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Safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation with *Corynebacterium glutamicum* KCCM 80216 as feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Pier Sandro Cocconcelli, Boet Glandorf, Lieve Herman, Miguel Prieto Maradona, Maria Saarela, Montserrat Anguita, Jaume Galobart, Orsolya Holczknecht, Paola Manini, Fabiola Pizzo, Jordi Tarrés-Call and Elisa Pettenati

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 concentrated liquid L-lysine (base, minimum 50%) and L-lysine monohydrochloride (HCl, minimum 99%) produced by fermentation with a genetically modified strain of *Corynebacterium glutamicum* (KCCM 80216) as nutritional additives for all animal species. Neither the production strain nor its recombinant DNA was detected in the final products. The additives do not pose any safety concern associated with the genetic modification of the production strain. Concentrated liquid L-lysine (base) and L-lysine HCl produced by *C. glutamicum* KCCM 80216 do not represent a risk for the target species, the consumer and the environment. From the results of studies on the safety for the user of concentrated liquid L-lysine (base) and L-lysine HCl produced by a different production strain, it was possible to conclude on the safety for the user of the products under assessment. The concentrated liquid L-lysine (base) and the L-lysine HCl are not irritant to skin or eyes or skin sensitiser. L-lysine HCl is not hazardous by inhalation. L-lysine HCl and concentrated liquid L-lysine (base) 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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Correspondence: feedap@efsa.europa.eu

Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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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 Daesang Europe B. V.² for authorisation of the product L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation with *Corynebacterium glutamicum* KCCM 80216, when used as a feed additive for all animal species (category: nutritional additives; 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 3 June 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 L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation with *C. glutamicum* KCCM 80216, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

L-Lysine produced by fermentation using different production strains is currently authorised for use in all animal species as a nutritional additive.³

L-Lysine is also authorised for use in food,⁴ cosmetics⁵ and as a veterinary medicinal product.^{6,7}

L-Lysine hydrochloride is described in a monograph of the European Pharmacopoeia (PhEur 9th edition, 2017) 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, 2015a, 2016a, 2017a, 2019a–e, 2020a–c), an opinion on the safety and efficacy of concentrated liquid L-lysine (base) and L-lysine monohydrochloride produced by fermentation with *Corynebacterium casei* KCCM 80190 for all animal species (EFSA FEEDAP Panel, 2020d) and others on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and/or L-lysine monohydrochloride produced by *Escherichia coli* for all animal species (EFSA FEEDAP Panel, 2013, 2014, 2015a–c, 2016b, 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 Daesang Europe B. V., Van Heuven Goedhartlaan 935, 1181 LD, Amsterdam, The Netherlands.

³ 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.

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 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.

⁶ 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.

⁷ 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. OJ 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 dossier⁸ in support of the authorisation request for the use of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation with *C. glutamicum* KCCM 80216 as feed additives.

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 the L-lysine in animal feed. The executive summary of the EURL report can be found in Annex A.⁹

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation with *C. glutamicum* KCCM 80216 is in line with the principles laid down in Regulation (EC) No 429/2008¹⁰ and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), 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 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 product subject of this application is L-lysine in the forms of L-lysine monohydrochloride (HCl) and concentrated liquid L-lysine (base) produced by fermentation with a genetically modified strain of *C. glutamicum* (KCCM 80216). This product is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed for all animal species.

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 at the Korean Culture Collection of Microorganisms under the number KCCM 80216.¹¹

A bioinformatic analysis based on whole genome sequence (WGS)¹² confirmed the taxonomic identification of the production strain as *C. glutamicum*.¹³

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 Panel, 2018a) for '*Corynebacterium* and other Gram +' was assessed by broth microdilution method

⁸ FEED dossier reference: FAD-2020-0008.

⁹ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2020-0008-l-lysine.pdf.

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

¹¹ Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2.a.

¹² Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2.d.

¹³ Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2.c.

(streptomycin was assessed by agar dilution method).¹⁴ All the minimum inhibitory concentration (MIC) values were lower than the corresponding cut-off values except for streptomycin which was exceeded by three dilutions (64 mg/L vs. 8 mg/L). Therefore, the strain is considered resistant to streptomycin.

The whole genome sequence (WGS) of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes

[REDACTED]

genes of concern were identified.

3.1.1.1. Information related to the genetically modified microorganism

Description of the genetic modification

[REDACTED]

3.1.1.2. Manufacturing process

L-Lysine is produced by fermentation using *C. glutamicum* KCCM 80216.¹⁶

[REDACTED]

3.1.1.3. 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

¹⁴ Technical dossier/Section II/Annexes Section II/Annex 2.2.2.2.

¹⁵ Technical dossier/Section II/Annexes Section II/Annex 2.2.1.2.b and c.

¹⁶ Technical dossier/Section II/Annexes Section II/Annex 2.3.1.a.

¹⁷ Technical dossier/Section II/Annexes Section II/Annex 2.3.1.b.

Substances (EINECS) No 200-294-2, has a molecular weight of 146.2 g/mol. The molecular formula is $C_6H_{14}N_2O_2$. The molecular structure is given in Figure 1.

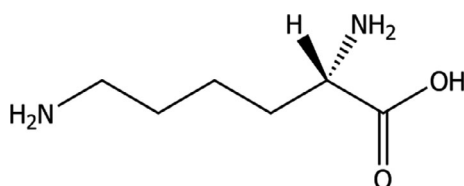


Figure 1: Molecular structure of L-lysine

The product is specified to contain $\geq 50\%$ L-lysine and $\leq 48\%$ water. Compliance with the specification was shown in five batches in which lysine was on average 51.6% (range 51.0–52.3%) on 'as is' basis and moisture was on average 46.5% (range 45.5–47.1%).¹⁸ The amount of unidentified material was on average 1.9% (1.1–2.8%).

3.1.3.1. Impurities

Five batches were analysed for undesirable substances. Specifications are set for lead (< 10 mg/kg), cadmium (< 1 mg/kg), arsenic (< 2 mg/kg) and mercury (< 0.1 mg/kg). Compliance with the specifications was demonstrated in five batches tested.¹⁹ Analysis in three of these batches showed that arsenic and cadmium concentrations were below their corresponding limits of detection (LOD), those of lead ranged from $< LOD$ to 0.03 mg/kg and of mercury were 0.003 mg/kg (in all batches tested).²⁰ Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) and dioxin-like polychlorinated biphenyls (PCBs) levels were below the corresponding limits of quantification (LOQ) in three batches of the additive.²¹ The levels of dioxins and the sum of dioxins and dioxin-like-PCBs (upper bond) were calculated to be 0.14 ng WHO-PCDD/F-TEQ/kg and 0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. Regarding the mycotoxin content, the analysis of those batches showed that the levels of aflatoxin (not specified), ochratoxin A, zearalenone, fumonisins (B1, B2, B3) and deoxynivalenol were below the LOD. Citrinin ranged from 112 to 163 $\mu\text{g}/\text{kg}$.²²

Regarding the microbiological contamination, the specifications are as follows: yeast and filamentous fungi $< 10^3$ colony-forming units (CFU)/g, *Salmonella* spp. not detected in 25 g sample and *Escherichia coli* not detected in 1 g sample. Five batches of the additive analysed complied with the specifications.²³ Enterobacteriaceae were analysed in three of these batches and not detected in any of the batches tested.

The presence of viable cells of the production strain in the final additive was investigated in three batches of concentrated liquid L-lysine (base).²⁴

[REDACTED]

No viable cells of the production strain were found in three independent batches of the final product concentrated liquid L-lysine (base).

The presence of DNA from the production strain was tested in three batches of concentrated liquid L-lysine (base), each tested in triplicate.²⁵

¹⁸ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 f-j. Lysine analysed following the method described in Section 2.2.56 (method I) of the European Pharmacopoeia 9th edition.

¹⁹ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 f-j.

²⁰ Technical dossier/Supplementary information August 2020/Annex 2.1.4.b. LOD in mg/kg were 0.01 for arsenic and lead, 0.002 for cadmium.

²¹ Technical dossier/Section II/Annexes Section II/Annex 2.1.4 f-h.

²² Technical dossier/Supplementary information August 2020/Annex 2.1.4.b. LOD in $\mu\text{g}/\text{kg}$ were 1.7 for aflatoxin B1, 5 for ochratoxin A, 17 for zearalenone, 25 for fumonisins, 134 for deoxynivalenol and 15 for citrinine.

²³ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 f-j and Supplementary information August 2020/Annex 2.1.4.b and SIn_220720 Answers 80216 200814/Reply to question 10. LOD was 1 CFU/g for *E. coli*, Enterobacteriaceae, yeasts and moulds.

²⁴ Technical dossier/Spontaneous information October 2020/Annex 2.

²⁵ Technical dossier/Section II/Annexes Section II/Annex 2.1.4.I.

No DNA of the production strain was detected.

3.1.3.2. Physical characteristics

The additive is an odourless, dark brown liquid.²⁶ The specific gravity (at 20°C) was measured in five batches of the additive and was 1,132 kg/m³ (range 1,131–1,133 kg/m³).¹⁹ The pH (measured in five batches) was on average 10.4 (range 10.3–10.5).²⁷

3.1.3.3. Stability and homogeneity

No data were provided on the shelf-life or on the stability in feedingstuffs of the concentrated liquid L-lysine (base) under assessment. The technical dossier contains data on the shelf-life of nine batches of concentrated liquid L-lysine (base) (production strains are unknown) when stored in closed containers at 20 and 25°C.²⁸ The recovery after 12 months was nearly complete.

The applicant provided a study to investigate if concentrated liquid L-lysine (base) (one batch) produced by a different *C. glutamicum* strain can be distributed homogeneously in a compound feed for chickens for fattening.²⁹ The compound feed was based on wheat, soybean meal and maize and contained a total content of lysine of 9.8%. The supplemental lysine was not described. Ten subsamples were analysed for supplemental lysine and the coefficient of variation (CV) calculated was 8%. As the manufacturing process, the composition and characteristics of the concentrated liquid L-lysine (base) tested were similar to the concentrated liquid L-lysine (base) under assessment, the FEEDAP Panel considers that the results of this study in compound feed for chickens for fattening can be used to support that the concentrated liquid L-lysine (base) under assessment can be distributed homogeneously in compound feed.

3.1.4. Characterisation of L-lysine monohydrochloride (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 CAS No 657-27-2, 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 chemical formula is C₆H₁₅ClN₂O₂. The structural formula is given in Figure 2.

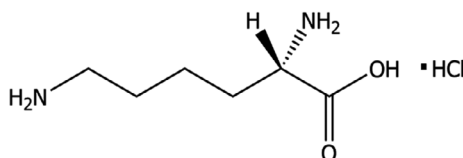


Figure 2: Molecular structure of L-lysine HCl

The specifications are for an additive containing $\geq 99\%$ L-lysine HCl on dry matter (DM) basis and $\leq 1\%$ water.

The applicant provided data of five batches of the additive.^{30,31} Lysine HCl was on average 99.2% (range 99.1–99.3%) on DM basis, loss on drying was on average 0.2% (range 0.2–0.4%) and ash content was on average 0.15% (0.08–0.2%). The values are in compliance with the specifications. The content of lysine in those five batches was on average 78.6% (range 78.3–79.1%) on DM basis.^{32,33} The amount of unidentified material was $< 1\%$ on DM basis.

²⁶ Technical dossier/Section II/Annexes Section II/Annex 2.5.2.b.

²⁷ Technical dossier/Supplementary information August 2020/Annex 1.a-e.

²⁸ Technical dossier/Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to question 3; letter of access AMAC FEFANA 200812 and Section II identity L-lysine AMAC dossier.

²⁹ Technical dossier/Section II/Annexes Section II/Annex 2.4.2.b and 2.4.2.c.

³⁰ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 a-e. Lysine analysed following the method described in section 2.2.56 (method I) of the European Pharmacopoeia 9th edition and Lysine HCl was calculated by stoichiometry.

³¹ Technical dossier/Supplementary information August 2020/Annex 2.1.3.d reissued.

³² Technical dossier/Section II/Annexes Section II/Annex 2.1.4.a.

³³ Technical dossier/Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to question 8.

The specific optical rotation of those batches was analysed by the Food Chemical Codex method and it was on average + 21.1° (range + 21.0 to + 21.3°) (reference values + 18 to + 21.5°).^{34, 31} This confirms the L-enantiomer of lysine in the additive.

3.1.4.1. Impurities

Five batches were analysed for undesirable substances. Specifications are set for lead (< 10 mg/kg), cadmium (< 1 mg/kg), arsenic (< 2 mg/kg) and mercury (< 0.1 mg/kg). Compliance with the specifications was demonstrated in five batches tested.^{34,31} Analysis in three of these batches showed that arsenic, lead and cadmium concentrations were below their corresponding LOD, and those of mercury ranged 0.007–0.008 mg/kg.³⁵ PCDD/FPCBs levels were below the corresponding LOQ in three batches of the additive.³⁶ The levels of dioxins and the sum of dioxins and dioxin-like-PCBs (upper bond) were calculated to be 0.14 ng WHO-PCDD/F-TEQ/kg and 0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. Regarding the mycotoxin content, the analysis of those batches showed that the levels of aflatoxins (not specified), ochratoxin A, zearalenone, fumonisins (B1, B2, B3) and deoxynivalenol were below the LOD. Citrinin levels were 32.5, 35 and < 15 µg/kg in the three batches tested, respectively.³⁷

Regarding the microbiological contamination, the specifications are as follows: yeast and filamentous fungi < 10³ CFU/g, *Salmonella* spp. not detected in 25 g sample and *E. coli* not detected in 1 g sample.³⁸ Five batches of the additive analysed complied with the specifications. Enterobacteriaceae were analysed in three of these batches and not detected in any of the batches tested.

The presence of viable cells of the production strain in the final additive was investigated in three batches of L-lysine HCl.³⁹

No viable cells of the production strain were found in three independent batches of the final product L-lysine HCl.

The presence of DNA from the production strain was tested in three batches of L-lysine HCl, each tested in triplicate.⁴⁰

No DNA of the production strain was detected.

3.1.4.2. Physical characteristics

The additive is an odourless, beige to brown crystalline powder with a solubility in water of about 600 g/L (at 20°C).⁴¹ The average bulk density analysed in five batches was 658 kg/m³ (range from 630 to 690 kg/m³).^{31,34}

The dusting potential was analysed (Stauber-Heubach method) in three batches of the final product.⁴² The values ranged from 2.7 to 3.1 g/m³. The particle size distribution of three batches of the final product was measured (laser diffraction).⁴³ The fractions of particles with diameters < 50 µm and < 100 µm ranged 2–4% and 4–6%, respectively.

³⁴ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 a-e.

³⁵ Technical dossier/Section II/Annexes Section II/Annex 2.1.4 a. LOD in mg/kg were 0.01 for arsenic and lead, 0.002 for cadmium.

³⁶ Technical dossier/Section II/Annexes Section II/Annex 2.1.4 c1-e1.

³⁷ Technical dossier/Section II/Annexes Section II/Annex 2.1.4 a. LOD in µg/kg were 1.7 for aflatoxins, 5 for ochratoxin A, 17 for zearalenone, 25 for fumonisins, 134 for deoxynivalenol and 15 for citrinine.

³⁸ Technical dossier/Section II/Annexes Section II/Annex 2.1.3 a-e and 2.1.4.a and Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to question 10. LOD was 1 CFU/g for *E. coli*, Enterobacteriaceae, yeasts and moulds.

³⁹ Technical dossier/Spontaneous information October 2020/Annex 1.

⁴⁰ Technical dossier/Section II/Annexes Section II/Annex 2.1.4.k.

⁴¹ Technical dossier/Section II/Annexes Section II/Annex 2.5.2.a.

⁴² Technical dossier/Section II/Annexes Section II/Annex 2.1.5 a-c.

⁴³ Technical dossier/Section II/Annexes Section II/Annex 2.1.4 c2-e2.

3.1.4.3. Stability and homogeneity

No data were submitted on the shelf-life of L-lysine HCl under assessment. The technical dossier contains data on 10 batches of L-lysine HCl (origin not described) showing losses of 0.6% of its initial lysine content when stored in the original package under ambient conditions (20–25 °C) for 24 months.⁴⁴

The applicant submitted a study on the stability of L-lysine HCl (one batch) produced by a different *C. glutamicum* production strain in a premixture and in a compound feed for pigs.⁴⁵ A vitamin premixture was supplemented with 10% L-lysine HCl, packed in sewed lined paper bags and stored at ambient temperature (18–35°C) for 3 months. At the end of the storage period, the L-lysine loss observed was of 2.5%. The FEEDAP Panel notes that the storage period tested is only half of the one requested in the corresponding guidance.

The premixture described above was used to produce a mash and pelleted compound feed for pigs. A basal diet consisting of maize and dehulled soya beans after oil extraction was supplemented up to a level of 0.4% L-lysine HCl. Pelleting was performed at 83 °C and it represented a loss of 2% lysine. Samples of mash and pelleted feed were packed in paper bags and stored at 18–35°C for 3 months. At the end of the storage period, no loss was detected in mash feed and a loss of 4% was observed in the pelleted feed. As the manufacturing process and the composition and characteristics of the L-lysine HCl tested were similar to the L-lysine HCl under assessment, the FEEDAP Panel considers that the results of the stability study in the vitamin premixture and in the compound feed for pigs can be used to support the stability of the L-lysine HCl under assessment.

The stability of three batches of the L-lysine HCl under assessment in water was studied at a concentration of 3 g/L water for drinking when stored for 48 h.³² No losses were detected.

The applicant submitted a study showing that L-lysine HCl (one batch) produced by a different *C. glutamicum* strain can be distributed homogeneously in a compound feed for chickens for fattening.⁴⁶ The compound feed was based on wheat, soybean meal and maize and contained a total content of lysine of 9.8%. The supplemental lysine was not described. Ten subsamples were analysed for supplemental lysine and the CV calculated was 9%. As the manufacturing process, the composition and characteristics of the L-lysine HCl tested were similar to the L-lysine HCl under assessment, the FEEDAP Panel considers that the results of this study in the compound feed for chickens for fattening can be used to support that the L-lysine HCl under assessment can be distributed homogeneously in compound feed.

3.1.5. Conditions of use

The additives are intended to be used in feed for all animal species. Both forms of the additive can be added directly to the feed, complementary feed or via premixture. The liquid form is not foreseen for addition via premixtures. No 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, as well as the amino acid composition of the unsupplemented diet.

3.2. Safety of L-lysine HCl and concentrated liquid L-lysine (base)

3.2.1. Safety aspects of the production organism

The production organism *C. glutamicum* KCCM 80216 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 genes inserted during the genetic modification do not raise safety concerns and the production strain does not carry acquired antimicrobial resistance genes. [REDACTED]

[REDACTED] 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.

⁴⁴ Technical dossier/Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to question 13; letter of access AMAC FEFANA 200812 and Section II identity L-lysine AMAC dossier.

⁴⁵ Technical dossier/Section II/Annexes Section II/Annex 2.4.1b and Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to questions 11, 12, 13.

⁴⁶ Technical dossier/Section II/Annexes Section II/Annex 2.4.2.a and 2.4.2.c and Supplementary information August 2020/SIn_220720 Answers 80216 200814/Reply to question 15.

3.2.2. Safety for the target species, consumer and environment

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 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 80216 belongs to a species, *Corynebacterium glutamicum*, which is eligible for the QPS approach (EFSA, 2007b) when used for production purposes (EFSA BIOHAZ Panel, 2020). Consequently, no safety concerns for target animal, 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 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 these additives in animal nutrition would not lead to any localised increase in its concentration in the environment and do not represent a risk to the environment.

The FEEDAP Panel concludes that both forms of L-lysine produced by *C. glutamicum* KCCM 80216 are safe for the target species, for the consumer and for the environment.

3.2.3. Safety for user

No studies were submitted to assess the safety of the additives under assessment for the user. The applicant has submitted studies on the safety for the user performed with concentrated liquid L-lysine (base) and L-lysine HCl originating from *C. glutamicum* KCTC 12307BP. Those studies have already been assessed in a previous opinion (EFSA FEEDAP Panel, 2019a). As the production strain is considered safe, the manufacturing process and the composition and characteristics of the concentrated liquid L-lysine (base) and L-lysine HCl under assessment are the same as of those assessed in the above-mentioned opinion, the FEEDAP Panel considers that the results of the studies performed with concentrated liquid L-lysine (base) and L-lysine HCl produced by *C. glutamicum* KCTC 12307BP can be used to support the safety for the user for the additives under assessment.

3.2.3.1. Concentrated liquid L-lysine (base)

Effects on skin and eyes

From an acute dermal irritation study performed in rabbits in accordance with OECD Guideline 404, compliant with good laboratory practice (GLP), concentrated liquid L-lysine (50%) produced by *C. glutamicum* KCTC 12307BP appeared not to be a skin irritant and had no corrosive effect on skin.⁴⁷

From an acute eye irritation study in rabbits, in accordance with OECD Guideline 405, GLP compliant, concentrated liquid L-lysine (50%, pH 9.95) produced by *C. glutamicum* KCTC 12307BP was found not to be an eye irritant.⁴⁸

From a skin sensitisation study in guinea pigs in accordance with OECD Guideline 406 (Guinea pig maximisation test), GLP compliant, the concentrated liquid L-lysine (50%) produced by *C. glutamicum* KCTC 12307BP was found not to be a skin sensitiser.⁴⁹

3.2.3.2. L-Lysine HCl

Effects on the respiratory system

The dusting potential of the additive under assessment may be up to 3.1 g/m³, and the fractions of particles with diameters < 50 µm and < 100 µm may range 2–4% and 4–6%, respectively.

⁴⁷ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.b.

⁴⁸ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.d.

⁴⁹ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.f.

From a previous acute inhalation toxicity study in accordance with OECD Guideline 436, GLP compliant, L-lysine HCl (99.4% pure) produced by *C. glutamicum* KCTC 12307BP showed an inhalation median lethal dose > 5.1 mg/L air and the test item required no classification.⁵⁰

Effects on skin and eyes

From an acute dermal irritation/corrosion study performed according to OECD Guideline 404, GLP compliant, L-lysine HCl (99.5% pure) produced by *C. glutamicum* KCTC 12307BP had no irritant effect on the skin.⁵¹

From an acute eye irritation/corrosion test performed in accordance with OECD Guideline 405, GLP compliant, L-lysine HCl (99.5% pure) produced by *C. glutamicum* KCTC 12307BP was found not to be an eye irritant.⁵²

From a skin sensitisation study in guinea pigs in accordance with OECD Guideline 406 (Guinea pig maximisation test), GLP compliant, L-lysine HCl (99.5%) produced by *C. glutamicum* KCTC 12307BP was found not to be a skin sensitiser.⁵³

3.2.3.3. Conclusions on safety for the user

Neither the concentrated liquid L-lysine (base) nor the L-lysine HCl are irritant to skin or eyes and they are not skin sensitisers. L-lysine HCl is not hazardous by inhalation.

3.3. Efficacy of concentrated liquid L-lysine (base) and L-lysine HCl

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 (EFSA FEEDAP Panel, 2013, 2014). 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 Regulation⁵⁴ and Good Manufacturing Practice.

4. Conclusions

Both forms of L-lysine, concentrated liquid L-lysine (base) and L-lysine HCl, are produced by the genetically modified strain *C. glutamicum* KCCM 80216. Neither the production strain nor its recombinant DNA was detected in the final products. The additives do not pose any safety concern associated with the genetic modification of the production strain.

Concentrated liquid L-lysine (base) and L-lysine HCl produced by the strain *C. glutamicum* KCCM 80216 do not represent a risk for the target species, consumers, the user and for the environment.

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
24/01/2020	Dossier received by EFSA. L-lysine monohydrochloride and concentrated liquid L-lysine (base) for all animal species. Submitted by Daesang Europa B.V
06/02/2020	Reception mandate from the European Commission
03/06/2020	Application validated by EFSA – Start of the scientific assessment

⁵⁰ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.1.

⁵¹ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.a.

⁵² Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.c.

⁵³ Technical dossier/Section III/Annexes Section III/Annex 3.3.1.2.e.

⁵⁴ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Date	Event
22/07/2020	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, safety for the user</i>
18/08/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
31/08/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
03/09/2020	Comments received from Member States
12/10/2020	Reception of spontaneous information from the applicant
18/11/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony-forming units
FCC	Food Chemical Codex
FEEDAP	the Panel on Additives and Products or Substances used in Animal Feed
GLP	good laboratory practice
LOD	limits of detection
LOQ	limits of quantification
PCBs	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
QPS	qualified presumption of safety
WGS	whole genome sequence

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 concentrated liquid L-lysine (base) produced by *Corynebacterium glutamicum* KCCM 80216

In the current application, authorisation is sought under Article 4(1) for *L-lysine monohydrochloride* and *concentrated liquid L-lysine produced by fermentation with Corynebacterium glutamicum* KCCM 80216 (LU13544), 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) of 99%, while the *concentrated liquid L-lysine* contains at least 50% of *L-lysine* and a maximal water content of 48%.

The two forms of the *feed additive* are intended to be added directly into *feedingstuffs* or through *premixtures* (for *L-lysine monohydrochloride*). 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 (RSD_r) ranging from 0.7 to 1.7% and a relative standard deviation for reproducibility (RSD_R) 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*.

For the quantification of *lysine* in *premixtures* and *feedingstuffs*, 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*: RSD_r ranging from 2.1 to 2.8% and RSD_R ranging from 3.0 to 6.7%.

In the frame of this authorisation, 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 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*) and (iii) the European Union method based on IEC-VIS for the quantification of *lysine* in *premixtures* and *feedingstuffs*.

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.