

Concentrated liquid L-lysine (base) produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, not irritant to the skin and eyes and it is not a skin sensitiser. L-Lysine HCl produced by *C. glutamicum* KCCM 80183 is considered hazardous by inhalation, it is not irritant to skin but mildly irritant to eyes and it is not a skin sensitiser.

Concentrated liquid L-lysine (base) and L-lysine HCl 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, it would require protection against degradation in the rumen.

5. Documentation as provided to EFSA/Chronology

Date	Event
22/02/2019	Dossier received by EFSA. L-Lysine monohydrochloride/Concentrated liquid L-Lysine/L-Lysine sulphate produced by fermentation with <i>Corynebacterium glutamicum</i> KCCM80183 for all animal species. Submitted by CJ Europe GmbH.
11/06/2019	Reception mandate from the European Commission
23/07/2019	Application validated by EFSA – Start of the scientific assessment
23/10/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additive, conditions of use.</i>
23/10/2019	Comments received from Member States
29/10/2019	The applicant requests a partial withdrawal: to remove the L-lysine sulfate from the application
23/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
06/12/2019	The European Commission accepts the proposed partial withdrawal
14/12/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AMR	antimicrobial resistance
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
DON	deoxynivalenol
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
KCCM	Korean Culture Collection for Microorganisms
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organization 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
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
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 on the analytical methods L-lysine monohydrochloride, concentrated liquid L-lysine and L-lysine sulphate produced by *Corynebacterium glutamicum* KCCM80183; and L-lysine monohydrochloride produced by *Corynebacterium glutamicum* DSM32932

In the current applications authorisation is sought under Article 4(1) for L-lysine monohydrochloride, concentrated liquid L-lysine and L-lysine sulphate produced by *Corynebacterium glutamicum* KCCM80183 and L-lysine monohydrochloride produced by *Corynebacterium glutamicum* DSM32932, 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 Applicants, L-lysine monohydrochloride contains a minimum (mass fraction) of 78 % of L-lysine as active substance, while the concentrated liquid L-lysine and the L-lysine sulphate contain a minimum of 50 and 55 % of L-lysine, respectively.

The different forms of the feed additive are intended to be added directly into feedingstuffs or through premixtures. L-lysine monohydrochloride, concentrated liquid L-lysine and L-lysine sulphate produced by *Corynebacterium glutamicum* KCCM80183 can also be included in water for drinking. However, the Applicants did not propose any minimum or maximum content of L-lysine in feedingstuffs.

For the quantification of lysine in the feed additive, the Applicants submitted the European Union (EU) method dedicated for the determination of amino acids in premixtures and feedingstuffs. However, for the quantification of lysine in the feed additive the EURL previously evaluated and recommended the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IECVIS/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 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 %.

For the quantification of L-lysine in premixtures and feedingstuffs one Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled with photometric detection (IEC-VIS), which was previously recommended by the EURL. 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 to 6.7 %.

The different forms of Lysine produced by *Corynebacterium glutamicum* KCCM80183 can also be included in water for drinking. However, the corresponding Applicant did not provide any experimental data to determine lysine in water. Nevertheless, as concluded in previous amino acids reports of the EURL, the IEC-VIS procedure described in the European Union method is considered fit-for-purpose for the determination of lysine in water.

In addition, the EURL found 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. 20301) for the identification of sulphate ion in L-lysine sulphate.

In the frame of these authorisations the EURL recommends for official control (i) the "L-lysine monohydrochloride monograph" of the Food Chemical Codex (FCC) based on infrared absorption 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 the sulphate ion in L-lysine sulphate; (iii) 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 lysine in the feed additive and premixtures (containing more than 10 % lysine); and (iv) the European Union method based on IEC-VIS for the quantification of lysine 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.