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# Safety and efficacy of concentrated liquid L-lysine (base) and L-lysine monohydrochloride produced by fermentation with *Corynebacterium casei* KCCM 80190 as feed additives for all animal species

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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 casei* KCCM 80190 when used as nutritional additives in feed and water for drinking for all animal species. The active substance is L-lysine. The production strain is genetically modified. It does not carry acquired antimicrobial resistance genes and no viable cells of the production strain nor its DNA were detected in the final products. Therefore, the additives do not pose any safety concern regarding the genetic modifications. Concentrated liquid L-lysine (base) and L-lysine HCl produced by *C. casei* KCCM 80190 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. Concentrated liquid L-lysine (base) produced by *C. casei* KCCM 80190 is considered hazardous by inhalation, not irritant to skin and eyes and it is not a skin sensitiser. L-Lysine HCl produced by *C. casei* KCCM 80190 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 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.



**Keywords:** nutritional additive, 'amino acids, their salts and analogues', concentrated liquid L-lysine (base), lysine monohydrochloride, *Corynebacterium casei*, Safety, efficacy

**Requestor:** European Commission

**Question number:** EFSA-Q-2019-00195

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# 1. Introduction

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

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

The European Commission received a request from CJ Europe GmbH[2] for authorisation of the products concentrated liquid L-lysine (base) and L-lysine monohydrochloride, when used as feed additives 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 7 May 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 concentrated liquid L-lysine (base) and L-lysine monohydrochloride, produced by fermentation using *Corynebacterium casei* KCCM 80190 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

The additives under assessment are concentrated liquid L-lysine (base) and L-lysine monohydrochloride, produced by fermentation using *C. casei* KCCM 80190. 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, 2007; EFSA FEEDAP Panel, 2015b, 2016b, 2017a, 2019a,–d, 2020a,b); and other opinions 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).

L-Lysine produced using other microorganisms and/or 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] cosmetics[5] and as a veterinary medicinal product.[6,7]

L-Lysine hydrochloride is described in a monograph (01/2008:0930) of the European Pharmacopoeia (PhEur 10th edition, 2019).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] CJ Europe GmbH. Unterschweinstiege 2 – 14, 60549 Frankfurt am Main, Germany.
[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 (EU) 2019/701 of 5 April 2019 establishing a glossary of common ingredient names for use in the labelling of cosmetic products. OJ L 128, 8.5.2019, p 1–370.
[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. 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[8] in support of the authorisation request for the use of concentrated liquid L-lysine (base) and L-lysine monohydrochloride (HCl) as 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 the concentrated liquid L-lysine (base) and L-lysine HCl produced using *C. casei* KCCM 80190 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 concentrated liquid L-lysine (base) and L-lysine HCl is in line with the principles laid down in Regulation (EC) No 429/2008[10] 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) or monohydrochloride (HCl) produced by fermentation with a genetically modified strain of *C. casei*. 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 microorganism

The additive is produced by a genetically modified strain of *C. casei* which is deposited [redacted] with accession number KCCM 80190.[11] A bioinformatic analysis of the whole genome sequence (WGS) [redacted] of the production strain KCCM 80190 confirmed its identity as *C. casei*. [redacted]

The susceptibility of the production strain to relevant antibiotics was tested [redacted] All measured minimum inhibitory concentration (MIC) values were lower than or equal to the cut off values specified in such guidance.

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

[8] FEED dossier reference: FAD-2019-0014.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2019-0014-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.

[redacted]

[redacted] No relevant hits were identified.

*Characterisation of the recipient or parental microorganism*

[redacted]

*Description of the genetic modification*

[redacted]

### 3.1.2. Manufacturing process

[redacted]

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

L-Lysine (International Union of Pure and Applied Chemistry (IUPAC) name (2*S*)-2,6 diaminohexanoic acid; synonym (S)-2,6-diaminocaproic acid) is a compound identified with the Chemical Abstracts Service (CAS) 56−87−1 and the EC-No 201−300−6. It has a molecular weight of 146.2 g/mol and the molecular formula is $C_6H_{14}N_2O_2$. The molecular structure is given in Figure 1.

[redacted]

[17] Technical dossier/Section II/Annex Conf II.2.3.
[18] Technical dossier/Supplementary information April 2020/Annex/Annex_SIN_CONFID_05.
[19] Technical dossier/Section II.3.
[20] Technical dossier/Section II/Annex Conf II.3.19.

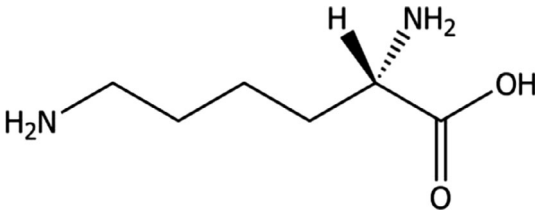


**Figure 1:** Molecular structure of L-lysine

The product is specified to contain ≥ 50% lysine 'as is' and ≤ 50% water. Compliance with the specification was shown in five batches in which L-lysine was on average 50.2% on 'as is' basis (range 50.1–50.2%). Water content was 47.2% (range 46.8–47.8%).[21]

Five additional batches were analysed and showed an average content of lysine of 51.4% (range 50.1–51.8%) 'as is', 46.7% water (range 46.2–47.1%), 0.9% chloride, 0.17% sulfate, 0.14% potassium, 0.1% of free amino acids other than lysine (glutamic acid, alanine, isoleucine and arginine), 0.07% sodium and 0.03% ammonium. Ash was on average 0.38% (range 0.35–0.40%).[22] Total identified material on 'as is' basis was on average 99.6% (range 99.2–99.9%). Total identified material on dry matter basis was on average 99.23% (range 98.5–99.9%).

### 3.1.3.1. Impurities

Two sets of three batches of the additive were analysed for undesirable substances in two different laboratories (one set in each lab).[23] Levels of heavy metals (cadmium, lead, mercury) and arsenic were below the limit of detection (LOD).[24] Microbial contaminants were either absent in 25 g (*Salmonella* spp.), reported as negative (*Escherichia coli*, coliforms), below the specification ($< 1 \times 10^3$ CFU/g for total plate count and < 50 CFU/g for yeast and moulds) or below the LOD (Enterobacteriaceae).[25] Mycotoxins (aflatoxins B1, B2, G1, G2; ochratoxin A, zearalenone, deoxynivalenol (DON) and fumonisins B1, B2) were found below the respective limits of detection.[26] Pesticides (358 species) were analysed in three batches of concentrated liquid L-lysine (base) and found below the LOD of the analytical method.[27]

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

The absence of viable cells of the production strain was tested in three batches of concentrated liquid L-lysine (base). [redacted] No colonies were detected.

The presence of DNA from the production strain in the final product was tested in three batches of concentrated liquid L-lysine (base) [redacted] No DNA from the production strain was detected in the samples.

[21] Technical dossier/Section II/Annex II.1.5 and Supplementary information April 2020/Document/03_SIN CJ L-lys 80190 20200423. Analytical method to measure lysine was HPCL with refractive index detector.
[22] Technical dossier/Section II/Annex II.1.8.
[23] Technical dossier/Section II/Annex II.1.9 and Supplementary information April 2020/Annex SIN_05.
[24] From the analysis submitted, LOD (in μg/kg) were (depending on the laboratory making the analyses): 1 and 0.015 for lead, 1 and 0.01 cadmium, 1 and 0.04 for arsenic and 5 and 0.01 for mercury (Technical Dossier/Section II/Annex II.1.9 and Technical dossier/Supplementary information April 2020/Annex SIN_05).
[25] Technical dossier/Supplementary information April 2020/Document/03 Sin L-lys 80190 20200423. LOD in CFU/g was 100 for total plate count, 10 for yeasts and molds, and 1 for *E. coli* and coliform bacteria. The method used Is according to the Korean Food Standards Codex (Annex II.1.9). LOD for *Enterobacteriaceae*, *E. coli*, yeasts and moulds was 10 CFU/g (Annex SIN_5).
[26] 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 (Annex II.1.9); and 0.2 for aflatoxins, 0.5 for ochratoxin A, 5 for zearalenone and 10 for deoxynivalenol (Annex SIN_05).
[27] Technical dossier/Section II/Annex II.1.11. LOD ranged from 0.5 to 8 μg/kg depending on the pesticide considered.
[redacted]

#### 3.1.3.2. Physical characteristics

Concentrated liquid L-lysine base is presented as a dark brown liquid product of a particular odour. It is highly soluble in water. It has a pH ranging from 9 to 11, a bulk density of 1,120–1,170 kg/m$^3$ and a viscosity ranging from 73 to 87 cp at 25°C.[30]

#### 3.1.3.3. Stability and homogeneity

The shelf-life of three batches of concentrated liquid L-lysine (base) under assessment was studied when stored in aluminium vacuum bags at 25°C for 18 months and at 40°C for 6 months.[31] No losses were observed.

The stability of concentrated liquid L-lysine (base) under assessment (three batches) was studied in water for drinking at a concentration of 0.1% (corresponding to 0.05% lysine), at 25 and 40°C for 48 h.[32] Losses ranged from 0% to 4% at 25°C and from 0% to 5% at 40°C, depending on the batch considered.

The studies provided on the stability in premixtures and in mash feed for chicken for fattening and on the capacity of the additive to distribute homogeneously in premixtures, mash and pelleted feed of chicken for fattening were not performed with the additive under assessment but with concentrated liquid L-lysine (base) produced by a different species (*C. glutamicum* KCCM 10227). Those studies had been assessed in a previous opinion of the FEEDAP Panel, and the results were described as follows: 'Losses ranged from 0% to 4.2% in the premixtures, depending on the batch considered; losses in mash feed (6%) were observed in only one batch; regarding the homogeneous distribution in feed, the coefficients of variation were of 3% in the premixture, 13% in the mash feed and 4% in the pelleted feed' (EFSA FEEDAP Panel, 2019a–f). As the production process is the same and the product characteristics in terms of composition and physical properties are very similar,[33] 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 monohydrochloride

L-Lysine HCl (IUPAC name: (2*S*)-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 European Inventory of Existing Commercial chemical Substances (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 2.

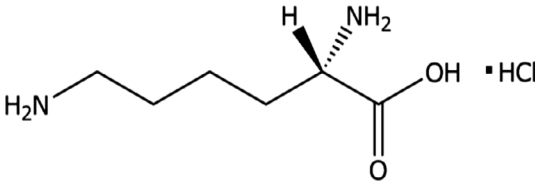


**Figure 2:** Molecular structure of L-lysine HCl

The specification is for an additive containing ≥ 78% L-lysine on 'as is' basis, ≤ 1% water and ≤ 0.3% ash.

The applicant provided data of five batches of the additive. The average content of lysine was 79.5% (range 79.4–79.6%) on 'as is' basis. Loss on drying was on average 0.16% (range 0.14–0.18%).[34]

Analytical data of five additional bathes showed an average of lysine of 79.6% (range 79.5–79.6%) on 'as is' basis; chloride average was 19.5% (range 19.4 –19.6%); loss on drying was 0.10% (range 0.07–0.12%); ash was on average 0.04% (range 0.02–0.06%); sulfate was 0.02%; sodium, potassium and ammonium 0.01% each. The amount of identified material on dry matter basis was on average 99.2% (range 99.1–99.2%).[35]

[30] Technical dossier/Section II/Annex II.1.14.
[31] Technical dossier/Section II/Annexes II.4.1a and b.
[32] Technical dossier/Section II/Annex II.4.4.
[33] Technical dossier/Supplementary information April 2020/Document/03_SIN_Lys.
[34] Technical dossier/Section II/Annex II.1.4 and Supplementary information December 2019/Annex 3. Method of analysis of lysine was HPCL using refractive index detector.
[35] Technical dossier/Section II/Annex II.1.7.

The specific optical rotation was measured in three batches (European Pharmacopoeia method 2.2.7) and ranged from +21.5 to +21.7°. 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.

#### 3.1.4.1. Impurities

Two sets of three batches of the additive were analysed for undesirable substances in two different laboratories (one set in each lab).[36] Levels of heavy metals (cadmium, lead, mercury) and arsenic were below the LOD.[37] In the second data set, the sum of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), the dioxin-like polychlorinated biphenyls (PCB) and the non-dioxin like PCBs were also below the LOD.[38] Microbial contaminants were either absent in 25 g (*Salmonella* spp.), reported as negative (*E. coli*, coliforms) below the specification ($< 1 \times 10^3$ CFU/g for total plate count and < 50 CFU/g for yeast and moulds) or below the LOD (Enterobacteriaceae).[25] Mycotoxins (aflatoxins B1, B2, G1, G2; ochratoxin A, zearalenone, DON and fumonisins B1, B2) were found below the respective limits of detection.[39] Pesticides (358 species) were analysed in three batches of L-lysine HCl and found below the LOD.[40]

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

For confirming the absence of viable cells of the production strain samples were taken from [redacted] No colonies were detected.

For confirming the absence of recombinant DNA of the production strain samples were taken from [redacted] No DNA from the production strain was detected in the samples.

#### 3.1.4.2. Physical characteristics

L-Lysine HCl is presented as a pale brownish free flowing solid crystalline powder of characteristic odour. It has a bulk density of 550–750 kg/m$^3$, a solubility in water of 642 g/L (at 30°C). Its dusting potential (Stauber–Heubach method, three batches analysed) ranged from 0.46 to 0.69 g/m$^3$.[43] There is no information on the particle size distribution for fractions < 100, 50 and 10 μm diameter. Data obtained by sieving (three batches analysed) indicate that the fraction < 250 μm diameter ranged from 11% to 21% (w/w).[44]

#### 3.1.4.3. Stability and homogeneity

The shelf-life of three batches of L-lysine HCl under assessment was studied when stored in aluminium vacuum bags at 25°C for 18 months and at 40°C for 6 months.[31] No losses were observed.

The stability of L-lysine HCl under assessment (three batches) was studied in water for drinking at a concentration of 0.063% (corresponding to 0.05% lysine), at 25 and 40°C for 48 h.[45] No losses were detected.

[36] Technical dossier/Section II/Annex II.1.7 and Supplementary information April 2020/Annex SIN_04.

[37] From the analysis submitted, LOD (in μg/kg) were: < 1 and < 0.015 for lead, < 1 and < 0.01 cadmium, < 1 and < 0.04 for arsenic and < 5 and < 0.01 for mercury (Technical Dossier/Section II/Annex II.1.7 and Technical dossier/Supplementary information April 2020/Annex SIN_04).

[38] Technical dossier/Supplementary information April 2020/Annex Sin 04. LOD in ng TEQ-WHO, 2005/kg additive were 0.06 for PCDD/F, 0.06 for PCBs and 0.53 for non-dioxin like PCBs.

[39] LOD in μg/kg was 0.1 for aflatoxins (B1, B2, G1, G2) and ochratoxin A, 0.5 for DON and 5 for fumonisins (Annex II.1.7); and < 0.2 for aflatoxins, < 0.5 for ochratoxin A, < 5 for zearalenone and < 10 for deoxynivalenol (Annex SIN_04).

[40] Technical dossier/Section II/Annex II.1.10. LOD ranged from 0.5 to 8 μg/kg depending on the pesticide considered.

[redacted]

[43] Technical dossier/Section II/Annex II.1.13.

[44] Technical dossier/Section II/Annex II.1.12.

[45] Technical dossier/Section II/Annex II.4.3.

No information on the stability in premixtures and feedingstuffs, and on the capacity to distribute homogeneously in feed of the additive under assessment was provided. The applicant provided studies on the stability in premixtures and in mash feed for chicken for fattening, as well as on the capacity of L-lysine HCl to distribute homogeneously in premixtures, mash and pelleted feed of chicken for fattening. 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 of the FEEDAP Panel, and the results were described as follows: 'No losses were observed in the premixtures; losses in mash feed ranged from 0% to 3% depending on the batch considered; and regarding the homogeneous distribution in feed, the coefficients of variation were of 6% in the premixture, 8% in the mash feed and 2% in the pelleted feed for chicken for fattening' (EFSA FEEDAP Panel, 2019a–f). As the production process is the same and the product characteristics in terms of composition and physical properties are very similar,[46] 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 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 feedingstuffs or via premixture. Both forms of the additive are also proposed for use in water for drinking.[47] 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, as well as the amino acid content of the unsupplemented diet.

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

### 3.2.1. Safety of the production microorganism

[redacted] The production strain does not carry acquired antimicrobial resistance genes and no viable cells of the production strain nor its DNA were detected in the final products. Therefore, the additives do not pose any safety concern regarding the genetic modification of the production strain.

### 3.2.2. Safety for the target species, consumers and the environment

Both forms of the additive are highly purified (> 99% on a dry matter (DM) basis) and are produced by fermentation using a strain that is considered safe. Concerns from the use of the additive would not derive from the amino acid L-lysine, which is considered safe, but may arise from residues of the fermentation process/production strain remaining in the final product. Since the products under assessment have a high purity and the unidentified fraction contributes with < 1% on DM basis, the production strain is susceptible to relevant antimicrobials used in human and veterinary medicine, and no viable cells/DNA of the production strain are in the final products, both forms of L-lysine produced by *C. casei* KCCM 80190 are considered safe for the target species provided that it is supplemented in appropriate amounts to the diets. 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 simultaneous oral administration of lysine-containing additives via feed and water for drinking.

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 use of both forms of the additive in animal nutrition does not raise safety concerns for the consumers.

The amino acid L-lysine is a physiological and natural component of the proteins of living organisms. When used in feed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass or excreted as such. The absorbed L-lysine will be incorporated into body

[46] Technical dossier/Supplementary information December 2019/Annex 3.
[47] Technical dossier/Supplementary information April 2020/Document/03 Sin CJ L-lys 80190 20200423.

proteins or excreted as urea/uric acid and as 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 any localised increase in the concentration of L-lysine or its metabolites in the environment.

The FEEDAP Panel concludes that both forms of L-lysine produced by *C. casei* KCCM 80190 are safe for the target species, for the consumer and for the environment.

### 3.2.3. Safety for the user

No studies were submitted to support the safety for the user using the additive under assessment as test item. The applicant submitted acute inhalation toxicity studies, *in vitro* eye irritation studies, *in vitro* skin irritation studies and skin sensitisation studies performed with L-lysine HCl or concentrated liquid L-lysine (base) produced by a different species of microorganism (*C. glutamicum* KCCM 10227). Those studies had been assessed in a previous opinion (EFSA FEEDAP Panel, 2019d). As the production strain is considered safe, the production process is the same and the product characteristics are very similar,[46] the FEEDAP Panel considers that the results of those studies can be applicable to the products under assessment.

#### 3.2.3.1. Concentrated liquid L-lysine (base)

**Effects on respiratory system**

From a previous acute inhalation toxicity study performed in accordance with OECD Guideline 403 and testing concentrated liquid L-lysine (base) produced using *C. glutamicum* KCCM 10227, it was concluded that the additive was hazardous by inhalation.[48]

**Effects on skin and eyes**

From an *in vitro* skin irritation test (Human Skin Model Test) performed according to OECD Guideline 439 and testing concentrated liquid L-lysine (base) produced using *C. glutamicum* KCCM 10227, the additive was classified as non-irritant for the skin.[49]

From an *in vitro* eye irritation test (Bovine Corneal Opacity and Permeability Assay) according to OECD Guideline 437 and testing concentrated liquid L-lysine (base) produced using *C. glutamicum* KCCM 10227, it was concluded that the additive was non-irritant to eyes.[50]

From a skin sensitisation study using local lymph node assay in the mouse, in accordance with OECD Guideline 429 and testing concentrated liquid L-lysine (base) produced using *C. glutamicum* KCCM 10227, it was concluded that the additive was not a skin sensitiser.[51]

#### 3.2.3.2. L-lysine HCl

**Effects on the respiratory system**

The dusting potential of the additive may be up to 0.7 g/m$^3$. Data obtained by sieving (three batches analysed) indicate that the fraction < 250 μm diameter ranged from 11% to 21% (w/w). The studies to support the safety for the user were performed with an L-lysine HCl (produced using *C. glutamicum* KCCM 10227) having a dusting potential up to 1.7 g/m$^3$; and the fraction of particles having a diameter < 100, 50 and 10 μm diameter were 0.49–1.9%, 0–0.2% and 0%, respectively.

In a previous acute inhalation toxicity study performed in accordance with OECD Guideline 403 and testing L-lysine HCl produced using *C. glutamicum* KCCM 10227,[52] persistent alterations were observed in almost all exposed animals at the end of the 14-day post-treatment observation period, indicating a potential for inhalation toxicity.

[48] Technical dossier/Section III/Annex III.3.5. Good Laboratory Practice (GLP)-compliant study.
[49] Technical dossier/Section III/Annex III.3.9. GLP-compliant study.
[50] Technical dossier/Section III/Annex III.3.7. GLP-compliant study.
[51] Technical dossier/Section III/Annex III.3.11. GLP-compliant study.
[52] Technical dossier/Section II/Annex III.3.4. GLP-compliant study.

**Effects on skin and eyes**

From an *in vitro* skin irritation test (Human Skin Model Test) according to OECD Guideline 439, testing L-lysine HCl produced using *C. glutamicum* KCCM 10227, it was concluded that the additive was non-irritant for the skin.[53]

From an *in vitro* eye irritation test (Bovine Corneal Opacity and Permeability Assay) according to OECD Guideline 437, testing L-lysine HCl produced using *C. glutamicum* KCCM 10227, no prediction on eye irritation could be made.[54] According to the irritant classification using the classification scheme adopted by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, 2006) the *in vitro* irritancy score obtained in the study (5.3) corresponded to mild irritation for the eyes.

From a skin sensitisation study using local lymph node assay in the mouse, in accordance with OECD Guideline 429, testing L-lysine HCl produced using *C. glutamicum* KCCM 10227, it was concluded that the additive was not a skin sensitiser.[55]

#### 3.2.3.3. Conclusions on safety for the user

Based on the results of studies on the safety for the user of L-lysine HCl and concentrated liquid L-lysine (base) produced by a different production strain it was possible to conclude on the safety for the user of the products under assessment.

Concentrated liquid L-lysine (base) produced by *C. casei* KCCM 80190 is considered hazardous by inhalation, not irritant to skin and eyes and it is not a skin sensitiser.

L-Lysine HCl produced by *C. casei* KCCM 80190 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 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, 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 Regulation[56] and Good Manufacturing Practice.

## 4. Conclusions

The production strain does not carry acquired antimicrobial resistance genes and no viable cells of the production strain nor its DNA were detected in the final products. Therefore, the additives do not pose any safety concern regarding the genetic modification of the production strain.

Concentrated liquid L-lysine (base) and L-lysine HCl produced by the strain *C. casei* KCCM 80190 do not represent a risk for the target species, for the consumer and for the environment.

Concentrated liquid L-lysine (base) produced by *C. casei* KCCM 80190 is considered hazardous by inhalation, not irritant to skin and eyes and it is not a skin sensitiser. L-Lysine HCl produced by *C. casei* KCCM 80190 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.

[53] Technical dossier/Section III/Annex III.3.8. GLP-compliant study.
[54] Technical dossier/Section III/Annex III.3.6. GLP-compliant study.
[55] Technical dossier/Section III/Annex III.3.10. GLP-compliant study.
[56] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

## 5. Documentation as provided to EFSA/Chronology

| Date | Event |
|---|---|
| 22/02/2019 | Dossier received by EFSA. L-Lysine HCl and concentrated liquid L-lysine (base) produced using *Corynebacterium casei* for all animal species. Submitted by CJ Europe GmbH |
| 20/03/2019 | Reception mandate from the European Commission |
| 07/05/2019 | Application validated by EFSA – Start of the scientific assessment |
| 08/07/2019 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the production strain, characterisation of the additive, conditions of use* |
| 07/08/2019 | Comments received from Member States |
| 06/09/2019 | Reception of the evaluation report of the European Union Reference Laboratory for feed additives |
| 29/04/2020 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 25/05/2020 | 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* |
| 19/06/2020 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 30/09/2020 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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## Abbreviations

| | |
|---|---|
| CAS | Chemical Abstracts Service |
| CFU | colony forming unit |
| CV | coefficient of variation |
| DON | deoxynivalenol |
| DM | dry matter |
| EURL | European Union Reference Laboratory |
| FCC | Food chemical codex |
| FEEDAP | Panel on additives and products or substances used in animal feed |
| IEC-VIS/FLD | Ion exchange chromatography coupled to visible or fluorescence detection |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MIC | minimum inhibitory concentration |
| PCB | polychlorinated biphenyls |
| PCDD/F | polychlorinated dibenzodioxins/dibenzofurans |
| RSDr | relative standard deviation for repeatability |
| RSDR | relative standard deviation for reproducibility |
| TEQ | toxic equivalents |
| VDLUFA | Association of German agricultural analytic and research institutes |
| WHO | World Health Organization |

# Annex A – Executive summary of the evaluation report of the European Union Reference Laboratory on the methods of analysis of L-lysine monohydrochloride and concentrated liquid L-lysine produced by fermentation with *Corynebacterium casei* KCCM 80190

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 Casei* KCCM80190, 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 98.5% (minimum of 78% of L-lysine), while the concentrated liquid L-lysine contains a minimum of 50% of L-lysine.

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

For the quantification of lysine in the feed additive the Applicant submitted a slightly modified protocol of the European Union method dedicated for the determination of amino acids in feed. However, the EURL previously evaluated lysine dossiers and recommended for the quantification of lysine in the feed additives and premixtures (containing more than 10% lysine) the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). This standard method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. It applies for products containing more than 10% of amino acid. The following performance characteristics 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.

For the quantification of lysine in premixtures and feedingstuffs the Applicant suggested using the ring-trial validated VDLUFA 4.11.6 method. However, the EURL previously evaluated lysine dossiers and recommended for the quantification of lysine in premixtures and feedingstuffs 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 to 6.7%.

In the frame of the stability studies the Applicant presented experimental data obtained analysing lysine in water with a slightly modified protocol of the VDLUFA 4.11.6 method based on IEC-VIS/FLD. The results presented are considered sufficient to demonstrate the suitability of the procedure for the analysis of the amino acid in water.

In the frame of this authorisation 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 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); (iii) the European Union method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; and (iv) the slightly modified VDLUFA 4.11.6 method based on IEC-VIS/FLD to quantify 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.