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Safety and efficacy of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *Corynebacterium glutamicum* strains NRRL-B-67439 or NRRL B-67535 for all animal species

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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 genetically modified strains of *Corynebacterium glutamicum* (NRRL-B-67439 or NRRL B-67535). They are intended to be used in feed or water for drinking for all animal species and categories. Neither viable cells of the production strains *C. glutamicum* strains NRRLB-67439 or NRRL B-67535; nor their recombinant DNA were detected in the final products. Therefore, those products do not pose any safety concern associated with the genetic modification of the production strains. L-Lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* strains NRRLB-67439 or NRRL B-67535 are considered safe for the target species, for the consumer and for the environment. L-Lysine HCl produced by *C. glutamicum* strains NRRL B-67439 or NRRL B-67535 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* strains NRRL B-67439 or NRRL B-67535. Concentrated liquid L-lysine (base) produced by *C. glutamicum* strains NRRL B-67439 or NRRL B-67535, due to its high pH (10.7 and 10.9, respectively) 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* strains NRRLB-67439 or NRRL B-67535 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 two requests from Archer Daniels Midland Company² for authorisation of the products L-lysine monohydrochloride and concentrated liquid L-lysine (base), produced by two different strains of *Corynebacterium glutamicum*, when used as a 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 applications to the European Food Safety Authority (EFSA) as applications under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the two applications were considered valid by EFSA as of 8 of May 2018 and as of 1 October 2018, respectively.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the products L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using genetically modified strains of *Corynebacterium glutamicum* (NRRL B-67439 or NRRL B-67535), when used under the proposed conditions of use (see Section 3.2.5).

1.2. Additional information

The active substance of the two products under application, L-lysine, is produced by genetically modified strains of *C. glutamicum* (NRRL B-67439 or NRRL B-67535).

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

L-Lysine is authorised for use in food,⁴ 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: 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, 2017a); 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, 2015c, 2016a), produced by fermentation using different strains of *E. coli* or *C. glutamicum* (EFSA FEEDAP Panel, 2016b, 2019b,c,d).

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 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.

The Scientific 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, 2008; EFSA AFC Panel, 2010). The Scientific 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 Scientific 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 volumes/anticipated production volumes have been submitted on request by DG SANCO, including L-lysine (FLAVIS No 17.026) as a flavouring compound (EFSA CEF Panel, 2011).

Bacterial protein from *C. glutamicum* as well as by-products from the production of amino acids with *C. glutamicum* (the cells of the micro-organisms have to be inactivated or killed) are listed in the Catalogue of feed materials (Commission Regulation (EU) 2017/2017).⁸

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of two different technical dossiers⁹ in support of the authorisation request for the use of L-lysine monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using genetically modified strains of *C. glutamicum* (NRRL B-67439 or NRRL B-67535) 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, other scientific reports 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 monohydrochloride and concentrated liquid L-lysine (base) produced by fermentation using *C. glutamicum* NRRL B-67439 or NRRL B-67535 in animal feed. The Executive Summaries of the EURL reports can be found in Annexes A and B.¹⁰

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 using *C. glutamicum* NRRL B-67439 or NRRL B-67535 is in line with the principles laid down in Regulation (EC) No 429/2008¹¹ 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, 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 for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

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 genetically modified strains of *C. glutamicum*. The product under application is intended to be used in feed and water for drinking for all animal species and categories as a nutritional additive, under the functional group 'amino acids, their salts and analogues'.

⁸ Commission Regulation (EU) No. 2017/2017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. OJ L 159, 21.6.2017, p. 48.

⁹ FEED dossiers reference: FAD-2018-0012 and FAD-2018-0037.

¹⁰ The full reports are available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0012_L-lysine.pdf and https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0037-lysine.pdf

¹¹ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3.1. Manufacturing process

[REDACTED]

3.2. Characterisation of L-lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67439

3.2.1. Characterisation of the production microorganism

The additive is produced by a genetically modified strain of *C. glutamicum*, which is deposited [REDACTED] as *C. glutamicum* NRRL B-67439. [REDACTED]

The susceptibility of the production strain to the antibiotics listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) for '*Corynebacterium* and other Gram +' was tested [REDACTED]. All of the minimum inhibitory concentration (MIC) values found well below the cut-off values of the above mentioned guidance. According to the guidance, the whole genome sequence (WGS) of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [REDACTED].

[REDACTED] No AMR genes were found.

3.2.1.1. Information related to the genetically modified microorganism

a) Characteristics of the recipient or parental microorganism¹⁷

[REDACTED]

b) Characterisation of the donor organism

[REDACTED]

[REDACTED]

c) Description of the genetic modification process

3.2.2. Characterisation of L-lysine HCl

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 $\text{NH}_2\text{-(CH}_2\text{)}_4\text{-CH(NH}_2\text{)-COOH-HCl}$ and the molecular structure is given in Figure 1.

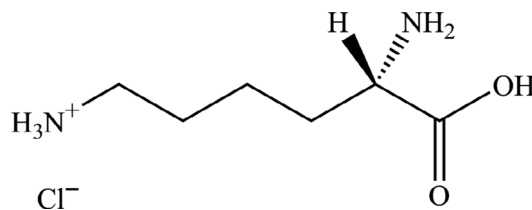


Figure 1: Molecular structure of L-lysine HCl

The specification is for an additive containing $\geq 98.5\%$ L-lysine HCl, $\leq 1.5\%$ water and $< 1\%$ unidentified material.²²

The average lysine content analysed in five batches was 79.3% (range 79.2–79.5%) on an 'as is' basis.²³ The content of chloride was on average 18.6% (range 18.5–18.7%). The water content was in

²² FAD-2018-0012/Technical dossier/Section 2.1.3 and annex 2.1.3.

²³ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.3b. Lysine analysed using AOAC official method 999.13.

the range 0.15–0.24%. Other analysed components were (average values of five batches) 0.02% cadaverine, 0.09% crude fat, 0.01% sulfate, 0.06% other free amino acids, 0.07% protein (hydrolysed amino acids minus free amino acids other than lysine) and 0.29% of other components identified by liquid chromatography/mass spectrometry (LC/MS).²⁴ The concentration of lysine HCl in the additive was on average 98.1% on a dry matter basis.²⁵ The amount of identified material was 98.9% on a dry matter basis.

The applicant submitted analytical data on specific optical rotation of five batches of the additive and the values was on average 19.4 (range +17.5 to +20.7).²⁶ Three of the five batches tested were outside of the reference range specified in the food chemicals code of the United States Pharmacopoeia (2010) (+20.3 to +21.5°).

3.2.2.1. Impurities of L-lysine HCl

Heavy metals (cadmium, lead and mercury) and arsenic were analysed in five batches of the final product and all values were below the limit of detection (LOD) except one batch that contained 19 µg lead/kg.²⁷ Dioxins (polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (co-planar PCBs) were analysed in five batches of the final product. PCDD/F concentrations were on average 0.0884 TEQ-WHO ng/kg, PCBs were on average 0.0032 TEQ-WHO ng/kg and the sum of PCDD/F and PCBs were on average 0.0915 TEQ-WHO ng/kg.²⁶ The amount of the above-mentioned impurities does not raise safety concerns.

As regards the microbial contamination, five batches were analysed and in all batches *Salmonella* spp. was absent (100-g samples); aerobic plate counts were < 100 colony forming units (CFU)/g; coliforms and *E. coli* were < 3 CFU/g; yeasts, moulds and *Staphylococcus* coagulase positive were < 100 CFU/g; and *Pseudomonas aeruginosa* was absent (25-g samples). Mycotoxin concentrations were measured in three batches of the additive and all batches yielded the same result: aflatoxin B1 was < 0.5 µg/kg, aflatoxin B2 was < 0.3 µg/kg, aflatoxin G1 was < 0.7 µg/kg, aflatoxin G2 was < 0.2 µg/kg, Deoxynivalenol (DON) and fumonisin B2 were < 10 µg/kg, fumonisin B1 was < 4 µg/kg, fumonisin B3 was < 8 µg/kg, ochratoxin was < 0.8 µg/kg, T-2 toxin was < 9 µg/kg and zearalenone was < 7 µg/kg.²⁸

The presence of viable cells of the production strain was investigated

and no colonies were detected.

The absence of DNA was confirmed

3.2.2.2. Physical characteristics of L-lysine HCl

The additive is a tan coloured granulate with a slight fermentation odour. Its water solubility is 500–600 g/L at 25°C.³⁰ The density (three batches analysed) ranged from 562.5 to 607.3 g/L.³¹

The dusting potential was measured in three batches of the final product (Stauber–Heubach method) and the values ranged from 0.1 to 0.6 g/m³.³² The particle size distribution (three batches analysed by laser diffraction) showed that the fractions < 100, < 52 and < 18 µm diameter ranged 1.1–3.7, 0.6–2.8 and 0–1.4%, respectively.³³

3.2.2.3. Stability and homogeneity of L-lysine HCl

The applicant did not provide data on the shelf life, stability in premixtures and in feedingstuffs and on the capacity of the L-lysine HCl under assessment to distribute homogeneously in feed. The data

²⁴ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.3a. 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.

²⁵ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.3b.

²⁶ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 4a to 4e.

²⁷ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.4c. LOD (in µg/kg) was 10 for arsenic, cadmium and lead and 0.17 for mercury.

²⁸ FAD-2018-0012/Technical dossier/Supplementary information October 2019.

³⁰ FAD-2018-0012/Technical dossier/Section II/Annex 2.5.2a.

³¹ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 3a1 to 3c1.

³² FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 2a to 2c.

³³ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 3a2 to 3c2.

provided corresponded to a L-lysine HCl produced with different strains of *C. glutamicum* (i.e. strain NRRL B-50775 for the stability (EFSA FEEDAP Panel, 2019b); and strain NRRL B-50547 for the homogeneity (EFSA FEEDAP Panel, 2016b)).

Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considers the previous data as representative for the product under assessment.

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

L-Lysine (IUPAC name (2S)-2,6 diaminohexanoic 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 $\text{NH}_2\text{-(CH}_2\text{)}_4\text{-CH(NH}_2\text{)-COOH}$. The molecular structure is given in Figure 2.

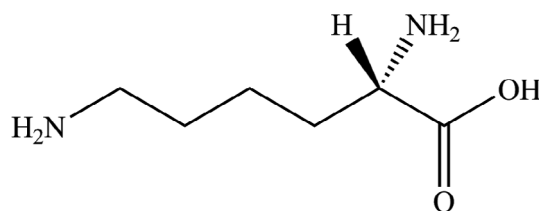


Figure 2: Molecular structure of L-lysine

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

The specification was confirmed by analytical data from five batches which contained on average 50.8% lysine 'as is' (range 50.3–51.3%).³⁴ The water content was 46.6% (range 45.5–47.7%). Other analysed constituents were (average values) 0.7% protein (hydrolysed amino acids minus free amino acids other than lysine), 0.3% organic acids, 0.3% ash, 0.3% other amino acids, 0.2% crude fat, 0.05% sugars, 0.03% sulfate, 0.02% chlorine, 0.02% cadaverine, 0.01% ammonia and 1.2% of substances identified by LC/MS.³⁵

On a dry matter basis, the sum of quantified components was on average 100.5% (range 98.8–102.7%). L-Lysine represented on average 95.2% (range 94.0–96.6%) of the total dry matter content.

3.2.3.1. Impurities

Heavy metals (cadmium, lead and mercury) and arsenic were analysed in five batches of the final product and all values were below the LOD.³⁶ PCDDs, PCDFs and co-planar PCBs were analysed in five batches of the final product. PCDD/F concentrations were on average 0.1200 TEQ-WHO ng/kg, PCBs were on average 0.0068 TEQ-WHO ng/kg and the sum of PCDD/F and PCBs were on average 0.1266 TEQ-WHO ng/kg.³⁷ The amount of the abovementioned impurities does not raise safety concerns.

As regards the microbial contamination, five batches were analysed and in all batches *Salmonella* spp. was absent (100-g samples); aerobic plate counts were < 100 CFU/g; coliforms and *E. coli* were < 3 CFU/g; yeasts, moulds and *Staphylococcus* coagulase positive were < 100 CFU/g; and *Pseudomonas aeruginosa* was absent (25-g samples). Mycotoxin concentrations were measured in three batches of the additive and all batches yielded the same result: aflatoxin B1 was < 0.5 $\mu\text{g/kg}$, aflatoxin B2 was < 0.3 $\mu\text{g/kg}$, aflatoxin G1 was < 0.7 $\mu\text{g/kg}$, aflatoxin G2 was < 0.2 $\mu\text{g/kg}$, DON and fumonisin B2 were < 10 $\mu\text{g/kg}$, fumonisin B1 was < 4 $\mu\text{g/kg}$, fumonisin B3 was < 8 $\mu\text{g/kg}$, ochratoxin was < 0.8 $\mu\text{g/kg}$, T-2 toxin was < 9 $\mu\text{g/kg}$ and zearalenone was < 7 $\mu\text{g/kg}$.²⁸

The presence of viable cells of the production strain was investigated

³⁴ FAD-2018-0012/technical dossier/Annex 2.1.3.a. Lysine analysed using AOAC 999.13 method.

³⁵ FAD-2018-0012/Technical dossier/Annex 2.1.3.a. LC/MS identified material includes 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.

³⁶ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.4c. LOD (in $\mu\text{g/L}$) was 10 for arsenic, cadmium and lead and 0.17 for mercury.

³⁷ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 6a to 6e.

██████████ and no colonies of the production strain were detected. ██████████

The absence of DNA was confirmed ██████████

3.2.3.2. Physical characteristics

The additive is a dark brown liquid with fermentation odour.³⁸ The pH (measured in three batches) was 10.7 in all them.³⁹

Analytical data of three batches was provided on viscosity and density at 20°C. The viscosity ranged from 55 to 71 mm²/s and the density from 1.137 to 1.143 g/cm³.⁴⁰ Vapour pressure analysed in three batches ranged from 16 to 66 kPa.⁴¹

3.2.3.3. Stability and homogeneity

The applicant did not provide data on the shelf life, stability in premixtures and in feedingstuffs and on the capacity of the product under assessment to distribute homogeneously in feed. The data provided corresponded to a concentrated liquid L-lysine base produced with different strains of *C. glutamicum*, i.e. NRRL B-50775 for the stability (EFSA FEEDAP Panel, 2019b), and NRRL B-50547 for the homogeneity in pelleted feed for chickens for fattening (EFSA FEEDAP Panel, 2016b).

Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considers the previous data as representative for the product under assessment.

3.2.4. Physico-chemical incompatibilities in feed

No physicochemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

3.2.5. Conditions of use

According to the applicant, both forms of the additive can be added directly to complete feed, or complementary feedingstuffs. Only L-lysine HCl can be used via premixtures. Both forms of the additive are aimed for all animal species. No proposed inclusion levels are provided as the optimal daily allowance in quantitative terms depends on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid composition of the unsupplemented diet.

The applicant states that the additive (both forms) can be used in water for drinking but should not be simultaneously administered via water for drinking and feed.⁴²

3.3. Characterisation of L-lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67535

3.3.1. Characterisation of the production microorganism

The additive is produced by a genetically modified strain of *C. glutamicum*, which is deposited ██████████ as *C. glutamicum* NRRL B-67535. ██████████ The production strain was confirmed as *C. glutamicum* ██████████

The susceptibility of the production strain to the antibiotics listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) for 'Corynebacterium and other Gram +' was tested ██████████ All of the MIC values found fell below the FEEDAP cut-off values of the above mentioned guidance. The

³⁸ FAD-2018-0012/Technical dossier/Section II/Annex 2.5.2b.

³⁹ FAD-2018-0012/Technical dossier/Section II/Annex 2.1.4m.

⁴⁰ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 7a to 7c.

⁴¹ FAD-2018-0012/Technical dossier/Supplementary information May 2019/Annexes 7d to 7e.

⁴² FAD-2018-0012/Technical dossier/Section 2.5.1.

WGS of the production strain was interrogated for the presence of AMR genes [REDACTED]
[REDACTED] No AMR genes were found.

3.3.1.1. Information relating to the genetically modified microorganism

Characteristics of the recipient or parental microorganism⁴⁶

[REDACTED]

Characterisation of the donor organism

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

[REDACTED]

[REDACTED]

3.3.2. Characterisation of the L-lysine monohydrochloride

The specification is for an additive containing $\geq 98.5\%$ L-lysine HCl, $\leq 1.5\%$ water, and $< 1\%$ unidentified material.⁴⁹ The minimum lysine content is 78%.⁵⁰

The average lysine content analysed in five batches was 79.8% (range 79.5–80.1%) on an 'as is' basis.⁵¹ The content of chloride was on average 18.9% (range 18.8–19.0%). The water content was in the range 0.12–0.17%. Other analysed components were (average values of five batches) 0.003% cadaverine, 0.05% crude fat, 0.02% sulfate, 0.09% other free amino acids, 0.05% protein (hydrolysed amino acids minus free amino acids other than lysine) and 0.25% of other substances identified by LC/MS.⁵² The concentration of lysine HCl in the additive was on average 98.9% on a dry matter basis (range 98.6–99.3%).⁵³ The amount of identified material was 100.4% on a dry matter basis.

The applicant submitted analytical data on specific optical rotation of five batches and the values ranged from +20.4 to +20.7°, confirming the presence of the L-isomer.⁵⁴

3.3.2.1. Impurities of L-lysine HCl

Heavy metals (cadmium, lead and mercury), arsenic and antimony were analysed in five batches of the final product and all values were below the LOD.⁵⁵ PCDDs, PCDFs and co-planar PCBs were analysed in five batches of the final product. PCDD/F concentrations were on average 0.0860 TEQ-WHO ng/kg, PCBs were on average 0.0026 TEQ-WHO ng/kg and the sum of PCDD/F and PCBs were on average 0.0885 TEQ-WHO ng/kg.⁵⁶

Aflatoxins (B1, B2, G1, G2), ochratoxin A, zearalenone, DON, fumonisins B1, B2 and B3 and T-2 toxin were below the corresponding LOD.⁵⁷

Microbiological contamination was analysed in five batches and showed that *Salmonella* spp. was absent in 100 g, *E. coli* and coliforms were < 3 CFU/g and aerobic plate count, *Pseudomonas*, staphylococci coagulase positive, yeasts and filamentous fungi were < 10 CFU/g.⁵⁸

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

The presence of viable cells of the production strain was investigated [REDACTED]

[REDACTED] and no growth was found.

The absence of DNA of the production strain was confirmed [REDACTED]

3.3.2.2. Physical characteristics of L-lysine HCl

The additive is a tan coloured granulate with slight fermentation odour, with a water solubility of 500–600 g/L at 25°C.⁶⁰ Its bulk density (three batches) ranged from 595 to 635 kg/m³.⁶¹

Analytical data on dusting potential of three batches of the final product showed values ranging from 0.6 to 4.6 g/m³.⁶² Particle size distribution was measured by laser diffraction in three batches and the fractions of particles with a size < 100 , < 50 and < 10 μm (w/v) of diameter ranged 0.6–2.8, 0.6–1.8 and 0.5–0.9%, respectively.⁶³

⁴⁹ Technical dossier/Section 2.1.3 and Annex 2.1.3.

⁵⁰ Technical dossier/Supplementary information May 2019/FAD-2018-0037 Sin 261018 and Add 180119 Answers 190506.

⁵¹ Technical dossier/Section II/Annex 2.1.3b. AOAC official method 999.13.

⁵² Technical dossier/Section II/Annex 2.1.3a. 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 aminocaprolactam.

⁵³ Technical dossier/Section II/Annex 2.1.3b.

⁵⁴ Technical dossier/Supplementary information May 2019/Annexes 2a to 2e. The range specified in the food chemicals code of the United States Pharmacopoeia (2010) is from +20.3 to +21.5°.

⁵⁵ Technical dossier/Section II/Annex 2.1.4b. LOD (in $\mu\text{g}/\text{kg}$) was 10 for arsenic, cadmium lead and antimony; and 0.17 for mercury.

⁵⁶ Technical dossier/Supplementary information May 2019/Annexes 2a to 2e.

⁵⁷ Technical dossier/Section II/Annex 2.1.4a. LOD in $\mu\text{g}/\text{kg}$ were 0.5 for aflatoxin B1, 0.3 for aflatoxin B2, 0.07 for aflatoxin G1, 0.09 for aflatoxin G2, 0.8 for ochratoxin A, 0.7 for zearalenone, 4 for fumonisin B1, 10 for fumonisin B2, 8 for fumonisin B3, 10 for DON and 9 for T-2 toxin.

⁵⁸ Technical dossier/Section II/Annex 2.1.4a.

⁶⁰ Technical dossier/Section II/Annex 2.5.2a.

⁶¹ Technical dossier/Supplementary information May 2019/Annexes 3a1 to 3c1.

⁶² Technical dossier/Supplementary information May 2019/Annexes 4a to c.

⁶³ Technical dossier/Supplementary information May 2019/Annexes 3a1 to 3c2.

3.3.2.3. Stability and homogeneity of L-lysine HCl

The applicant did not provide data on the shelf-life, stability in premixtures and in feedingstuffs and on the capacity of the L-lysine HCl under assessment to distribute homogeneously in feed testing the additive under assessment. The data provided were the same as mentioned above for L-lysine HCl produced using *C. glutamicum* NRRL B-67439 (Section 3.2.2.3). Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considers the previous data as representative for the product under assessment.

The applicant provided information on the stability of the additive under assessment in water for drinking. Three batches were tested at 0.1, 0.5, 1, 5 and 10 g/kg when stored at 16–23 °C for 3 days.⁶⁴ No losses were detected.

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

The product is specified to contain $\geq 50\%$ lysine, $\leq 50\%$ water and 1% unidentified material.⁶⁵

The specification was confirmed by analytical data from 5 batches which contained on average 50.5 % lysine 'as is' (range 50.1–51.7 %).⁶⁶ The average water content was 44.9% (range 44.5–45.2%). Other analysed constituents (average values) were 0.4% protein (hydrolysed amino acids minus free amino acids other than lysine), 0.3% organic acids, 0.3% ash, 0.6% other amino acids, 0.2% crude fat, 0.04% sugars, 0.09% sulfate, 0.09% chloride, 0.02% cadaverine, 0.01% ammonia, 0.01% phosphate and 1.7% of other identified material.⁶⁷

On a dry matter basis, the sum of quantified components was on average 98.3 % (range 97.7–98.8%). L-Lysine represented on average 91.6% (range 90.2–92.4%) of the total dry matter.

3.3.3.1. Impurities

Heavy metals (cadmium, lead and mercury), arsenic and antimony were analysed in five batches of the final product and all values were below the LOD.⁶⁸ PCDDs, PCDFs and co-planar PCBs were analysed in five batches of the final product. PCDD/F concentrations were on average 0.1694 TEQ-WHO ng/kg, PCBs were on average 0.0056 TEQ-WHO ng/kg and the sum of PCDD/F and PCBs were on average 0.175 TEQ-WHO ng/kg.⁶⁹ Mycotoxins (Aflatoxins B1, B2, G1, G2, ochratoxin A, zearalenone, fumonisins B1, B2 and B3, DON and T-2 toxin) were found below the LOD.⁷⁰

As regards the microbial contamination, *Salmonella* spp. was negative in 100 g samples, coliforms and *E. coli* were < 3 CFU/g, and aerobic plate count, *Pseudomonas*, *Staphylococcus* coagulase positive, yeasts and moulds were < 10 CFU/g.⁵⁸

The amount of the aforementioned contaminants/impurities does not raise safety concerns.

The presence of viable cells of the production strain was investigated [REDACTED]

[REDACTED] and no growth was found.

The absence of DNA of the production strain was confirmed [REDACTED]

3.3.3.2. Physical characteristics

The additive is a dark brown liquid with fermentation odour.⁷¹ The pH measured in three batches ranged from 10.91 to 10.92.⁷² Analytical data of three batches was provided on viscosity and specific

⁶⁴ Technical dossier/Section II/Annex 2.4.1h.

⁶⁵ Technical dossier/Section 2.1.3.

⁶⁶ Technical dossier/Section II/Annex 2.1.3a. L-lysine analysed by AOAC method 999.13.

⁶⁷ Technical dossier/Section II/Annex 2.1.3a. 1.7% of liquid chromatography–mass spectrometry (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.

⁶⁸ Technical dossier/Section II/Annex 2.1.4b. LOD (in $\mu\text{g/L}$) was 10 for arsenic, cadmium, lead and antimony; and 0.17 for mercury.

⁶⁹ Technical dossier/Supplementary information May 2019/Annexes 6a to e.

⁷⁰ Technical dossier/Section II/Annex 2.1.4a. LOD (in $\mu\text{g/L}$) was 0.07 for aflatoxin G1, 0.09 for aflatoxin G2, 0.3 for aflatoxin B2, 0.5 for aflatoxin B1, 0.8 for ochratoxin A, 4 for fumonisin B1, 7 for zearalenone, 8 for fumonisin B3, 9 for T-2 toxin and 10 for fumonisin B2 and for deoxynivalenol.

⁷¹ Technical dossier/Section II/Annex 2.5.2b.

⁷² Technical dossier/Section II/Annex II.1.4k.

weight at 20°C. Viscosity ranged from 58 to 72 mm²/s, specific weight ranged 1.14–1.15 g/cm³. Vapour pressure (three batches analysed) ranged from 13 to 67 kPa.⁷³

3.3.3.3. Stability and homogeneity

The applicant did not provide data on the shelf life, stability in premixtures and in feedingstuffs and on the capacity of the product under assessment to distribute homogeneously in feed testing the product under assessment. The data provided were the same as mentioned above for concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67439 (Section 3.2.3.3). Since the physical characteristics, the purity and the production process are similar, the FEEDAP Panel considers the previous data as representative for the product under assessment.

The applicant provided information on the stability of the additive under assessment in water for drinking. Three batches were tested at 0.1, 0.5, 1, 5 and 10 g/kg when stored at 16–23°C for 3 days.⁶⁴ No losses were detected.

3.3.4. Physico-chemical incompatibilities in feed

No physicochemical incompatibilities in feed are expected with other additives, medicinal products or other feed materials.

3.3.5. Conditions of use

The conditions of use are the same as described above for L-lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67439 (Section 3.2.5).

3.4. Safety of L-lysine HCl and concentrated liquid L-lysine (base) produced by *C. glutamicum* strains NRRL B-67439 or NRRL B-67535

3.4.1. Safety of the production strains

For both strains, the recipient organism belongs to a species, *C. glutamicum*, that is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment when used for production purposes (EFSA, 2007; EFSA BIOHAZ Panel, 2019).

The genetic modifications introduced in those strains are [REDACTED] considered to be safe. No AMR [REDACTED] genes [REDACTED] are present in the final production strains.

The identity of the strains has been established, the strains are susceptible to the relevant antimicrobials, and the genetic modifications do not raise safety concerns. Therefore, the production strains can be presumed safe when used for production purposes. No viable cells and DNA of the production strain were detected in the additive.

3.4.2. Safety for the target species, consumer and environment

L-Lysine requirements of different species (non-ruminant and ruminant) and animal categories, absorption and metabolic fate of L-lysine, and tolerance to L-lysine excess in the diet were described in previous opinions (EFSA FEEDAP Panel, 2013, 2014).

Both forms of the additive are highly purified. Safety concerns from the additive may derive either from the amino acid or from the residues of the fermentation process/production strain remaining in the final product. The production strains NRRL B-67439 or NRRL B-67535 are presumed safe for production purposes and no viable cells or DNA of the production strains were found in the final products; consequently, no safety concerns for target animal, consumers and the environment would rise 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 products do not pose any environmental safety concern associated with the production strains. 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

⁷³ Technical dossier/Supplementary information May 2019/Annexes 7a to f.

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.

The use of amino acids in water for drinking, in addition to complete diets with a well-balanced amino acid profile may represent a risk for the target species due to nutritional imbalances and hygienic reasons (EFSA FEEDAP Panel, 2010). Moreover, it may result in an increased nitrogen excretion via urine. Therefore, the FEEDAP Panel has concerns on the safety of the use of lysine-containing additives via water for drinking.

The FEEDAP Panel concludes that both forms of L-lysine produced either by *C. glutamicum* NRRL B-67439 or NRRL B-67535 are safe for the target species, for the consumer and for the environment.

3.4.3. Safety for the user

No studies were submitted using the products under assessment as test items to support the safety for the user.

3.4.3.1. Effects on the respiratory system of L-lysine HCl

As regards L-lysine HCl produced using strain NRRL B-67439, the dusting potential may be up to 0.6 g/m³ and the fractions of particles < 100, < 50 and < 10 µm diameter may be up to 3.7%, 2.8% and 1.4%, respectively (Section 3.2.2.2). The user may be exposed by inhalation. No study on acute inhalation toxicity was submitted.

Considering L-lysine HCl produced using strain NRRL B-67535, the dusting potential may be up to 4.6 g/m³ and the fractions of particles < 100, < 50 and < 10 µm diameter may be up to 2.8%, 1.8% and < 1%, respectively (Section 3.3.2.2). Although exposure via inhalation is possible, no study on acute inhalation toxicity was submitted.

In the absence of data is not possible to conclude on its potential to be toxic by inhalation.

3.4.3.2. Effects on skin and eyes of L-lysine HCl and concentrated liquid L-lysine (base)

The applicant provided acute dermal irritation/corrosion studies (in accordance with OECD Guideline 404), eye irritation/corrosion studies (in accordance with OECD 405) and skin sensitisation studies (in accordance with OECD Guideline 406) testing L-lysine HCl and concentrated liquid L-lysine base (3 studies per each form of the additive), where the test item was produced by a different production strain (*C. glutamicum* KCTC 12307BP).^{74,75} Those studies had been previously assessed (EFSA FEEDAP Panel, 2019a).

As both production strains qualify for the QPS approach and there are no safety concerns related to their genetic modifications; the characteristics of the products and the manufacturing process are very similar, the FEEDAP Panel considers that the results of those studies are applicable to the products L-lysine HCl under assessment. L-lysine HCl produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535 is considered not irritant to skin and eyes and not a skin sensitiser.

Regarding the products concentrated liquid L-lysine (base) under assessment, however, the 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 the concentrated liquid L-lysine (base) produced using *C. glutamicum* strains NRRL B-67439 or NRRL B-67535, because the pH of these products is one point higher (10.7 and 10.9, respectively). The FEEDAP Panel considers that the products concentrated liquid L-lysine (base) under assessment are corrosive to skin and eyes and pose a risk by inhalation.

3.4.3.3. Conclusions on the safety for the user

L-lysine HCl produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535 is considered not irritant to skin and eyes and not a skin sensitiser. Due to the absence of data is not possible to conclude on its potential to be toxic by inhalation.

Concentrated liquid L-lysine (base) produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535, due to its high pH (10.7 and 10.9, respectively), is anticipated to be corrosive to skin and eyes and poses a risk by inhalation.

⁷⁴ FAD-2018-0012/Technical dossier/Section III/Annex 3.3.1.2.a, annex 3.3.1.2.b and annex 3.3.1.2.c.

⁷⁵ FAD-2018-0037/Technical dossier/Section III/Annex 3.3.1.2.a, annex 3.3.1.2.b and annex 3.3.1.2.c; and Supplementary information May 2019/Annexes 5b to 5c.

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

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.

3.6. Post-marketing 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 Regulation⁷⁶ and Good Manufacturing Practice.

4. Conclusions

The production strains (*C. glutamicum* strain NRRL B-67439 and strain NRRL B-67535) or their recombinant DNA were not detected in the respective final products. Therefore, the final products do not pose any safety concern associated with the genetic modification of these production strains.

L-Lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535 do not represent a risk for the target species, for the consumer and for the environment. The Panel has concerns for the target species of the use of the additive in water for drinking.

L-Lysine HCl produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535 are considered not irritant to skin and eyes and not skin sensitisers. In the absence of data, the FEEDAP Panel cannot conclude on the potential toxicity by inhalation of L-lysine HCl produced using these strains. Concentrated liquid L-lysine (base) produced using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535, due to their high pH (10.7 and 10.9, respectively), 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 using *C. glutamicum* strain NRRL B-67439 or strain NRRL B-67535 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.

Chronology of the application for L-lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67439

Date	Event
21/03/2018	Dossier received by EFSA: L-Lysine HCl and concentrated liquid L-lysine (base) produced using <i>Corynebacterium glutamicum</i> for all animal species. Submitted by ADM Speciality Ingredients (Europe) B.V.
21/03/2018	Reception mandate from the European Commission
08/05/2018	Application validated by EFSA – Start of the scientific assessment
20/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additive and safety for the user.</i>
08/08/2018	Comments received from Member States
07/08/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
20/06/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – <i>Issues: Characterisation of the production strain</i>
14/05/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
07/10/2019	Spontaneous submission of supplementary information
07/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

⁷⁶ Regulation (EC) No 1831/2005 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.

Chronology of the application for L-lysine HCl and concentrated liquid L-lysine (base) produced using *C. glutamicum* NRRL B-67535

Date	Event
08/06/2018	Dossier received by EFSA: L-Lysine HCl and concentrated liquid L-lysine (base) produced using <i>Corynebacterium glutamicum</i> for all animal species. Submitted by ADM Speciality Ingredients (Europe) B.V.
19/06/2018	Reception mandate from the European Commission
01/10/2018	Application validated by EFSA – Start of the scientific assessment
26/10/2018	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 the formulated additives, safety for the user.</i>
13/12/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
01/01/2019	Comments received from Member States
14/05/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
07/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

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Abbreviations

AFC	EFSA Panel on Flavourings, Processing Aids and Materials in Contact with Food
AMR	antimicrobial resistance
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Contact materials, Enzymes, Flavourings and Processing Aids
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
DON	deoxynivalenol
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FCC	Food Chemical Codex
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
IEC-VIS/FLD	ion exchange chromatography coupled to visible or fluorescence detection
IUPAC	International Union of Pure and Applied Chemistry
LC/MS	liquid chromatography/mass spectrometry
LOD	limit of detection

LOQ	limit of quantification
MIC	minimum inhibitory concentration
NDA	EFSA Panel on Food additives and Nutrient Sources added to Food
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyls
PCDDs	polychlorinated dibenzodioxins
QPS	Qualified Presumption of Safety
RSDr	relative standard deviation for repeatability
RSDR	relative standard deviation for reproducibility
TEQ	toxic equivalent
WGS	whole genome sequence
WHO	World Health Organization

Annex A – Executive summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL B-67439

In the current application, authorisation is sought under Article 4(1) for L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL B-67439, 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.

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 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 L-lysine in premixtures, feedingstuffs and water the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on Ion Exchange Chromatography coupled with photometric detection (IECVIS). 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 the slightly modified AOAC official method 999.13 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. Hence, the EURL recommends for official control this method to quantify lysine 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 Community method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; and (iv) the modified AOAC 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) is not considered necessary.

Annex B – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL B-67535

In the current application authorisation is sought under Article 4(1) for L-lysine monohydrochloride and concentrated liquid L-lysine produced by *Corynebacterium glutamicum* NRRL-B-67535, 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, the dry crystalline powdered L-lysine monohydrochloride has a minimum purity (mass fraction) of 98.5% (minimum of 78.5% of L-lysine) and 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 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 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 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 by analysing lysine in water with the slightly modified AOAC official method 999.13 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 Community method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; and (iv) the modified AOAC 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.