Safety and efficacy of a feed additive consisting of concentrated liquid L-lysine, L-lysine monohydrochloride and concentrated liquid L-lysine monohydrochloride produced by Escherichia coli NITE BP-02917 for all animal species (Metex NoovistaGo)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fasmon Durjava, Maryline Koubi, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Debora Glandorf, Luca Tosti, Montserrat Anguita, Rosella Brozzi, Joana Firmino, Jaume Galobart, Yolanda García Cazorla, Jordi Ortuño Casanova, Elisa Pettenati, Joana Revez and Jordi Tarrés-Call

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of concentrated liquid L-lysine, L-lysine monohydrochloride and concentrated liquid L-lysine monohydrochloride produced by Escherichia coli NITE BP-02917 as nutritional and as sensory (flavouring compound) feed additives for all animal species. The production strain did not carry antimicrobial resistance genes and no viable cells of the production strain were detected in the final products. However, since no sequences of concern remained in the production strain, the potential presence of that DNA did not raise safety concerns. The use of the three forms of L-lysine produced by E. coli NITE BP-02917 in supplementing feed to compensate for L-lysine deficiency in feedingstuffs was safe for the target species. This conclusion would also cover the use as a sensory additive. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking. The use of the three forms of L-lysine produced by E. coli NITE BP-02917 in animal nutrition was considered safe for the consumers and for the environment. Concentrated liquid L-lysine, L-lysine HCl and concentrated liquid L-lysine HCl were not considered to have the potential to cause respiratory toxicity, or skin sensitisation. L-Lysine HCl and concentrated liquid L-lysine HCl were not considered skin and eye irritants. Concentrated liquid L-lysine, due to its high pH, might be corrosive for skin and eyes. The three forms were considered 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. The three forms of the additive were also considered efficacious as feed flavouring compounds under the proposed conditions of use.

Keywords: nutritional additives, amino acid, L-lysine, Escherichia coli NITE BP-02917, safety, efficacy

Requestor: European Commission
Question number: EFSA-Q-2021-00462
Correspondence: feedap@efsagoogle
Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fasmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Metex NoovistaGo2 for the authorisation of the additives consisting of three forms of lysine, concentrated liquid L-lysine (minimum 50% L-lysine), solid L-lysine monohydrochloride (minimum 78% L-lysine), and concentrated liquid L-lysine monohydrochloride (minimum 22.4% L-lysine), produced by Escherichia coli NITE BP-02917 when used in feed for all animal species as nutritional additives (functional group: amino acids, their salts and analogues) and as sensory additives (functional group: flavouring compounds).

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 22 October 2021.

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 feed additive consisting of concentrated liquid L-lysine, L-lysine monohydrochloride, and concentrated liquid L-lysine monohydrochloride, produced by Escherichia coli NITE BP-02917, when used under the proposed conditions of use (see Section 3.1.7).

1.2. Additional information

Concentrated liquid L-lysine (minimum 50% L-lysine), L-lysine monohydrochloride (minimum 78% L-lysine) and concentrated liquid L-lysine monohydrochloride (minimum 22.4% L-lysine) produced by E. coli NITE BP-02917 are not currently authorised in the European Union (EU). L-Lysine produced using different microbial strains is currently authorised for its use in all animal species as a nutritional additive and as a sensory 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, 2019).

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 Corynebacterium glutamicum, Escherichia coli and Corynebacterium casei for all animal species.

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1 Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
2 METEX NOOVISTAGO (MNG), 32 Rue Guersant, 75,017 Paris, France.
3 See the European Union Register of Feed Additives, available online https://ec.europa.eu/food/system/files/2021-12/animal-feed_additives_eu-register_1831-03.pdf
4 Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35.
5 Commission Decision (EU) 2019/701 of 5 April 2019 establishing a glossary of common ingredient names for use in the labelling of cosmetic products. OJ L 121, 8.5.2019, pp. 1–370.
6 Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.
7 Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OL L 152, 16.6.2009, p. 11.
2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier in support of the authorisation request for the use of concentrated liquid L-lysine, L-lysine monohydrochloride and concentrated liquid L-lysine monohydrochloride produced by fermentation with E. coli NITE BP-02917 as nutritional or as sensory feed additives for all animal species.

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 concentrated liquid L-lysine, L-lysine monohydrochloride and concentrated liquid L-lysine monohydrochloride produced by fermentation with E. coli NITE BP-02917 in animal feed. The Executive Summary of the EURL report can be found in Annex A.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of concentrated liquid L-lysine, L-lysine monohydrochloride and concentrated liquid L-lysine monohydrochloride produced by fermentation with Escherichia coli NITE BP-02917 is in line with the principles laid down in Regulation (EC) No 429/2008 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

Concentrated liquid L-lysine (minimum 50% L-lysine), L-lysine monohydrochloride (minimum 78% L-lysine) and concentrated liquid L-lysine monohydrochloride (minimum 22.4% L-lysine), produced by Escherichia coli NITE BP-02917 are intended to be used as nutritional additives (functional group: amino acids, their salts and analogues) and as sensory additives (functional group: flavouring compounds) in feed and water for drinking for all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The L-lysine in the three forms of the additive is produced by a genetically modified strain of Escherichia coli that is deposited in the Japanese National Institute of Technology and Evaluation with accession number NITE BP-02917.

The production strain was identified as an E. coli K-12 derivative.

The susceptibility of the production strain to the relevant antibiotics was tested against the list of antimicrobials described for Enterobacteriaceae in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

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8 FEED dossier reference: FAD-2021-0075.
9 The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/fnrep_fad-2021-0075_l-lysine.pdf.
10 Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
11 Technical dossier/Section II/An Sect II Supp/An Supp II.1.
12 Technical dossier/Section II/An Sect II Supp/An Supp II.2–2.
Therefore, the production strain is considered to be susceptible to all relevant antimicrobials.

The WGS data of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes. The FEEDAP Panel notes, however, that the results of the WGS search for AMR genes were poorly reported and only in the form of a statement. Considering that the production strain was susceptible to the relevant antibiotics tested, the data suggest that the production strain does not carry any acquired antibiotic resistance genes of concern.

The WGS data of the production strain was also interrogated for the presence of known toxins and virulence factor genes. None of these genes were present in the genome of the production strain.

3.1.1.1. Information related to the genetically modified microorganism

3.1.1.1.1. Characterisation of the recipient or parental microorganism

3.1.1.1.2. Characterisation of the donor organism(s)

3.1.1.1.3. Description of the genetic modification

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13 Technical dossier/Section II/An Sect II Supp/An Supp II.2–1.
14 Technical dossier/Section II/An Sect II Supp/An Supp II.2–2 and supplementary information May 2022/Annex supp_info_An_2_Weill_2022.
15 Technical dossier/Section II/An Sect II Supp/ Ann Supp II 80.
3.1.2. Manufacturing process

The three forms of l-lysine under assessment are produced by fermentation using E. coli NITE BP-02917 as production strain. The applicant stated that no antibiotics are used during the manufacturing process.17

3.1.3. Characterisation of the concentrated liquid l-lysine

L-Lysine (International Union of Pure and Applied Chemistry (IUPAC) name: (2S)-2,6 diaminohexanoic acid; synonym: (S)-2,6-diaminocaproic acid), a compound identified with the Chemical Abstracts Service (CAS) No 56-87-1 and the European Inventory of Existing Commercial chemical Substances (EINECS) No 200-294-2, has a molecular weight of 146.2 g/mol. The molecular formula is C₆H₁₄N₂O₂ and the molecular structure is given in Figure 1.

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16 Technical dossier/Section II/An Sect II Supp/An Supp II 80.
17 Technical dossier/Section II/Subsection 2.3.1.2.
The product is specified to contain ≥ 50% L-lysine. Compliance with the specification was shown in eight batches in which L-lysine was on average 51.1% on ‘as is’ basis (range 50.4–51.9%).

Water content was on average 46.8% (range 46.3–47.5%). The calculated amount of lysine on dry matter (DM) basis was on average 96.1% (range 95.1–97.0%). Other analysed constituents (3 batches analysed) were ammonium (0.01%), nitrates (range 0.001–0.002%) and free amino acids other than lysine (summing up 0.05–0.06%).

3.1.3.1. Impurities

Three batches of the additive were analysed for impurities. Concentration of arsenic was < 0.1 mg/kg; cadmium < 0.01 mg/kg, lead < 0.05 mg/kg and mercury < 0.005 mg/kg. From additional elements analysed, of relevance were the levels of iron (3–4 mg/kg), manganese (2–3 mg/kg), sulfur (1,300–1,800 mg/kg), sodium (404–527 mg/kg), potassium (706–769 mg/kg), magnesium (6.5–7.0 mg/kg) and phosphorus (12–17 mg/kg). Fluorine was < 20 mg/kg, melamine < 0.05 mg/kg, and polycyclic aromatic hydrocarbons were < 0.5 μg/kg except for benzo(a)pyrene which was 0.6–0.8 μg/kg.

Mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisins B1 and B2, deoxynivalenol, T-2 toxin, HT-2 toxin and zearalenone) were below the limit of quantification (LOQ). Pesticides (organochlorides including pyrethroids and organophosphorus) were below the LOQ. Biogenic amines (cadaverine, putrescine, spermidine and tyramine) represented 14–23 mg/kg.

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (co-planar PCBs) were analysed in three batches and found below the corresponding LOQ. The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs ranged 0.032–0.036 ng WHO-PCDD/F-TEQ/kg and 0.049–0.056 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. For no dioxin-like PCBs (ICES6) the calculated upper bound levels ranged 0.030–0.035 μg/kg additive.

The detected amounts of the above-described impurities do not raise safety concerns.

Five batches of the final product were analysed for microbiological contamination. Salmonella spp. was not detected in 25 g samples; total aerobic count was < 400 CFU/g; Bacillus cereus count was < 100 CFU/g except in one batch that was < 400 CFU/g; coagulase positive Staphylococcus were < 100 CFU/g; and coliforms, Enterobacteriaceae, yeasts and moulds were < 10 CFU/g.

Endotoxin activity was analysed in three batches and ranged from 360 to 408 IU/mL.

The presence of viable cells of the production strain in the final product was tested in three batches of the additive in triplicate.

Figure 1: Molecular structure of L-lysine

The product is specified to contain ≥ 50% L-lysine. Compliance with the specification was shown in eight batches in which L-lysine was on average 51.1% on ‘as is’ basis (range 50.4–51.9%).

Water content was on average 46.8% (range 46.3–47.5%). The calculated amount of lysine on dry matter (DM) basis was on average 96.1% (range 95.1–97.0%). Other analysed constituents (3 batches analysed) were ammonium (0.01%), nitrates (range 0.001–0.002%) and free amino acids other than lysine (summing up 0.05–0.06%).

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Endotoxin activity was analysed in three batches and ranged from 360 to 408 IU/mL.

The presence of viable cells of the production strain in the final product was tested in three batches of the additive in triplicate.

18 Technical dossier/Section II/Annexes II.10 and II.14. Analytical method to measure lysine was NF EN ISO 17180.
19 Technical dossier/Section II/Annex II.11.
20 Technical dossier/Section II/Annex II.11. LOQ in μg/kg was 0.1 for aflatoxins B1, B2 and G1; 0.2 for aflatoxin G2 and ochratoxin A; 20 for fumonisins B1 and B2, for T-2 toxin, HT-2 toxin and for zearealenone; and 50 for deoxynivalenol.
21 Technical dossier/Section II/Annex II.11. LOQ in mg/kg ranged from 0.002 to 0.1 in organochlorides and pyrethroids; and from 0.01 to 0.03 for organophosphorus pesticides, depending on the parameter analysed.
22 Technical dossier/Section II/Annex II.12.
23 Analytical method recombinant factor C (rFC) assay: approved as an alternative method to the conventional Limulus amoebocyte lysate assay.
24 Technical dossier/Section II/Annex II.42.
25 Technical dossier/Section II/An Supp/An Supp II.84.
The presence of recombinant DNA from the production strain was tested in three batches of the additive in triplicate.\textsuperscript{25}

3.1.3.2. Physicochemical properties

Concentrated liquid \(\text{L-lysine}\) is a dark brown liquid. Its density and dynamic viscosity were analysed in five batches and were on average 1,136 kg/m\(^3\) and 43.4 mPa.s, respectively.\textsuperscript{26} The pH (20°C) is 10–11 and the boiling point is stated to be 110–120°C.\textsuperscript{27}

3.1.4. Characterisation of the solid \(\text{L-lysine monohydrochloride}\)

\(\text{L-lysine monohydrochloride}\) (\(\text{L-lysine HCl}\)) (IUPAC name: \((2S)-2,6\text{-diaminohexanoic acid monohydrochloride};\) synonym: \(\text{\((+)-2,6\text{-diamino-}\text{N-caproic acid monohydrochloride, a compound identified with the CAS No. 657-27-2 and the EINECS No. 211-519-9)}\)), has a molecular weight of 182.65 g/mol. The theoretical content of lysine in \(\text{L-lysine HCl}\) is 80%. The molecular formula is \(\text{C}_6\text{H}_{15}\text{ClN}_2\text{O}_2\) and the molecular structure is given in Figure 2.

Figure 2: Molecular structure of \(\text{L-lysine HCl}\)

The product is specified to have a minimum purity (mass fraction on DM basis) of 99% and a minimum \(\text{L-lysine}\) content of 78%.

Compliance with the specification was shown in eight batches in which lysine HCl was on average 99.6% on DM basis (range 99.3–99.8%).\textsuperscript{28} Water content was 0.7% (range 0.5–0.9%). Lysine content on DM was calculated to be on average 79.7% (range 79.5–79.9%). Chloride, analysed in three batches, ranged 18.8–20.4% on a DM basis, and sulfate was 0.1%. Other constituents were free amino acids other than lysine (3 batches analysed) ranging from 0.001 to 0.003%.

The specific optical rotation (5 batches analysed by EuPh method) ranged from +21.3 to +22.3°.\textsuperscript{29} These values are within the reference range specified in the European Pharmacopoeia monograph for this substance (+21.0 to +22.5°) and confirm the \(\text{\text{l-enantiomer}}\) of lysine.

3.1.4.1. Impurities

Three batches of \(\text{L-lysine HCl}\) were analysed for impurities.\textsuperscript{30} Cadmium, lead, mercury and arsenic concentrations were below the LOQ.\textsuperscript{31} From additional elements analysed, of relevance were the levels of iron (1.3–2.3 mg/kg), manganese (0.5–0.6 mg/kg), sulfur (310–350 mg/kg), sodium (105–135 mg/kg), potassium (132–155 mg/kg) and phosphorus (< 3 to 6.9 mg/kg). Fluorine was < 20 mg/kg, melamine < 0.05 mg/kg, and polycyclic aromatic hydrocarbons < 0.5 µg/kg except for benzo(a)pyrene which was 0.7–0.8 µg/kg.

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\textsuperscript{26} Technical dossier/Section II/Annexes II.10 and II.14.
\textsuperscript{27} Technical dossier/Section II/Annex II.39.
\textsuperscript{28} Technical dossier/Section II/Annexes II.4 and II.8. Lysine determined using method NF EN ISO 17180.
\textsuperscript{29} Technical dossier/Supplementary information May 2022/Supp_Info_Annex 6.
\textsuperscript{30} Technical dossier/Section II/Annexes II.5 and II.4.
\textsuperscript{31} LOQ in mg/kg was 0.1 for arsenic, 0.01 for cadmium, 0.05 for lead and 0.005 for mercury.
Mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisins B1 and B2, deoxynivalenol, T-2 toxin, HT-2 toxin and zearalenone) were below the LOQ. Pesticides (organochlorides including pyrethroids and organophosphorus) were below the LOQ. Biogenic amines were below the limit of quantification except for cadaverine, which ranged from 3 to 6 mg/kg.

PCDDs, PCDFs and co-planar PCBs were analysed in three batches and found below the corresponding LOQ. The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs ranged 0.054–0.056 ng WHO-PCDD/F-TEQ/kg and 0.082–0.085 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. For no dioxin-like PCBs (ICES6), the calculated upper bound levels ranged 0.053–0.059 μg/kg additive.

The detected amounts of the above-described impurities do not raise safety concerns.

Five batches of the final product were analysed for microbiological contamination. Salmonella spp. was not detected in 25 g samples; total germ count, Bacillus cereus and coagulase positive Staphylococcus were < 100 CFU/g; and coliforms, Enterobacteriaceae, yeasts and moulds were < 10 CFU/g.

Endotoxin activity was measured in three batches and ranged from 10 to 29 IU/g.

The presence of viable cells of the production strain in the final product was tested in three batches of the additive in triplicate. Growth of the production strain was neither detected.

Absence of DNA from the production strain was tested in three batches of the additive in triplicate.

3.1.4.2. Physicochemical properties

L-Lysine HCl is a white to pale yellow crystalline powder, soluble in water (64.2 g/L at 20°C) and with a pH ranging from 5.6 to 5.9 (5% w/w solution at 20°C). Bulk density (3 batches analysed) ranged from 608–615 kg/m³. Packed bulk density ranged 667–687 kg/m³.

The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed values ranging 4,977–7,452 mg/m³. The particle size distribution of this form of the additive (5 batches by sieving) indicates that most of particles are in the range of 100–1,400 μm diameter, being the fraction of particles < 100 μm from 5 to 10%. Particle size distribution of the dust was analysed by laser-diffraction method; the results showed that 100% of particles had a diameter < 25 μm; the fraction of particles ≤ 10 μm ranged 74–76%; and the fraction of particles < 0.5 μm ranged 1.7 to 2.1%.

32 Technical dossier/Section II/Annex II.5.
33 Technical dossier/Section II/Annex II.5. LOQ in μg/kg was 0.1 for aflatoxins B1, B2 and G1; 0.2 for aflatoxin G2 and ochratoxin A; 20 for fumonisins B1 and B2, for T-2 toxin, HT-2 toxin and for zearalenone; and 50 for deoxynivalenol.
34 Technical dossier/Section II/Annex II.5. LOQ in mg/kg ranged from 0.002 to 0.1 in organochlorides and pyrethroids; and from 0.01 to 0.03 for organophosphorus pesticides, depending on the parameter analysed.
35 Technical dossier/Section II/Annex II.5.
36 Technical dossier/Section II/Annex II.5.
37 Approved as an alternative method to the conventional Limulus amoebocyte lysate assay.
3.1.5. Characterisation of the concentrated liquid L-lysine monohydrochloride

The product is specified to contain ≥ 22.4% of L-lysine.

Compliance with the specification was shown in eight batches in which L-lysine was on average 22.8% on ‘as is’ basis (range 22.5–23.0%). Water content was 63.5% (range 59.5–65.3%). Free amino acids other than lysine

3.1.5.1. Impurities

Three batches of concentrated liquid L-lysine HCl were analysed for impurities. Cadmium, mercury and arsenic concentrations were below the LOQ. Lead ranged from 0.05 to 0.15 mg/kg. From additional elements analysed, of relevance were the levels of iron (20–24 mg/kg), manganese (10–12 mg/kg), chromium (0.14–0.16 mg/kg), nickel (0.2–0.3 mg/kg), cobalt 0.2 mg/kg, zinc (0.8–1.6 mg/kg), sodium (4,170–8,190 mg/kg), calcium (11–16 mg/kg), potassium (3,000–3,540 mg/kg), magnesium (27–35 mg/kg) and phosphorus (67–79 mg/kg). Anionic salts analysed included chloride (38,000–41,000 mg/kg), nitrates (126–171 mg/kg), phosphates (192 and 202 mg/kg in only 2 batches), and sulfates (21,000–26,000 mg/kg). Melamine was <0.05 mg/kg, and polycyclic aromatic hydrocarbons < 0.5 µg/kg.

Mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisins B1 and B2, deoxynivalenol, T-2 toxin, HT-2 toxin and zearalenone) were below the LOQ. Pesticides (organochlorides including pyrethroids and organophosphorus) were below the LOQ. Regarding biogenic amines, analytic values were provided for cadaverine (29–42 mg/kg), putrescine (20–24 mg/kg), 2-phenylethylamine (9–22 mg/kg), tyramine (20–24 mg/kg), 2-phenylethylamine (9–22 mg/kg), tryptamine were below the LOQ. Organic acids analysed were acetic acid (1,600–2,600 mg/kg), succinic acid (77–200 mg/kg), butyric acid (<600–780 mg/kg), citric acid (<90–140 mg/kg), lactic acid (47–200 mg/kg), formic acid (150–200 mg/kg), oxalic acid (20–200 mg/kg), propionic acid (720–1,000 mg/kg) and pyruvic acid (<3–10 mg/kg).

PCDDs, PCDFs and co-planar PCBs were analysed in three batches and found below the LOQ. The calculated (upper bound) levels of dioxins and the sum of dioxins and dioxin-like-PCBs ranged 0.052–0.16 ng WHO-PCDD/F-TEQ/kg and 0.077 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. For no dioxin-like PCBs (ICES6) the calculated upper bound levels ranged 0.041–0.042 µg/kg additive.

The detected amounts of the above-described impurities do not raise safety concerns.

Five batches of the final product were analysed for microbiological contamination. Salmonella spp. was not detected in 25 g samples; total germ count (at 30°C) was <100 CFU/g except in three batches where it ranged from <400 to 600 CFU/g; Bacillus cereus (at 30°C) were <100 CFU/g except in one batch that indicated values <400 CFU/g; coagulase positive Staphylococcus (at 37°C) were <100 CFU/g; and presumptive coliforms (at 30°C), Enterobacteriaceae (at 37°C), yeasts (at 25°C) and moulds (at 25°C) were <10 CFU/g.

Endotoxin activity of three batches of this form of the additive was analysed (method recombinant factor C (rFC) assay) and ranged 290–340 IU/mL. The presence of viable cells of the production strain in the final product was tested in three batches of the additive in triplicate, using two methods.

Growth of the

42 Technical dossier/Section II/Annexes II.15 and II.19. Analytical method for measuring lysine derived from NF EN ISO 17180.
43 Technical dossier/Section II/Annexes II.15 and II.16.
44 Technical dossier/Section II/Annex II.16. LOQ in mg/kg was 0.1 for aflatoxins B1, B2 and G1; 0.2 for aflatoxin G2 and ochratoxin A; 20 for fumonisins B1 and B2, for T-2 toxin, HT-2 toxin and for zearalenone; and 50 for deoxynivalenol.
45 Technical dossier/Section II/Annex II.16. LOQ in mg/kg from 0.002 to 0.1 in organochlorides and pyrethroids; and from 0.01 to 0.03 for organophosphorus pesticides, depending on the parameter analysed.
46 Technical dossier/Section II/Annex II.16. LOQ in mg/kg was 1 for spermine and histamine; and 5 for tryptamine.
47 Technical dossier/Section II/Annex II.16.
48 Technical dossier/Section II/Annex II.16.
49 Technical dossier/Section II/Annex II.17.
50 Technical dossier/Section II/Annex II.85.
production strain was neither detected in the additive.50 The absence of DNA from the production strain was tested in three batches of the additive in triplicate.

3.1.5.2. Physicochemical properties

Concentrated liquid L-lysine HCl is a dark brown liquid with a pH at 20°C of 7.5–8.5 and a boiling point of 110–120°C.51 Density (measured in 6 batches) and dynamic viscosity (measured in 5 batches) averaged 1.128 kg/m³ and 5.4 mPa.s, respectively.52

3.1.6. Stability and homogeneity of all three forms of the additive

Data submitted on stability (shelf life, stability in premixtures and stability in feedingstuffs) and on the capacity of the additive to homogeneously distribute in feed were obtained using similar L-lysine products originating from another production strain (E. coli FERM BP-10941). Since there are no essential differences between the L-lysine products made by fermentation with the two different E. coli strains in terms of composition, manufacturing process and physicochemical properties, the stability and homogeneity outcomes of the previous opinion (EFSA FEEDAP Panel, 2013) are considered applicable to the products under assessment.

The stability of each form of the additive (3 batches/form) in water for drinking was studied when supplemented at 0.5, 2.5 and 5 g/L of tap water (each batch tested in a different concentration).53 Samples were stored at 25 and at 40°C in glass containers for ≥ 50 h. Losses at the end of the storage period were only observed in the samples stored at 25°C. In concentrated liquid L-lysine and solid L-lysine HCl, losses ranged 0–2%; in concentrated liquid L-lysine HCl losses ranged 0–6%.

3.1.7. Conditions of use

When used as a nutritional additive (amino acids), the three forms of the additive are proposed for all animal species, to be incorporated either directly into the final feed or via premixtures. Liquid forms are added to feed via spraying during feed conditioning. All three forms of the additive can be used in water for drinking. No inclusion levels are proposed as the optimal dietary requirement in quantitative terms depends on the species as well as on the physiological state of the animal, performance level and environmental conditions.

When used as sensory additives (flavouring compound), any of the three forms of the additive can be added to feed via flavouring premixtures at a recommended inclusion level of 25 mg/kg complete feed without time limits. No specific use levels are proposed for use in water.54

3.2. Safety

3.2.1. Safety of the production micro-organism

The parental strain is an E. coli K-12 derivative. The genetic modifications performed to obtain the production strain NITE BP-02917 have the purpose to increase the production of L-lysine. None of the introduced modifications raises a safety concern. The production strain is free of antibiotic resistance genes used during the genetic modification process. The production strain was not detected in the additive.51 since no sequences of concern remain in the final production strain, the potential presence of recombinant DNA in the final products does not raise any safety concern.

51 Technical dossier/Section II/Annex II.40.
52 Technical dossier/Section II/Annexes II.15 and II.19.
53 Technical dossier/Section II/Annex II.62.
54 Technical dossier/Supplementary information May 2022/Lys_NITE_BP_02917_Supp_Info_15022022_CONF.
3.2.2. Toxicological studies

According to the manufacturing process (see Section 3.1.2), concentrated liquid L-lysine HCl contains the highest concentration of substances other than L-lysine (about 37%). Consequently, tests using concentrated liquid L-lysine HCl are considered by the FEEDAP Panel to be appropriate (as a worst-case scenario) for the safety assessment of the other two products concentrated liquid L-lysine and solid L-lysine HCl (containing about 4% substances other than lysine, and < 1% substances other than lysine HCl, respectively).

The applicant provided three toxicity studies testing the concentrated liquid L-lysine HCl under assessment: a bacterial reverse mutation test; an in vitro micronucleus test; and a repeated dose 90-day oral toxicity study.

3.2.2.1. Bacterial reverse mutation test

An Ames test was performed to assess the potential of concentrated liquid L-lysine-HCl (containing 22.95% of the active substance lysine) to induce gene mutations in bacteria.55 The study was performed in Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and Escherichia coli WP2 uvrA in accordance with OECD TG 471 (1997) and GLP principles. A stock solution was prepared based on the purity of the active substance of 22.95% and corrected for the volume of the test substance (the additive). Five concentrations of the additive were tested ranging from 62 to 5,000 μg/plate in the absence and presence of metabolic activation. No toxicity and precipitation were reported. No biologically relevant increase in the number of revertant colonies was observed in any strain at any of the concentrations tested. Liquid L-lysine HCl did not induce gene mutations in bacteria under the experimental conditions applied in the study.

3.2.2.2. In vitro mammalian cell micronucleus test

To evaluate the potential of concentrated liquid L-lysine HCl (containing 22.95% of the active substance lysine) to induce chromosome damage, an in vitro micronucleus test was carried out in whole blood human lymphocytes according to OECD Test Guideline 487 (2016) and GLP principles.56 A stock solution was prepared based on the purity of the active substance of 22.95% and corrected for the volume of the test substance (the additive). Cells were treated with 500, 1,000, 2000 μg/mL of the test substance in the short treatment (4 h + 20 h of recovery) in the presence and absence of metabolic activation and 500, 1,000, 1,500, 2000 μg/mL in the continuous treatment (24 h) in the absence of metabolic activation. Cytotoxicity up to 51% compared to vehicle control values was induced by continuous treatment with 2000 μg/mL of the test substance. No increase of the frequency of micronuclei was observed in binucleated cells in any experimental condition. Liquid L-lysine HCl did not induce clastogenic and aneugenic effects in human lymphocytes under the experimental conditions employed in this study.

3.2.2.3. Repeated dose 90-day toxicity study

Groups of ten animals/sex of Crl CD (SD) rats were administered by gavage with concentrated liquid L-lysine HCl formulation (lot 9,021, containing 22.81% lysine) at nominal dose levels of 0, 400, 1,350 or 4,128 mg additive/kg body weight (bw) per day (corresponding to nominal dose levels of 0, 251, 846 and 2,587 mg lysine/kg bw per day, respectively) for 13 weeks.57 The study was performed following the OECD TG 408 and was GLP compliant.

No mortalities were reported during the study. Ptyalism in all males and half of females were observed at 4,128 mg/kg bw per day, occurring on several occasions.

As regards blood parameters, a statistically significant decrease of platelets count compared to controls was reported in males and an increase of reticulocytes was observed in females at 4,128 mg/kg bw per day. However, the platelets change fell within the normal biological variability. In the absence of effects on red blood cell counts, reticulocyte increase was considered of doubtful relation to treatment and of no toxicological significance. An increase of blood sodium was reported in both males and females; however, it was not dose-related. A statistically significant decrease of blood potassium level was reported in males and females at 4,128 mg/kg bw per day of 8% and 11%, respectively. In females, a statistically significant increase of blood urea was reported at the highest dose tested. At the same dose, a statistically significant increase of alanine aminotransaminase (ALT) was observed in

55 Technical dossier/Section III/Annex III.56.
56 Technical dossier/Section III/Annex III.63.
57 Technical dossier/Section III/Annex III.67.
both males and females. However, given the small magnitude of change in males and the absence of histopathological correlates, ALT changes were considered of no toxicological relevance.

Following urine analysis, a slight pH decrease was observed in males and females treated at doses \( \geq 1,350 \text{ mg/kg per day} \), with limited dose response in males and no clear dose response in females. In males, at the highest dose the pH decrease was accompanied by a slight specific gravity increase.

No treatment-related effects were observed upon gross pathology investigation. Statistically significant increase in mean kidneys weights (absolute and relative to body weight) was reported in males and females at 4,128 mg/kg per day. No histopathological findings were observed in males, while in females, an increased incidence of minimal mononuclear cell infiltration (4/10) was reported when compared to controls (1/10).

Overall, the FEEDAP Panel considered the effects observed at the highest level tested (e.g., increase in blood urea, decrease in urinary pH, ptyalism, increased relative/absolute weight of kidney) physiologically related with the nature of the active substance and identified a no observed adverse effect level (NOAEL) for the additive of 4,128 mg/kg body weight per day (the highest dose tested, corresponding to a lysine content of 2,587 mg/kg bw per day).

### 3.2.2.4. Conclusions of the toxicological studies

Concentrated liquid \( \alpha \)-lysine HCl produced by \( E. \ coli \) NITE BP-02917 shows no evidence of genotoxicity or sub-chronic oral toxicity. Considering that the other two products have a much higher purity, the Panel considers that these conclusions would apply to concentrated liquid \( \alpha \)-lysine and \( \alpha \)-lysine HCl under assessment.

### 3.2.3. Safety of concentrated liquid \( \alpha \)-lysine, solid \( \alpha \)-lysine HCl and concentrated liquid \( \alpha \)-lysine HCl for the target species, consumers and the environment

The \( \alpha \)-lysine requirements of different non-ruminant species and animal categories, the absorption and metabolic fate of \( \alpha \)-lysine, the tolerance to \( \alpha \)-lysine excess and 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). Safety concerns from the additives may derive either from the amino acid or the residues of the fermentation process/production strain remaining in the final product. The studies provided by the applicant allow to conclude that there is no evidence for genotoxicity or subchronic oral toxicity and the production strain NITE BP-02917 is a derivative of \( E. \ coli \) K-12 which is well characterised and its safety (non-pathogenicity) has been documented (Gorbach, 1978). Any potential presence of recombinant DNA in the final additives would not pose a safety concern. The use of the amino acid ‘per se’ will not raise safety concerns for the target animals provided it is supplemented in appropriate amounts to satisfy the nutritional requirements of the animals in \( \alpha \)-lysine-deficient diets. However, due to the risk of nutritional imbalances and hygienic reasons, associated to the use of amino acids via water for drinking (EFSA FEEDAP Panel, 2010), the FEEDAP Panel has concerns on the safety of the use of the amino acid via water for drinking.

As regards the endotoxin activity (lipopolysaccharides) in the final products (up to 408 IU/mL, up to 29 IU/g or up to 340 IU/mL in the concentrated liquid \( \alpha \)-lysine, solid \( \alpha \)-lysine HCl and concentrated liquid \( \alpha \)-lysine HCl, respectively) is more than three orders of magnitude lower than that commonly observed in feedingstuffs (1,000 IU/mg; Cort et al., 1990) and is, therefore, of no concern for the target species.

Since the levels proposed for the three forms of the additive when used as flavouring compound (up to 25 mg/kg complete feed) are substantially lower than the animal requirements, the FEEDAP Panel considers that the three forms of \( \alpha \)-lysine produced by \( E. \ coli \) NITE BP-02917 are safe when used as a flavouring compound.

The absorption, distribution, metabolism and excretion of \( \alpha \)-lysine were described in a previous scientific opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2013). The use of the amino acid \( \alpha \)-lysine itself in animal nutrition is considered safe for consumers. Potential concerns for consumers might arise from the fermentation process. However, the toxicological studies provided indicate that the three forms of the additive under assessment show no evidence of genotoxicity or subchronic oral toxicity (see Section 3.2.2) and the production strain is considered safe (see Section 3.2.1).

The amino acid \( \alpha \)-lysine is a physiological and natural component of animals and plants. When supplemented to feed, it will be incorporated into proteins of tissues and/or products of animal origin and any potential excess will be catabolised and excreted as urea/uric acid and carbon dioxide. The
use of amino acids in water for drinking, when given in addition to complete diets with a well-balanced amino acid profile, would disturb the nitrogen balance and increase nitrogen excretion via urine. The use of L-lysine in animal nutrition (either used as a nutritional additive or as a flavouring compound) would not lead to any localised increase in the concentration of L-lysine or its metabolites in the environment. Viable cells of the production strain *E. coli* NITE BP-02917 were not detected in the final products. Since no sequences of concern are present in the final production strain, a risk for the environment resulting from the use of the additives under assessment in animal nutrition is not foreseen.

### 3.2.3.1. Conclusions on safety for the target species, consumers and the environment

The use in animal nutrition of concentrated liquid L-lysine, solid L-lysine HCl and concentrated liquid L-lysine HCl produced by *E. coli* NITE BP-02917 is safe for the target species. The FEEDAP Panel has concerns on the use of amino acids in water for drinking for hygienic reasons, and due to the risk of imbalances when administered simultaneously via feed.

The use of the three forms of L-lysine produced by *E. coli* NITE BP-02917 in animal nutrition is considered safe for consumers and the environment.

### 3.2.4. Safety for the user

No specific information was submitted testing the products under assessment. The applicant submitted the same set of studies on the safety of the three forms of the additive for the user as in a previous opinion (EFSA FEEDAP Panel, 2013). These studies were considered valid also for a subsequent opinion (EFSA FEEDAP Panel, 2014). The studies consisted of acute inhalation toxicity, eye irritation, skin irritation and skin sensitisation tests conducted on formulations produced by other related *E. coli* K-12 strains (FERM BP-08659 or FERM BP-10941).

As the different forms of the additive under assessment are produced in a similar manufacturing process, have similar characteristics and purity, the FEEDAP Panel considers that the results of the studies testing concentrated liquid L-lysine, solid L-lysine HCl and concentrated liquid L-lysine HCl produced by *E. coli* FERM BP-10941 or *E. coli* K-12 FERM BP-08659 can be used to support the safety for the user of the additives under assessment. Therefore, concentrated liquid L-lysine, solid L-lysine HCl and concentrated liquid L-lysine HCl produced by *E. coli* NITE BP-02917 are considered not irritant to skin or eyes nor skin sensitisers. L-Lysine HCl solid is not hazardous by inhalation. However, the FEEDAP Panel notes that in the case of concentrated liquid L-lysine, due to its high pH, it may be corrosive for skin and eyes.

#### 3.2.4.1. Exposure to endotoxins by inhalation

The solid L-lysine HCl showed a dusting potential up to 7,452 g/m³ and the particle size distribution indicated that the fraction of the additive having particles of a diameter < 100 μm ranged 5–10% (see Section 3.1.4.2). Consequently, the user can be exposed by inhalation when handling the additive.

Concerning endotoxins, users can suffer from occupational respiratory disease depending on the level of endotoxins in air and dust (Rylander et al., 1999; Thorn, 2001). The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA guidance on user safety (EFSA FEEDAP Panel, 2012), is described in Appendix A. The threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from the provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based on calculations of the content of endotoxins in dust (up to 28.5 IU/g in the solid product), exposure by inhalation would be 118 IU per eight-hour working day, indicating no risk from exposure to endotoxins for people handling the additive.

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

Concentrated liquid L-lysine, L-lysine HCl and concentrated liquid L-lysine HCl are not considered to have the potential to cause respiratory toxicity, skin or eye irritation or skin sensitisation. However, the FEEDAP Panel notes that in the case of concentrated liquid L-lysine, due to its high pH, it may be corrosive for skin and eyes.
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 EFSA opinions (EFSA FEEDAP Panel, 2013, 2014). In general, the products concentrated liquid L-lysine, L-lysine HCl and concentrated liquid L-lysine HCl produced by E. coli NITE BP-02917 are regarded 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.

Since L-lysine [17.026] and L-lysine HCl [17.031] are used in food as flavouring compounds and their function in feed is essentially the same as that in food, no further demonstration of efficacy is necessary.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation59 and Good Manufacturing Practice.

4. Conclusions

Concentrated liquid L-lysine, L-lysine HCl, and concentrated liquid L-lysine HCl are produced by a genetically modified strain of Escherichia coli (E. coli NITE BP-02917). The production strain does not carry antimicrobial resistance genes and no viable cells of the production strain were detected in the final products. Since no sequences of concern remain in the production strain, the potential presence of recombinant DNA in the final products does not raise any safety concern.

The use of concentrated liquid L-lysine, L-lysine HCl, and concentrated liquid L-lysine HCl produced by the strain E. coli NITE BP-02917 in supplementing feed to compensate for L-lysine deficiency in feedingstuffs is safe for the target species. This conclusion would also cover the use as a sensory additive. The FEEDAP Panel identified risks of nutritional imbalances and hygienic concerns for amino acids when administered simultaneously in feed and in water for drinking.

The use of the three forms of L-lysine produced by fermentation using E. coli NITE BP-02917 in animal nutrition is considered safe for the consumers and for the environment.

Concentrated liquid L-lysine, L-lysine HCl and concentrated liquid L-lysine HCl are not considered to have the potential to cause respiratory toxicity, or skin sensitisation. L-Lysine HCl and concentrated liquid L-lysine HCl are not considered skin and eye irritants. However, the FEEDAP Panel notes that in the case of concentrated liquid L-lysine, due to its high pH, it may be corrosive for skin and eyes.

Concentrated liquid L-lysine, L-lysine HCl and concentrated liquid L-lysine HCl are considered 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. The three forms of the additive are also considered efficacious as feed flavouring compounds under the proposed conditions of use.

5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                                     |
|------------|--------------------------------------------------------------------------------------------|
| 06/04/2021 | Dossier received by EFSA. L-Lysine HCl, concentrated liquid L-lysine and concentrated liquid L-lysine HCl produced using strain Escherichia coli NITE BP-02917. Submitted by Ajinomoto Animal Nutrition Europe |

58 Commission implementing regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012. p 161.

59 Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
Lysine and its monohydrochloride salt for all animal species

References

Cort N, Fredriksson G, Kindahl H, Edqvist LE and Rylander R, 1990. A clinical and endocrine study on the effect of orally administered bacterial endotoxin in adult pigs and goats. Zentralbl Veterinärmed A., 37, 130–137. https://doi.org/10.1111/j.1439-0442.1990.tb00884.x

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010. Scientific Opinion on the use of feed additives authorised/applied for use in feed when supplied via water. EFSA Journal 2010;8(12):1956, 9 pp. https://doi.org/10.2903/j.efsa.2010.1956

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of concentrated liquid l-lysine (base), concentrated liquid L-lysine monohydrochloride and l-lysine monohydrochloride produced by Escherichia coli (FERM BP-10941) for all animal species, based on three dossiers submitted by Ajinomoto Eurolysine SAS. EFSA Journal 2013;11(10):3365, 22 pp. https://doi.org/10.2903/j.efsa.2013.3365

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of concentrated liquid L-lysine (base), concentrated liquid L-lysine monohydrochloride and L-lysine monohydrochloride technically pure produced using Escherichia coli (FERM BP-11355) for all animal species based on a dossier submitted by Ajinomoto Eurolysine S.A.S. EFSA Journal 2014;12(11):3895, 19 pp. https://doi.org/10.2903/j.efsa.2014.3895

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017a. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017b. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML and Martino L, 2017c. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML and Martino L, 2018a. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Giandolfi B, Herman L, Kärenlampi S, Aguiler J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Bastos M, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brock T, de Knecht J, Kolar B, van Beelen P, Padovani L, Tarres-Call J, Vettori MV and Azimonti G, 2019. Guidance on the assessment of the safety of feed additives for the environment. EFSA Journal 2019;17(4):5648, 78 pp. https://doi.org/10.2903/j.efsa.2019.5648

European Pharmacopeia (PhEur), 2019. Lysine hydrochloride, Monograph 01/2008:0930. 10th edn. Council of Europe (COE)-European Directorate for the Quality of Medicines, Strasbourg, France.

Gorbach SL, 1978. Recombinant DNA: an infectious disease perspective. The Journal of Infectious Diseases, 137, 615–623. Available online: http://www.jstor.org/stable/30111363

Health Council of the Netherlands, 2010. Endotoxins. Health-based recommended occupational exposure limit. Publication no 2010/04OSH, 100 pp.

HSE (Health and Safety Executive), 2013. Occupational Hygiene implications of processing waste at Materials Recycling Facilities (MRFs). RR977 Research Report, UK, 41 pp.

Rylander R, Thorn J and Attefors R, 1999. Airways inflammation among workers in a paper industry. European Respiratory Journal, 13, 1151–1157. https://doi.org/10.1034/j.1399-3003.1999.13e35.x

Thorn J, 2001. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. Inflammation Research, 50, 254–261. https://doi.org/10.1007/s000110050751

Abbreviations

| Abbreviation | Description |
|-------------|-------------|
| AMR         | antimicrobial resistance |
| CAS         | Chemical Abstracts Service |
| CFU         | colony forming unit |
| CV          | coefficient of variation |
| DM          | dry matter |
| EINECS      | European Inventory of Existing Chemical Substances |
| EURL        | European Union Reference Laboratory |
| FEEDAP      | EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed |
| FLAVIS      | The EU Flavour Information System |
| IUPAC       | International Union of Pure and Applied Chemistry |
| LOD         | limit of detection |
| LOQ         | limit of quantification |
| MCHC        | mean corpuscular haemoglobin concentration |
| MCV         | mean corpuscular volume |
| MIC         | minimum inhibitory concentration |
| MRL         | maximum residue limit |
| MW          | molecular weight |
| NOAEL       | no observed adverse effect level |
| OECD        | Organisation for Economic Co-operation and Development |
| PCB         | polychlorinated biphenyl |
| PCDD        | polychlorinated dibenzodioxin |
| PCDF        | polychlorinated dibenzofuran |
Appendix A – Calculation of user’s exposure to lipopolysaccharides

The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s = 40 × 20 = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s = 0.444 h/day. Again, assuming a respiration volume of 1.25 m³/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m³/day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900/0.556 = 1,619 IU/m³.

Calculation of endotoxin content of dust

Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m³, and the endotoxin activity of the dust, determined by the Limulus amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m³, then the content of endotoxins of the dust, c IU/m³, is obtained by simple multiplication, a × b. This resulting value is further used for calculation of the potential inhalation exposure of users to endotoxins from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012).

Table A.1: Estimation of user exposure to endotoxins from the additive l-lysine HCl produced by fermentation with a genetically modified microorganism E. coli NITE BP-02917

| Calculation | Identifier | Description | Amount | Source |
|-------------|------------|-------------|--------|--------|
| a           | a          | Endotoxin content IU/g product | 28.5   | Technical dossier |
| b           | b          | Dusting potential (g/m³)        | 7.452  | Technical dossier |
| a × b       | c          | Endotoxin content in the air (IU/m³) | 212.4  |        |
| d           | d          | No of premixture batches made/working day | 40     | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012) |
| e           | e          | Time of exposure (s) per production of one batch | 20     | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012) |
| d × e       | f          | Total duration of daily exposure/worker (s) | 800    |        |
| g           | g          | Uncertainty factor | 2      | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012) |
| f × g       | h          | Refined total duration of daily exposure/worker (s) | 1,600  |        |
| h/3,600     | i          | Refined total duration of daily exposure (h) | 0.44   |        |
| j           | j          | Inhaled air (m³) per eight-hour working day | 10     | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012) |
| j/8 × i     | k          | Inhaled air during exposure (m³) | 0.56   |        |
| c × k       | l          | Endotoxin inhaled (IU) during exposure per eight-hour working day | 118   |        |
| m           | m          | Health-based recommended exposure limit of endotoxin (IU/m³) per eight-hour working day | 90    | Health Council of the Netherlands (2010) |
| m × j       | n          | Health-based recommended exposure limit of total endotoxin exposure (IU) per eight-hour working day | 900   |        |
References
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539
Health Council of the Netherlands, 2010. Endotoxins. Health-based recommended occupational exposure limit. Publication no 2010/04OSH, 100 pp.
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for L-lysine monohydrochloride, concentrated liquid L-lysine and concentrated liquid L-lysine monohydrochloride produced by fermentation with *Escherichia coli* NITE BP-02917

In the current application authorisation is sought under Article 4 for L-lysine monohydrochloride, concentrated liquid L-lysine and concentrated liquid L-lysine monohydrochloride produced by fermentation with *Escherichia coli* NITE BP-02917, under the categories/functional groups 3(c) ‘nutritional additives’/’amino acids, their salts and analogues’ and 2(b) ‘sensory additives’/’flavouring compounds’ according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species.

According to the Applicant, L-lysine monohydrochloride has a minimum purity (mass fraction on dry matter basis) of 99% and a minimum L-lysine content of 78% while the concentrated liquid L-lysine and the concentrated liquid L-lysine monohydrochloride have a minimum L-lysine content of 50% and 22.4%, respectively.

When used as nutritional additive the solid form of the feed additive (L-lysine monohydrochloride) is intended to be added directly into feedingstuffs or through premixtures while the liquid forms (concentrated liquid L-lysine and concentrated liquid L-lysine monohydrochloride) are intended to be added directly into feedingstuffs. When used as sensory additive the three L-lysine forms are intended to be added directly into feedingstuffs and water for drinking.

The Applicant did not propose any minimum or maximum content of L-lysine in feedingstuffs when used as nutritional additive, however a recommended inclusion level of 25 mg/kg feedingstuffs was proposed by the Applicant when used as sensory additive.

For the quantification of lysine in the feed additive the Applicant proposed the ring-trial validated method EN ISO 17180:2013 based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). This standard method does not distinguish between the salts of amino acids and cannot differentiate between enantiomers. It applies for products containing more than 10% of amino acid. The following performance characteristics are reported: a relative standard deviation for repeatability (RSDr) ranging from 0.7 to 1.7% and a relative standard deviation for reproducibility (RSDR) ranging from 1.5 to 2.5%. In addition, the EURL identified the “L-lysine monohydrochloride monograph” of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive.

For the quantification of lysine in premixtures, feedingstuffs and water the Applicant proposed the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled with optical 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 3.0 to 6.7%. Furthermore, as concluded in previous amino acids reports, the EURL also considers the IEC-VIS/FLD procedure described above as fit-for-purpose for the determination of lysine in water.

Based on the performance characteristics available, the EURL recommends for official control (i) the “L-lysine monohydrochloride monograph” of the Food Chemical Codex (FCC) for the identification of L-lysine monohydrochloride in the feed additive (ii) the ring-trial validated method EN ISO 17180:2013 based on IEC-VIS/FLD to quantify free lysine in the feed additive and premixtures (containing more than 10% lysine); (iii) the European Union method based on IEC-VIS for the quantification of lysine in premixtures and feedingstuffs; (for feedingstuffs only when used as nutritional additive) and (iv) the ion-exchange chromatography methods coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) or coupled with post-column derivatisation and optical detection (IEC-VIS) for the quantification of lysine in water (only when used as nutritional additive).

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.