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Safety and efficacy of L-lysine monohydrochloride produced by fermentation with *Corynebacterium glutamicum* DSM 32932 for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-lysine monohydrochloride produced by fermentation with the genetically modified strain of *Corynebacterium glutamicum* DSM 32932. Neither the production strain nor its recombinant DNA were detected in the final product. The additive does not pose any safety concern associated with the genetic modification of the production strain. L-Lysine HCl produced by *C. glutamicum* DSM 32932 is considered safe for the target species, for the consumer and for the environment. L-Lysine HCl produced by *C. glutamicum* DSM 32932 is not toxic by inhalation; it is not irritant to skin and not a skin sensitiser. The additive is not corrosive to eyes but it should be considered as an eye irritant. In general, L-lysine HCl is considered as an efficacious source of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

Keywords: nutritional additive, amino acid, lysine monohydrochloride, safety, efficacy

Requestor: European Commission

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1. Introduction

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

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

The European Commission received a request from Evonik Nutrition & Care GmbH[2] for authorisation of the product L-lysine monohydrochloride produced by fermentation with *Corynebacterium glutamicum* DSM 32932, when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids).

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). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 22 August 2019.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product L-lysine monohydrochloride produced by fermentation with *Corynebacterium glutamicum* DSM 32932, when used under the proposed conditions of use (see Section 3.1.7).

1.2. Additional information

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 is authorised for use in food,[4] cosmetics[5] and as a veterinary medicinal product.[6,7]

L-Lysine hydrochloride is described in a monograph of the European Pharmacopoeia (PhEur 9th edition, 2017) monograph 01/2008:0930.

The Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has published several opinions on the safety and efficacy of L-lysine and/or its salts produced by fermentation using different strains of *C. glutamicum* or *Escherichia coli* for all animal species (EFSA, 2007a; EFSA FEEDAP Panel, 2013, 2014, 2015a,b,c, 2016a,b, 2017a, 2019a,b,c,d,e, 2020).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of L-lysine monohydrochloride produced by fermentation with *C. glutamicum* DSM 32932 as a feed additive.

[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] EVONIK Nutrition & Care GmbH, Rodenbacher Chaussee 4. 63457 Hanau, Germany.
[3] Commission Directive 88/485/EEC of 26 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition. OJ L 239, 30.8.88, 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. OL L 152, 16.6.2009, p. 11.
[8] FEED dossier reference: FAD-2019-0028.

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, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the L-lysine monohydrochloride in animal feed. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-lysine monohydrochloride is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017d), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019f).

3. Assessment

The product subject of this application is L-lysine in the form of monohydrochloride (HCl) produced by fermentation with a genetically modified strain of *C. glutamicum* (DSM 32932). This product is intended to be used as a nutritional additive (functional group: amino acids, their salts and analogues) in feed for all animal species.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *C. glutamicum* which is deposited at the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures under the number DSM 32932.[11] Since six copies of the 16S rRNA gene were sequenced, and are sharing 99% similarity with the type strain of *C. glutamicum,* identity of the production strain as a *C. glutamicum* species is confirmed.[12]

The antimicrobial susceptibility of the production strain was tested [redacted][13]

3.1.1.1. Characteristics of the recipient or parental microorganism

[redacted]

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

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

[11] Technical dossier/Section II/Annex II.17.

[12] Technical dossier/Section II/Supplementary information December 2019/File 4_Annex 31b.

[13] Technical dossier/Section II/Annex II.20.

[redacted] *Corynebacterium glutamicum* is recommended for the qualified presumption of safety (QPS) status for production purposes.

[redacted]

3.1.1.2. Characterisation of the modified sequences

[redacted]

3.1.2. Manufacturing process

The feed additive L-lysine monohydrochloride is produced by fermentation of *C. glutamicum* (DSM 32932). [redacted] The applicant stated that no antimicrobials are used during the production of the additive.[14]

3.1.3. Characterisation of the additive

L-Lysine HCl (International Union of Pure and Applied Chemistry (IUPAC) name: (2*S*)-2,6-diaminohexanoic acid monohydrochloride, synonym L-(+)-2,6-Diamino-*N*-caproic acid monohydrochloride, 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 chemical formula of L-lysine monohydrochloride is $C_6H_{15}ClN_2O_2$. The structural formula is given in Figure 1.

[14] Technical dossier/Section II/Annex II.11.

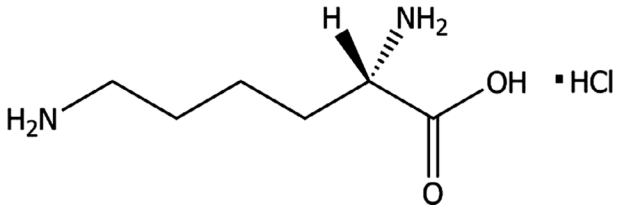

Figure 1: Molecular structure of L-lysine HCl

The specification is for an additive containing ≥ 98.5% L-lysine HCl, ≥ 78% L-lysine on 'as is' basis and ≤ 1.5% water.

The applicant provided data of five batches of the additive.[15] L-lysine was on average 79.5% (range 79.3–80 %) on 'as is basis', chloride was on average 19.5% (range 19.4–19.6), water was on average 0.38% (range 0.1–0.6%), crude ash was 0.04% (range 0.02–0.06 %). The calculated concentration of L-lysine HCl in the additive was on average 99.0% (range 98.7–99.5) on as is basis. The values are in compliance with the specifications.

The specific optical rotation was measured in five batches ranged from +21.0 to +21.1°.[16] This is within the range of the reference values established in the European Pharmacopoeia (range between +21.0 and +22.5°) and confirms the L-enantiomer of lysine in the additive.

3.1.4. Impurities

Three batches were analysed for undesirable substances. Levels of heavy metals and other impurities (cadmium, lead, mercury, chromium, copper, nickel, and zinc, aluminium and arsenic) were reported. All of them were below the limit of quantification (LOQ). Dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) amounted to 0.036 ng WHO-PCDD/F-TEQ/kg, and dioxin-like polychlorinated biphenyls (DL-PCBs) was 0.005 ng WHO-DL-PCB-TEQ/kg. Non-dioxin-like PCBs were 3 μg/kg. Melamine was not detected in any of the batches.[17] In reference to the microbiological contamination, analytical data (three batches) was submitted. *Salmonella* spp. was absent in 25 g sample; total viable count was < 2,000 CFU/g, *Bacillus* spp. ranged from 330 to 390 CFU/g in the 3 batches. Moulds, yeasts, *E. coli* and Enterobacteriaceae were below the LOD; *Clostridium* spp. was below 1.8 most probable number (MPN)/g. Regarding the mycotoxin content, analytical data of the same batches showed that the levels of ochratoxin A and aflatoxins (B1, B2, G1 and G2) were below the LOD. Measurement of endotoxin activity resulted in < 0.1 IU/mL.[18]

Although the parental strain is recommended for QPS approach to safety assessment, when used for the production of amino acids, three samples taken at the end of the downstream processing and representing three batches (final concentration 0.0016–0.8%) were tested for the presence of antimicrobial activity (reference strains used: *B. subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923). Positive controls were included. No inhibition was observed.[19]

No viable cells of the production strain were found in three batches of the additive.[20] [redacted] No colonies were detected.[21]

No recombinant DNA was detected in three batches of the additive [redacted]

[15] Technical dossier/Section II/Annex II.1. Lysine analysed by method ISO 17180. L-lysine HCl calculated by multiplying lysine concentration by 1.249.
[16] Technical dossier/Section II/Annex II.14.
[17] Technical dossier/Section II/Annex II.4. Annex II.5. and Annex II.6. LOQ (in mg/kg) were 0.5 for aluminium, iron and zinc, 0.2 for copper, lead and chromium, 0.01 for mercury and cadmium, and 0.04 for arsenic. The limit of detection (LOD) for melamine was 0.2 mg/kg.
[18] Technical dossier/Section II/Annex II.7 and 8. LODs (in CFU/g) were 100 for moulds and yeasts, 10 for Enterobacteriaceae and 1 *for E. coli*. LOD were 0.08 μg/kg for aflatoxins and 0.1 μg/kg for ochratoxin.
[19] Technical dossier/Section II/Annex II.12.
[20] Technical dossier/Section II/Annex II.10.
[21] Technical dossier/Supplementary information December 2019/File 5_Annex 10b.

[redacted][22]

3.1.5. Physical characteristics

The additive is a solid, free-flowing granular product with density of 1,300 kg/m^3 and water solubility of about 386 g/L.[23] The pH of the product was also determined and was in the range of 4.4–5.6; but the concentration of the test solution was not provided.

The dusting potential (Stauber–Heubach method) ranged from 0.13 to 0.8 g/m^3.[24] Concerning the particle size distribution, three batches were analysed by laser diffraction.[25] The fraction of particles < 10 μm, < 50 μm and < 100 μm of diameter ranged from 0.7 to 1.7%, 2.2 to 4.6% and 3.6 to 11.7%, respectively.

3.1.6. Stability and homogeneity

The shelf-life of L-lysine HCl (three batches) was studied when stored in commercial packaging for 9 months at 10–25°C/20–75% relative humidity (RH) and 40°C/75% RH; L-lysine HCl concentration showed no losses at both conditions. Additional tests at 45°C, 60°C and 85°C were conducted for 3 months, 3 weeks and 3 days, respectively. No losses were detected at the end of the test periods.[26]

The stability of the additive (three batches) in a vitamin/mineral premixture containing choline chloride (11.67%) was studied when supplemented at 9% with the product under assessment. Three samples per batch were collected in double-layer paper bags (inside polyethylene layer) stored at 10–25°C/20–75% RH for 6 months. Recoveries were in the range of 93–98%.[27]

Two different compound feeds for layer hens (based on maize, soy-bean meal and wheat) were supplemented with the additive at 0.15% and 0.13%. One of the feeds was also pelletised at 80°C. Thereafter the feed was packed and stored in double-layer paper bags (inside polyethylene layer) for 3 months at 10–25°C/20–75% RH. Recoveries were 100% (both mash and pelleted) supplemented at 0.15% and was 92% in the feed supplemented at 0.13% (only mesh feed).[28]

The capacity of the additive (one batch) to distribute homogeneously was studied in the premixture and in the mash feed described above for layers. Analyses of 10 subsamples yielded a coefficient of variation (CV) of 4.1% for the premixture and 5.6% for the mash feed.[29]

3.1.7. Conditions of use

L-Lysine is proposed to be used in feeds in order to achieve the adequate amino acid profile and meet the requirements on L-Lysine for all animal species. It can be added directly to the feedingstuffs, complementary feedingstuffs or via premixture. No inclusion levels are proposed as the requirements in quantitative terms depend on the species, the physiological state of the animal, the performance level and the environmental conditions, as well as the amino acid content of the unsupplemented diet.

3.2. Safety

3.2.1. Safety aspects of the production organism

The production organism *C. glutamicum* DSM 32932 was developed from [redacted] The production strain belongs to a species, *Corynebacterium glutamicum*, that qualifies for the QPS approach to safety assessment (EFSA, 2007b) when used for production purposes (EFSA BIOHAZ Panel, 2020). The genes inserted during the genetic modification do not raise safety concerns and the production strain does not carry acquired antimicrobial resistance genes. [redacted] The

[22] Technical dossier/Section II/Annex II.13.
[23] Technical dossier/Section II/Annex II.14.
[24] Technical dossier/Section II/Annex II.14.
[25] Technical dossier/Section II/Annex II.16.
[26] Technical dossier/Section II/Annex II.23.
[27] Technical dossier/Section II/Annex II.24.
[28] Technical dossier/Section II/Annex II.25.
[29] Technical dossier/Section II/Annex II.26.

production strain and its DNA were not detected in the additive. Therefore, the additive does not pose any safety concern regarding the genetic modification of the production strain.

3.2.2. Safety for the target species, consumer and environment

L-Lysine produced by fermentation with *C. glutamicum* DSM 32932 is a product with less than 1% unidentified material. 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 strain DSM 32932 belongs to a species, *C. glutamicum*, that qualifies for the QPS approach to safety assessment (EFSA, 2007b) for production purposes (EFSA BIOHAZ Panel, 2020). The strain was unambiguously identified as *C. glutamicum*, does not harbour acquired antimicrobial resistance genes and no viable cells were found in the final product, consequently no safety concerns for target animal, consumers and the environment would rise from the fermentation residues that may be present in the final additive.

Considering the safety of the amino acid for the target species, L-lysine hydrochloride produced by fermentation with *C. glutamicum* DSM 32932 is considered safe for the target species provided that it is supplemented in appropriate amounts to the diet to satisfy the nutritional requirements of the target species.

Regarding the safety for the consumer, L-lysine hydrochloride produced by fermentation with *C. glutamicum* DSM 32932 supplemented to feed will be incorporated into proteins of tissues and/or products of animal origin and any of their potential excess will be catabolised 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 the additive in animal nutrition.

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 produced by the strain *C. glutamicum* DSM 32932 does not represent a risk for the environment.

3.2.3. Safety for the user

The applicant submitted an acute inhalation toxicity study, an *in vitro* skin irritation study, an *in vitro* eye corrosion study, an *in vitro* eye irritation study and a skin sensitisation study performed with the product under assessment.

3.2.3.1. Effects on the respiratory system

The dusting potential may reach 0.8 g/m^3.[30] No information on the particle size distribution of the dust of the additive was provided. The fraction of particles in the additive < 10 μm, < 50 μm and < 100 μm of diameter ranged from 0.7 to 1.7%, 2.2 to 4.6% and 3.6 to 11.7%., respectively. The user can be exposed by inhalation.

The acute inhalation toxicity of the additive was tested in a valid study performed in accordance to OECD Guideline 403.[31] The particle size distribution of the aerosol showed that the mass median aerodynamic diameter was 3.29 μm. The acute inhalation median lethal concentration (4-h LC_{50}) of the additive in the Sprague–Dawley strain rat was greater than 5.25 mg/L.

3.2.3.2. Effects on skin and eyes

The skin irritation potential of L-Lysine HCl produced by *C. glutamicum* DSM 32932 was studied in an *in vitro* skin irritation test (human skin model test), according to OECD Guideline 439,[32] which showed that it is not a skin irritant.

In an *in vitro* bovine corneal opacity and permeability assay according to OECD Guideline 437,[33] the additive was not corrosive to eyes.

[30] Technical dossier/Section II/Annex II.14.
[31] Technical dossier/Section III/Annex III.1.
[32] Technical dossier/Section III/Annex III.4.
[33] Technical dossier/Section III/Annex III.3.

The eye irritation potential of the additive was studied in an *in vitro* eye irritation test (human cornea model test) according to OECD TG 492.[34] Under the experimental conditions reported, L-Lysine HCl possesses an eye irritating potential.

L-Lysine HCl produced by *C. glutamicum* DSM 32932 (formulated in 1% aqueous Pluronic®) was assessed for its possible skin sensitising potential according to OECD TG 429.[35] The test item is not a skin sensitiser under the test conditions of this study.

3.2.3.3. Conclusions on the safety for the user

L-Lysine HCl produced by *C. glutamicum* DSM 32932 is not toxic by inhalation; it is not irritant to skin and not a skin sensitiser. The additive is not corrosive to eyes but it should be considered as an eye irritant.

3.3. Efficacy

Efficacy studies are not required for amino acids naturally occurring in proteins of plants and animals. The nutritional role of the amino acid L-lysine is well established in the scientific literature. The efficacy of L-lysine for both non-ruminant and ruminant species was described in two previous opinions (EFSA FEEDAP Panel, 2013, 2014). In general, L-lysine HCl is considered as efficacious source 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.

4. Conclusions

L-Lysine HCl is produced by the genetically modified stain of *C. glutamicum* DSM 32932. Neither the production strain nor its recombinant DNA were detected in the final product. The additive does not pose any safety concern associated with the genetic modification of the production strain.

L-Lysine HCl produced by *C. glutamicum* DSM 32932 is considered safe for the target species, for the consumer and for the environment.

L-Lysine HCl produced by *C. glutamicum* DSM 32932 is not toxic by inhalation, it is not irritant to skin and not a skin sensitiser. The additive is not corrosive to eyes but it should be considered as an eye irritant.

In general, L-lysine HCl is considered as efficacious source of the essential amino acid L-lysine for non-ruminant animal species. For the supplemental L-lysine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

5. Documentation as provided to EFSA/Chronology

Date	Event
26/04/2019	Dossier received by EFSA. L-lysine monohydrochloride produced by fermentation with Corynebacterium glutamicum DSM 32932 for all animal species submitted by Evonik Nutrition and Care GmbH.
06/05/2019	Reception mandate from the European Commission
22/08/2019	Application validated by EFSA – Start of the scientific assessment
23/10/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation*
12/11/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
22/11/2019	Comments received from Member States
23/12/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
19/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

[34] Technical dossier/Section III/Annex III.2.
[35] Technical dossier/Section III/Annex III.5.

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Abbreviations

CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
LC_{50}	lethal concentration, median
LOD	limit of detection
LOQ	limit of quantification
MPN	most probable number
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
RH	relative humidity

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-lysine monohydrochloride produced by fermentation with *Corynebacterium glutamicum* DSM 32932

In the current applications authorisation is sought under Article 4(1) for *L-lysine monohydrochloride*, *concentrated liquid L-lysine* and *l-lysine sulphate produced by Corynebacterium glutamicum KCCM80183* and *L-lysine monohydrochloride produced by Corynebacterium glutamicum DSM32932*, under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species.

According to the Applicants, *L-lysine monohydrochloride* contains a minimum (mass fraction) of 78 % of *L-lysine* as active substance, while the *concentrated liquid L-lysine* and the *L-lysine sulphate* contain a minimum of 50 and 55 % of *L-lysine*, respectively.

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

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

For the quantification of *L-lysine* in *premixtures* and *feedingstuffs* one Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled with photometric detection (IEC-VIS), which was previously recommended by the EURL. This method, designed only for the analysis of amino acids in *premixtures* and *feedingstuffs*, does not distinguish between the salts and the amino acid enantiomers. The following performance characteristics were reported for the quantification of total *lysine*: RSDr ranging from 2.1 to 2.8 % and RSDR ranging from 3 to 6.7 %.

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

In addition, the EURL found the "L-lysine monohydrochloride monograph" of the Food Chemical Codex (FCC) for the identification of *L-lysine monohydrochloride* in the *feed additive* and the generic European Pharmacopoeia monograph (Ph. Eur. 20301) for the identification of sulphate ion in *L-lysine sulphate*.

In the frame of these authorisations the EURL recommends for official control (i) the "L-lysine monohydrochloride monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-lysine monohydrochloride* in the *feed additive*; (ii) the European Pharmacopoeia monograph (Ph. Eur. 01/2008:20301) for the identification of the sulphate ion in *L-lysine sulphate*; (iii) the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) to quantify free *lysine* in the *feed additive* and *premixtures* (containing more than 10 % *lysine*); and (iv) the European Union method based on IEC-VIS for the quantification of *lysine* in *premixtures*, *feedingstuffs* and *water*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005), as last amended by Regulation (EU) 2015/1761) is not considered necessary.