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Safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *Corynebacterium glutamicum* strain NRRL B-50775 for all animal species based on a dossier submitted by ADM

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Abstract

The European Commission asked EFSA for an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of a L-lysine monohydrochloride (HCl, minimum 98.5%) and of a concentrated liquid L-lysine (base, minimum 50%) produced by a genetically modified strain of *Corynebacterium glutamicum* (NRRL B-50775). They are intended to be used in feed or water for drinking for all animal species and categories. Neither the production strain *C. glutamicum* NRRL B-50775 nor its recombinant DNA was detected in the final product. Therefore, the product does not pose any safety concern associated with the genetic modification of the production strain. L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 are considered safe for the target species, for the consumer and for the environment. L-Lysine HCl produced by *C. glutamicum* NRRL B-50775 is considered not irritant to skin or eyes and not a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced by *C. glutamicum* NRRL B-50775. Concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775, due to its high pH (11) it is anticipated to be corrosive to skin and eyes and poses a risk by inhalation. L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 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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Summary

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 L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using a genetically modified *Corynebacterium glutamicum* strain (NRRL B-50775) for all animal species.

L-Lysine is an essential amino acid for all animal species. L-Lysine and its salts are widely used in the feed industry to optimise dietary protein.

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine monohydrochloride (HCl) and concentrated liquid L-lysine (base) was in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant EFSA guidance documents. The FEEDAP Panel used the data provided by the applicant together with data from other sources, to deliver the present output.

Neither the production strain *C. glutamicum* NRRL B-50775 nor its recombinant DNA was detected in the final product. Therefore, the product does not pose any safety concern associated with the genetic modification of the production strain.

L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 are considered safe for the target species, for the consumer and for the environment.

L-Lysine HCl produced by *C. glutamicum* NRRL B-50775 is considered not irritant to skin or eyes and not a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced by *C. glutamicum* NRRL B-50775.

Concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775, due to its high pH (11) it is anticipated to be corrosive to skin and eyes and poses a risk by inhalation.

L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 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

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 Archer Daniels Midland Company (ADM)² for authorisation of the product L-lysine monohydrochloride and concentrated liquid L-lysine (base), 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 28 July 2017.

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, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The active substance of the two products under application, L-lysine, is produced by a genetically modified strain of *Corynebacterium glutamicum* (NRRL B-50775).

L-Lysine is currently authorised for its use in all animal species as a nutritional additive.³ No maximum content in feedingstuffs is established in the European Union (EU). L-Lysine and L-lysine acetate are authorised for specific nutritional purposes in foods for particular nutritional uses.⁴ L-Lysine and its hydrochloride salt may be used in the manufacture of infant formulae and follow-on formulae in order to satisfy requirements on amino acids and other nitrogen compounds.⁵ Lysine hydrochloride is described in the European Pharmacopoeia (2016) monograph 01/2008:0930. Lysine is listed as pharmacologically active substance in veterinary medicinal products and is not subject to maximum residue limits when used in food-producing animals.⁶

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has published several opinions on the safety and efficacy of L-lysine: some of them on L-lysine sulphate produced by fermentation using different strains of *C. glutamicum* for all animal species (EFSA FEEDAP Panel, 2007, 2015a,b, 2016b) or using a strain of *Escherichia coli* (EFSA FEEDAP Panel, 2017) and others on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and/or L-lysine monohydrochloride for all animal species (EFSA FEEDAP Panel, 2013, 2014, 2015b, 2016a), produced by fermentation using different strains of *E. coli* or *C. glutamicum* (EFSA FEEDAP Panel, 2016b).

The Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) issued an opinion on L-lysine and its monohydrochloride salt when used as a flavouring compound (EFSA, 2008a,b, 2010). The Panel on Dietetic Products, Nutrition and Allergies (NDA) issued three opinions on the substantiation of health claims related to L-lysine (EFSA NDA Panel, 2011a,b, 2014). The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) issued an opinion on consideration of 88 flavouring substances considered by EFSA for which EU production

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 ADM Speciality Ingredients (Europe) B.V. Kingsfordweg 43-117, 1043 GP 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, 30.8.88, pp. 36–39.

⁴ Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, pp. 9–19.

⁵ Commission Regulation (EC) No 1243/2008 of 12 December 2008 amending Annexes III and VI to Directive 2006/141/EC as regards compositional requirements for certain infant formulae. OJ L 335, 25, 13.12.2008, p. 18.

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, pp. 72.

volumes/anticipated production volumes have been submitted on request by DG SANCO, including L-lysine (FLAVIS No. 17.026) as a flavouring compound (EFSA, 2011b)

Bacterial protein from *C. glutamicum* as well as by-products from the production of amino acids with *C. glutamicum* (the cells of the microorganisms have to be inactivated or killed) are listed in the Catalogue of feed materials (Commission Regulation (EU) 68/2013).⁷ *C. glutamicum* is regarded as qualified presumption of safety (QPS) only when used as a production organism for amino acids, provided the susceptibility to antimicrobials has been demonstrated (EFSA, 2007; EFSA BIOHAZ Panel, 2013, 2017).

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 HCl and concentrated liquid L-lysine as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁹ and the applicable EFSA guidance documents.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of L-lysine monohydrochloride and concentrated liquid L-lysine produced by *C. glutamicum* NRRL B-50775 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 L-lysine HCl and concentrated liquid L-lysine is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a,b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Technical Guidance: Microbial Studies (EFSA, 2008a,b), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012d) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The product subject of this application is L-lysine in the forms of monohydrochloride (HCl) or concentrated liquid L-lysine (base) produced by fermentation with a genetically modified strain *C. glutamicum*. L-lysine is currently authorised for use as a nutritional additive, under the functional group 'amino acids, their salts and analogues'. The product under application is 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 two forms of the additive are produced by a genetically modified strain of *C. glutamicum*, which is deposited in the US Agricultural Research Service Patent Culture Collection as *C. glutamicum*

⁷ Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29/1, 30.1.2013, pp. 1–64.

⁸ FEED dossier reference: FAD-2017-0024.

⁹ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

¹⁰ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0024_lysine.pdf

NRRL B-50775.¹¹ *C. glutamicum* is a Gram-positive, non-pathogenic bacterium, which has been recommended for QPS when used for the production of amino acids (EFSA BIOHAZ Panel, 2013, 2017).

3.1.1.1. Information relating to the genetically modified microorganism

The production strain, *C. glutamicum* NRRL B-50775, was obtained from the strain [REDACTED]

[REDACTED]¹²

Characteristics of the recipient or parental microorganism

The recipient strain, [REDACTED] was developed from [REDACTED]. The strain is reported to be susceptible to the antibiotics listed in the technical guidance for the assessment of bacterial antimicrobial susceptibility (EFSA FEEDAP Panel, 2012d) to 'Other Gram +', except to kanamycin.¹³ The identity of the recipient strain [REDACTED] was confirmed as *C. glutamicum* [REDACTED]¹⁴

Characterisation of the donor organism

[REDACTED]¹⁵

[REDACTED]¹⁶

Description of the genetic modification process

3.1.2. Manufacturing process

L-Lysine is produced by [REDACTED] fermentation.

[REDACTED] The applicant states that no antimicrobials, including antibiotics, are used in the manufacturing processes of L-lysine HCl and concentrated liquid L-lysine 50%.¹⁷

3.1.3. Conditions of use

L-Lysine is proposed to be used in feeds for all animal species to achieve an adequate amino acid profile and to meet the L-lysine requirements. Both forms can be added directly to feedingstuffs or complementary feedingstuffs. Only L-lysine HCl is proposed to be used via premixture. Both forms of the additive can be added to water for drinking.¹⁸ Particular care should be given to an appropriate formulation to avoid amino acids imbalances. No inclusion levels have been proposed, as the

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

¹³ Technical dossier/Section II/Annex 2.2.2.2.a.

¹⁷ Technical dossier/Section II/Annex 2.3.1.7.

¹⁸ Technical dossier/Section 2.5.1.

requirements, in quantitative terms, depend on the species, the physiological state of the animal, the performance level, the environmental conditions and the amino acid composition of the unsupplemented diet.

3.1.4. Characterisation of the L-lysine monohydrochloride

L-Lysine HCl (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2,6-diaminohexanoic acid monohydrochloride, synonym L-lysine hydrochloride, a compound identified with the Chemical Abstracts Service (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 $\text{NH}_2-(\text{CH}_2)_4-\text{CH}(\text{NH}_2)-\text{COOH}-\text{HCl}$ and the molecular structure is given in Figure 1.

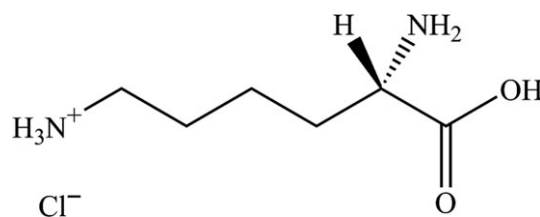


Figure 1: Molecular structure of L-lysine HCl

The specification is for an additive containing $\geq 98.5\%$ L-lysine HCl, $\leq 1.5\%$ water and $< 1\%$ unidentified material.¹⁹

The average lysine content analysed in five batches was 79.1% (range 79.0–79.2%) on an 'as is' basis.²⁰ The content of chloride was on average 19.1% (range 19.0–19.3%). The water content was in the range 0.1–0.2%. On a dry matter basis, the sum of lysine and chloride was on average 98.3%.²¹ Other constituents were proteins (0.11–0.16%), free amino acids other than lysine (0.06–0.1%), sulfate (0.03–0.04%), crude fat (0.05–0.07%), and liquid chromatography–mass spectrometry (LC–MS) identified material (0.5–0.6%).²² The amount of identified material on dry matter basis was 99.2%. The specification for lysine monohydrochloride is not reached.

The specific optical rotation was measured in three batches and ranged from +20.7 to +20.8° (reference values of the United States Pharmacopoeia range between +20.4 and +21.4°).²³

3.1.4.1. Impurities of L-lysine HCl

Analytical data (three batches) on the content of heavy metals (lead, cadmium and mercury) and arsenic were provided. Lead, cadmium and arsenic were $< 10 \mu\text{g}/\text{kg}$ and mercury was $< 0.17 \mu\text{g}/\text{kg}$.²⁴ Dioxins and the sum of dioxin plus dioxin-like polychlorinated biphenyls (PCBs) in three batches of the product were $< 0.14 \text{ ng}$ (WHO, 2005) PCDD/F TEQ per kg and 0.27 ng (WHO, 2005) PCDD/F-PCB TEQ/kg.²⁵ Aflatoxins (not specified), ochratoxin A, zearalenone, α -zearalenol, β -zearalenol and zeranone, deoxynivalenol (DON) and 3-acetyl DON were below the corresponding limit of detection (LOD).²⁶ Fumonisin B1, B2 and B3 ranged from 560 to 583 $\mu\text{g}/\text{kg}$ and citrinin from 30.5 to 35.4 $\mu\text{g}/\text{kg}$.

Microbiological contamination was analysed in five batches and showed that *Salmonella* spp. was absent in 100 g. *E. coli* and coliforms were < 3 colony forming unit (CFU)/g. Aerobic plate count, *Pseudomonas*, coagulase-positive staphylococci, yeasts and filamentous fungi were < 10 CFU/g.²⁷

The concentrations of the aforementioned contaminants/impurities do not raise safety concerns.

¹⁹ Technical dossier/Section 2.1.3 and Annex 2.1.3.

²⁰ Technical dossier/Supplementary information April 2018/Annex 1. Method of association of analytical communities (AOAC) 999.13.

²¹ Technical dossier/Section II/Annex 2.1.3.

²² LC/MS identified material including adenine, guanine, cytosine, thymine, uracil, valerolactam, 2,3-butanediol, *N*(alpha)-acetyl-lysine, *N*(epsilon)-acetyl-lysine, *N*-acetyl-cadaverine, *N,N*-diacetyl-cadaverine, phenyllactic acid, polypropylene glycol, 2-isopropylmalic acid, 2-isopropylmaleic acid, lysine dimer, hypoxanthine and aminocapro lactam.

²³ Technical dossier/Supplementary information April 2018/Annex 2.

²⁴ Technical dossier/Section II/Annex 2.1.4c.

²⁵ Technical dossier/Section II/Annexes 2.1.4e1, f1 and g1.

²⁶ Technical dossier/Section II/Annex 2.1.4d. LOD in $\mu\text{g}/\text{kg}$ were 1.7 for aflatoxins, 5 for ochratoxin A, 17 for zearalenone + α -zearalenol, β -zearalenol and zeranone, and 134 for DON + 3-ac DON.

²⁷ Technical dossier/Section II/Annex 2.1.4a.

No viable cells of the production strain were found in five batches of the L-lysine HCl (each tested in triplicate). [REDACTED]

No recombinant DNA was detected in three batches of L-lysine HCl [REDACTED]

3.1.4.2. Physical characteristics of L-lysine monohydrochloride

The additive is a tan coloured granulate, with a water solubility of 500–600 g/L at 25°C.³⁰ Its bulk density (three batches) ranged from 640 to 710 kg/m³.³¹

Particle size distribution (three batches) was analysed by laser diffraction and the fraction of particles < 10 µm diameter was < 1% in all three batches. The fractions of particles < 50 and < 100 µm diameter ranged from < 1% to 1.3% and from 1.2% to 1.7%, respectively (v/v).³² Mean particle size ranged from 586 to 716 µm. The dusting potential of these three batches (Stauber–Heubach method) ranged from 1.7 to 3.6 g/m³.³³

3.1.4.3. Stability and homogeneity

The shelf life of L-lysine HCl (three batches) was studied when stored in closed plastic bottles at 25–30°C protected from light for 24 months. Losses ranged from 0% to 1.2%.³⁴

The stability of the additive (three batches) in a vitamin–mineral premixture (without choline chloride) for pigs when supplemented at 10% was studied.³⁵ Three samples per batch were collected in paper bags with inner plastic liner and stored at 37–40°C for 6 months. Losses ranged from 0% to 7%, depending on the batch considered.

The stability of three batches of the additive in a complete feed for pigs (mash and a pelleted, with a basal diet containing maize and soybean meal) when supplemented at 0.5% lysine was studied. Pelleting was performed at 82°C. Three samples per batch and feed (mash or pelleted) were collected in paper bags as described above, stored in ambient conditions for 3 months.³⁵ Losses in mash feed ranged from 0% to 8% and those of pelleted feed from 0% to 3%. Loss during feed processing (comparing average values of mash and pelleted feed at T0) was 7%.

The stability of three batches of L-lysine HCl in water for drinking was studied at five different concentrations (0.1, 0.5, 1, 5 and 10 g/L) at 18–23°C for 3 days.³⁶ No losses were detected.

To prove the capacity of the L-lysine HCl to distribute homogeneously in feed, the applicant provided a homogeneity study performed with L-lysine HCl originating from a different production strain (*C. glutamicum* NRRL B-50547).³⁷ Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considered the previous data as representative for the product under assessment. A pelleted feed for chicken for fattening was tested when supplemented at 0.5% lysine.³⁸ The coefficient of variation (CV) of 10 analysed samples was 5%.

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

L-Lysine (IUPAC name (2S)-2,6 diaminoheptanoic acid; synonym α,ϵ -diaminocaproic acid), a compound identified with the CAS No 56-87-1 and the EINECS No 200-294-2, has a molecular weight of 146.2 g/mol. The molecular formula is NH₂–(CH₂)₄–CH(NH₂)–COOH. The molecular structure is given in Figure 2.

³⁰ Technical dossier/Section II/Annex 2.5.2a.

³¹ Technical dossier/Section 2.1.5.

³² Technical dossier/Section II/Annexes 2.1.4e1, e2, f1, f2, g1 and g2.

³³ Technical dossier/Section II/Annex 2.1.5.

³⁴ Technical dossier/Supplementary information April 2018/Annex 3.

³⁵ Technical dossier/Section II/Annex 2.4.1a, b and c; supplementary information April 2018/Annex 4a.

³⁶ Technical dossier/Supplementary information April 2018/Annex 6.

³⁷ FAD-2010-0067/Technical dossier/Supplementary information January 2015/Annexes/Annex Q1 homogeneity Lys HCl ADM.

³⁸ Technical dossier/Section II/Annex 2.4.2a.

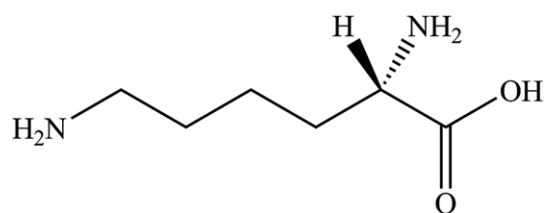


Figure 2: Molecular structure of L-lysine

The product is specified to contain $\geq 50\%$ lysine, $\leq 45\%$ water and about 1% unidentified material.¹⁹

The specification was confirmed by analytical data from five batches which contained on average 50.8% lysine as is (range 50.1–51.4%).³⁹ The water content was 43.5% (range 43.3–43.8%). Other relevant constituents were free amino acids other than lysine (range 0.5–0.6%), protein (without free amino acids other than lysine, range 0.7–0.9), ash (range 0.3–0.4%), organic acids (0.3–0.4%). The LC/MS miscellaneous fraction described above was on average 2.5% (range 2.4–2.6%).⁴⁰ The amount of lysine on dry matter basis was 89.8% (range 89.2–90.7%).

On a dry matter basis, the sum of quantified components was on average 98.3% (range 98.1–98.9%). The amount of the active substance (L-lysine) was less than 95% on a dry matter basis.

3.1.5.1. Impurities

Analytical data of three batches of concentrated liquid L-Lysine (base) were provided. Heavy metals (lead and cadmium) and arsenic were $< 10 \mu\text{g}/\text{kg}$ and mercury was $< 0.17 \mu\text{g}/\text{kg}$.⁴¹ Dioxins and the sum of dioxin plus dioxin-like PCBs were $< 0.14 \text{ ng}$ (WHO, 2005) PCDD/F TEQ per kg and 0.27 ng (WHO, 2005) PCDD/F-PCB TEQ per kg (in all three batches).⁴² Aflatoxins (not specified) ranged from below LOD to $2.1 \mu\text{g}/\text{kg}$; ochratoxin A ranged from 41 to $44 \mu\text{g}/\text{kg}$; zearalenone, α -zearalenol, β -zearalenol and zeranol ranged from 329 to $361 \mu\text{g}/\text{kg}$; DON and 3-acetyl DON were below the LOD.⁴³ Fumonisin B1, B2 and B3 ranged from 644 to $1,299 \mu\text{g}/\text{kg}$ and citrinin from 462 to $497 \mu\text{g}/\text{kg}$. The microbial contamination (analysed in five batches) showed that *Salmonella* spp. was absent in 100 mL, coliforms and *E. coli* were $< 3 \text{ CFU}/\text{mL}$, *Pseudomonas*, coagulase-positive staphylococci, yeasts and filamentous fungi were $< 10 \text{ CFU}/\text{mL}$ each.⁴⁴ The amount of the aforementioned contaminants/impurities does not raise safety concerns.

No viable cells of the production strain were found in five batches of the concentrated liquid L-lysine (base) (each tested in triplicate). [REDACTED]

No recombinant DNA was detected in three batches of concentrated liquid L-Lysine (base) [REDACTED]

3.1.5.2. Physical characteristics of concentrated liquid L-lysine (base)

Concentrated liquid L-lysine (base) is an aqueous solution of dark brown colour with a viscosity at 20°C ranging from 69 to $125 \text{ mm}^2/\text{s}$ (three batches analysed), a density at 20°C ranging from 1,140 to $1,150 \text{ kg}/\text{m}^3$ (three batches analysed).⁴⁷ It is soluble in water and has a boiling point of 115°C.⁴⁸ It has a pH (measured in three batches) of 11.⁴⁹

³⁹ Analytical method: AOAC 999.13.

⁴⁰ Technical dossier/Section II/Annex 2.1.3.

⁴¹ Technical dossier/Section II/Annex 2.1.4c.

⁴² Technical dossier/Section II/Annexes 2.1.4h1, 2.1.4h2 and 2.1.4h3.

⁴³ Technical dossier/Section II/Annex 2.1.4d. LOD in $\mu\text{g}/\text{kg}$ was 1.7 for aflatoxins, and 134 for DON + 3-ac DON.

⁴⁴ Technical dossier/Section II/Annex 2.1.4b.

⁴⁷ Technical dossier/Section II/Annexes 2.1.4h1 to 2.1.4h3.

⁴⁸ Technical dossier/Section II/Annex 2.5.2b.

⁴⁹ Technical dossier/Supplementary information April 2018/Annex 10.

3.1.5.3. Stability and homogeneity

The shelf life of three batches of concentrated liquid L-lysine (base) were studied when stored in closed plastic containers protected from light at 13°C for 12 months.⁵⁰ No losses were observed. No data were provided on stability at higher temperatures.

The stability of three batches of the additive in a complete feed for pigs (mash and a pelleted, with a basal diet containing maize and soy meal) when supplemented at 1% with concentrated liquid L-lysine (base) was studied. Pelleting was performed at 82°C. Three samples per batch and feed (mash or pelleted) were collected in paper bags with inner plastic liner, stored in ambient conditions for 3 months.⁵¹ Losses in mash feed ranged from 5% to 8%; and those of pelleted feed from 3% to 8%. No losses of lysine were observed during pelleting.

To prove the capacity of the concentrated liquid L-lysine (base) to distribute homogeneously in feed, the applicant provided an homogeneity study performed with concentrated liquid L-lysine (base) originating from a different production strain (*C. glutamicum* NRRL B-50547).⁵² Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considered the previous data as representative for the product under assessment. A pelleted feed for chicken for fattening was tested when supplemented at 0.19% lysine.⁵³ The CV of 10 analysed subsamples was 4%.

The stability of three batches of liquid L-lysine (base) in water for drinking was studied at five different concentrations (0.1, 0.5, 1, 5 and 10 g/L) at 18–23°C for 3 days.³⁶ No losses were detected.

3.1.6. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

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

3.1.7.1. Safety of the genetic modification

The recipient organism [REDACTED]. *C. glutamicum* is recommended for QPS when used for the production of amino acids (EFSA BIOHAZ Panel, 2017). [REDACTED]

[REDACTED] It also contains genes [REDACTED], inserted into its genome.

The presence of [REDACTED] genes would be considered as a hazard if present in the product. The applicant provided sufficient information that neither the production strain nor its recombinant DNA is present in the final product. Therefore, the product L-lysine, manufactured by fermentation with *C. glutamicum* NRRL B-50775, does not give rise to any safety concern with regard to the genetic modification of the production strain provided that the manufacturing process ensures the absence of viable cells and recombinant DNA of the production strain in the final products.

3.1.7.2. Safety for the target species

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. No safety concerns for ruminants would arise from ruminal lysine metabolism (EFSA FEEDAP Panel, 2013, 2014).

Tolerance studies with indispensable amino acids such as lysine cannot be designed according to the protocols of conventional toxicity experiments because high dietary concentrations of a certain amino acid will result in amino acid imbalances with depression of feed intake and hence impaired performance. Nevertheless, for nutritional additives produced by fermentation, the risk associated with the residues of the fermentation process in the final product needs to be assessed.

Both products are obtained by the same genetically modified strain of *C. glutamicum*, a species that qualifies for the QPS approach for safety assessment when used to produce amino acids. The

⁵⁰ Technical dossier/Supplementary information April 2018/Annex 3 and annex 11.

⁵¹ Technical dossier/Section II/Annex 2.4.1a, b and c.

⁵² FAD-2010-0067/Technical dossier/Supplementary information January 2015/Annexes/Annex Q1 homogeneity Lys base ADM.

⁵³ Technical dossier/Section II/Annex 2.4.2b.

identity of the strain has been established as *C. glutamicum* and the fermentation product would raise no safety concerns for the target species. The strain contains [REDACTED] genes and this could be seen as a safety concern. However, viable cells of the production strain and their DNA were not detected in the final products. Consequently, the FEEDAP Panel considers that L-lysine HCl and concentrated liquid L-lysine (base) produced by the strain *C. glutamicum* NRRL B-50775 do not represent a risk for the target species.

The FEEDAP Panel reiterates its previous statement that amino acids, their salts and analogues should generally not be used in water for drinking because of the risk of amino acid imbalances and for hygiene reasons (EFSA, 2010).

3.1.7.3. Safety for the consumer

Absorption, distribution, metabolism and excretion of L-lysine were described in a previous scientific opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The use of the amino acid L-lysine itself in animal nutrition is considered safe for consumers.

Both products are obtained by the same genetically modified strain of *C. glutamicum*, a species that qualifies for the QPS approach for safety assessment. Since the identity of the strain has been established as *C. glutamicum*, the FEEDAP Panel considers that no safety concerns for the consumer would arise from the production process. Although the production strain contains [REDACTED] genes, the absence of viable cells of the production strain or of its recombinant DNA in the final product has been demonstrated. Consequently, the FEEDAP Panel considers that L-lysine HCl and concentrated liquid L-lysine (base) produced by the strain *C. glutamicum* NRRL B-50775 do not represent a risk for the consumer.

3.1.7.4. Safety for the user

No data were submitted on the products under assessment.

L-Lysine HCl

- Effects on the respiratory system

Although the fractions of particles < 50 and < 100 µm diameter were up to 1% and 2%, respectively (v/v), the product has high dusting potential (up to 3.6 g/m³; see section 3.1.4.2).

The applicant provided an inhalation toxicity study performed with a different product (L-lysine sulfate) which is not further considered.⁵⁴

- Effects on skin and eyes

The applicant submitted an acute dermal irritation/corrosion study (in accordance with OECD Guideline 404), an eye irritation/corrosion study (in accordance with OECD 405) and a skin sensitisation study (in accordance with OECD Guideline 406) where the test item was an L-lysine HCl produced by a different production strain (*C. glutamicum* KCTC 12307BP). Those studies are summarised as follows:

An acute dermal irritation/corrosion study according to OECD Guideline 404 was carried out with L-lysine HCl (99.5% pure).⁵⁵ None of the three female albino rabbits used showed dermal irritation/corrosion at any observation time (1, 24, 48 and 72 h post-administration). Thus, the product L-lysine HCl is classified as not irritating to human skin.

An acute eye irritation/corrosion test in accordance with OECD Guideline 405 was performed using L-lysine HCl (99.5% pure).⁵⁶ No irritation/corrosion was observed in the eyes of the tested rabbits during the observation period. Thus the product L-lysine HCl is classified as not irritating to human eyes.

The skin sensitisation potential of the L-lysine HCl (99.5%) was studied in guinea pigs in accordance with OECD Guideline 406 (Guinea pig maximisation test).⁵⁷ No dermal reactions were observed at the 24- or 48-h post-challenge. Consequently, the test item has no sensitising properties.

As the L-lysine HCl under assessment is produced by a similar manufacturing process, it has similar characteristics and purity compared with the L-lysine HCl produced by *C. glutamicum* KCTC 12307BP, the FEEDAP Panel considers that the results of the acute dermal irritation/corrosion study, the eye

⁵⁴ Technical dossier/Section III/Annex 3.3.1.1.

⁵⁵ Technical dossier/Section III/Annex 3.3.1.2a.

⁵⁶ Technical dossier/Section III/Annex 3.3.1.2b.

⁵⁷ Technical dossier/Section III/Annex 3.3.1.2c.

irritation/corrosion study and the skin sensitisation study mentioned above are also applicable to the L-lysine HCl under assessment. So, L-lysine HCl produced by *C. glutamicum* NRRL B-50775 is considered not irritant to skin or eyes and not a skin sensitiser. The FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced by *C. glutamicum* NRRL B-50775.

Concentrated liquid L-lysine (base)

The applicant submitted an acute dermal irritation/corrosion study (in accordance with OECD Guideline 404), an eye irritation/corrosion study (in accordance with OECD 405) and a skin sensitisation study (in accordance with OECD Guideline 406) where the test item was a concentrated liquid L-lysine (base) produced by a different production strain (*C. glutamicum* KCTC 12307BP). Those studies are summarised as follows:

An acute dermal irritation study was performed with a concentrated liquid L-lysine (50%) in rabbits in accordance with OECD Guideline 404.⁵⁸ None of the rabbits showed any skin reaction. Consequently, the product is classified as not irritating to human skin.

An acute eye irritation study was conducted with a concentrated liquid L-lysine (50%, pH 9.95) in rabbits, in accordance with OECD Guideline 405.⁵⁹ Some redness of conjunctiva and chemosis was observed within 24 h post application in different grades in all three rabbits. As the recorded scores were lower than the threshold value for classifying a product as eye irritant (0.7 vs 2), the test material is considered not irritant for human eye.

The skin sensitisation potential of a concentrated liquid L-lysine (50%) was studied in guinea pigs in accordance with OECD Guideline 406 (Guinea pig maximisation test).⁶⁰ No dermal reactions were observed at the 24- or 48-h post-challenge. Consequently, the test item has no sensitising properties.

The FEEDAP Panel considers that the results of the acute dermal irritation/corrosion study, the eye irritation/corrosion study and the skin sensitisation study mentioned above cannot be extended to concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 because the pH of the product under assessment is one point higher. The FEEDAP Panel considers that the product under assessment is corrosive to skin and eyes and poses a risk by inhalation.

Conclusions on the safety for the user

L-Lysine HCl produced by *C. glutamicum* NRRL B-50775 is considered not irritant to skin or eyes and not a skin sensitiser. The FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced by *C. glutamicum* NRRL B-50775.

Concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775, due to its high pH (11), is corrosive to skin and eyes and poses a risk by inhalation.

3.1.7.5. Safety for the environment

Neither viable cells of the production strain *C. glutamicum* NRRL B-50775 nor its recombinant DNA were detected in the final product. The production strain contains [REDACTED] genes and this could be seen as a safety concern. However, viable cells of the production strain and their DNA were not detected in the final products. Therefore, the products do not pose any environmental safety concern associated with the genetic modification of 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 absorbed L-lysine will be incorporated into body protein or excreted as urea/uric acid and as carbon dioxide.

Consequently, the FEEDAP Panel considers that L-lysine HCl and concentrated liquid L-lysine (base) produced by the strain *C. glutamicum* NRRL B-50775 do not represent a risk for the environment.

3.1.8. 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. 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

⁵⁸ Technical dossier/Spontaneous supplementary information July 2018/Annexes 15a and 15b.

⁵⁹ Technical dossier/Spontaneous supplementary information July 2018/Annex 15c.

⁶⁰ Technical dossier/Spontaneous supplementary information July 2018/Annex 15d.

L-lysine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen (Chalupa, 1976; Broderick and Balthrop, 1979).

3.1.9. 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 Regulation⁶¹ and Good Manufacturing Practice.

4. Conclusions

Neither the production strain *C. glutamicum* NRRL B-50775 nor its recombinant DNA was detected in the final product. Therefore, the product does not pose any safety concern associated with the genetic modification of the production strain.

L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 are considered safe for the target species, for the consumer and for the environment.

L-lysine HCl produced by *C. glutamicum* NRRL B-50775 is considered not irritant to skin or eyes and not a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced by *C. glutamicum* NRRL B-50775.

Concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775, due to its high pH (11) it is anticipated to be corrosive to skin and eyes and poses a risk by inhalation.

L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* NRRL B-50775 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.

Documentation provided to EFSA

- 1) L-Lysine HCl and concentrated liquid L-lysine (base) produced using *Corynebacterium glutamicum* for all animal species. June 2017. Submitted by ADM Speciality Ingredients (Europe) B.V.
- 2) L-Lysine HCl and concentrated liquid L-lysine (base) produced using *Corynebacterium glutamicum* for all animal species. Supplementary information. April 2018. Submitted by ADM Speciality Ingredients (Europe) B.V.
- 3) L-Lysine HCl and concentrated liquid L-lysine (base) produced using *Corynebacterium glutamicum* for all animal species. Spontaneous supplementary information. July 2018. Submitted by ADM Speciality Ingredients (Europe) B.V.
- 4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis of L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL B-50775.
- 5) Comments from Member States.

Chronology

Date	Event
16/6/2017	Dossier received by EFSA
16/6/2017	Reception mandate from the European Commission
28/7/2017	Application validated by EFSA – Start of the scientific assessment
16/11/2017	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 L-lysine HCl and concentrated liquid L-lysine (base), stability and safety for the user.</i>
27/10/2017	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
28/10/2017	Comments received from Member States
20/04/2018	Reception of supplementary information from the applicant - Scientific assessment re-started

⁶¹ 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
4/7/2018	Spontaneous submission of information by the applicant. <i>Issues: safety for the user</i>
28/11/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CG	chemical group
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	EFSA Panel on Additives and Products or Substances used in Animal Feed
FID	fluorescence detection
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
IEC	ion exchange chromatography
IUPAC	International Union of Pure and Applied Chemistry
LC-MS	liquid chromatography-mass spectrometry
LOD	limit of detection
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo- <i>p</i> -dioxin/dibenzofuran
QPS	qualified presumption of safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalent
VIS	visible detection
WHO	World Health Organization

Annex A – Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL B-50775

In the current application, authorisation is sought under Article 4(1) L-lysine in the form of L-lysine monohydrochloride and concentrated liquid L-lysine produced both by *Corynebacterium glutamicum* NRRL B-50775, 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. L-Lysine is already authorised as feed additive under Commission Directive 88/485/EEC.

For the quantification of lysine in the feed additive, the Applicant submitted the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled with post-column derivatisation and visible or fluorescence detection (IEC-VIS/FD). 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 L-lysine in premixtures, feedingstuffs and water the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on IEC coupled with post-column derivatisation using an amino acid analyser or high-performance liquid chromatography with an ion exchange column and photometric detection (VIS). This method, designed only for the analysis of 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 3.5% and RSDR ranging from 3.0 to 13.1%.

The Applicant did not perform any validation/verification studies to demonstrate the suitability of the Community method for the determination of lysine in water. However, in the frame of the stability studies, the Applicant applied a slightly modified analytical method and demonstrated its applicability.

Based on the performance characteristics available, the EURL recommends for official control (i) the 'L-lysine monohydrochloride monograph' of the 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 coupled to visible or fluorescence detection (IEC-VIS/FD) to quantify free lysine in the feed additive and premixtures (containing more than 10% lysine); (iii) the Community method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; and (iv) the modified Community method based on IEC-VIS 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.