

SCIENTIFIC OPINION

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Safety of L-lysine monohydrochloride produced by fermentation with *Escherichia coli* CGMCC 7.57 for all animal species based on a dossier submitted by Feedway Europe NV

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

L-Lysine monohydrochloride is a feed additive produced by fermentation using a genetically modified strain of *Escherichia coli* (CGMCC 7.57). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the EFSA, in its opinion on the safety and efficacy of the product, could not conclude on the safety of the product for target animals, consumers, users and the environment. The European Commission asked EFSA to deliver an opinion on the safety of L-lysine monohydrochloride as a nutritional additive for all animal species based on additional data submitted by the applicant on the characterisation of the additive. No recombinant antibiotic resistance genes are present in the production strain and therefore in the final product. The L-lysine monohydrochloride manufactured by fermentation using *E. coli* CGMCC 7.57 does not raise safety concerns for the target species, consumers, users and the environment with regard to the genetic modification of the production strain. The levels of endotoxins present in the product and its dusting potential indicate no health risk for the user.

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on L-lysine monohydrochloride produced by a genetically modified strain of *Escherichia coli* (CGMCC 7.57) for all animal species.

In 2015, the FEEDAP Panel of the European Food Safety Authority (EFSA) adopted an opinion on the safety and efficacy of L-lysine monohydrochloride produced by fermentation using *E. coli* CGMCC 7.57. In that opinion, the FEEDAP Panel could not conclude on the safety of the product for target animals, consumers, users and the environment.

The applicant provided additional information in relation to the characterisation of the additive and the production process. The FEEDAP Panel has performed the assessment of those new data following an approach in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents.

This product was characterised in a previous scientific opinion (EFSA FEEDAP Panel, 2015).

No recombinant antibiotic resistance genes are present in the production strain and therefore in the final product. The L-lysine monohydrochloride manufactured by fermentation using *E. coli* CGMCC 7.57 does not raise safety concerns for the target species, consumers, users and the environment with regard to the genetic modifications of the production strain.

The levels of endotoxins present in the product and its dusting potential indicate no health risk for the user.

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1. Introduction

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

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Feedway Europe NV, is seeking a Community authorisation of L-lysine monohydrochloride (HCl) produced by fermentation with *Escherichia coli* to be used as a nutritional additive for all animal species. (Table 1)

Table 1: Description of the substances

| | |
|-------------------------------------|--|
| Category of additive | Nutritional additive |
| Functional group of additive | Amino acids, their salts and analogues |
| Description | L-Lysine in the form of L-lysine monohydrochloride (HCl), technically pure |
| Target animal category | All animal species |
| Applicant | Feedway Europe NV |
| Type of request | New opinion |

On 10 March 2015, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority, in its opinion on the safety and efficacy of the product, could not conclude on the safety of the product L-lysine HCl produced by fermentation with this recombinant *E. coli* strain for target animals, consumers, users and the environment. Regardless of the assessment of the genetic modification, the FEEDAP Panel has concerns regarding the safety of the simultaneous oral administration of the product L-lysine HCl via water for drinking and feed. FEEDAP Panel has concerns that the safety of the genetic modification may also have implications for the safety of the user.

The Commission gave the possibility to the applicant to submit complementary information to complete the assessment on the safety and to allow a revision of Authority's opinion.

The Commission has now received new data on the safety of L-lysine monohydrochloride produced by *Escherichia coli*.

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of L-lysine monohydrochloride produced by fermentation with *Escherichia coli* as nutritional additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

L-Lysine monohydrochloride (minimum content of L-lysine 78%, 'as is' basis) was first authorised for use in animal nutrition by Directive 88/485/EEC. It is currently included in the European Union as a nutritional additive (functional group amino acids, their salts and analogues) for use in all animal species without time limit and without maximum content in feed.

The applicant has provided additional information on the physical properties of the additive, the characterisation of the genetic modification and on the production process.

Although the additive was initially intended to be used in all animal species as a nutritional additive (functional group amino acids, their salts and analogues) in feed and water for drinking, during the assessment the applicant requested to withdraw the use of the additive in water for drinking.¹

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier² following a previous application on the same product.³

¹ Technical dossier/Correspondence and mandate/FAD-2015-0030 withdrawal partial app to EC and EFSA 150416.

² FEED dossier reference: FAD-2015-0030.

³ FEED dossier reference: FAD-2010-0281.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the characterisation and safety of L-lysine monohydrochloride (HCl) produced by *E. coli* CGMCC 7.57 is in line with the principles laid down in Regulation (EC) No 429/2008⁴ and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011).

3. Assessment

The current application is for the authorisation of L-Lysine monohydrochloride produced by a genetically modified (GM) strain of *E. coli* (CGMCC 7.57) which is a derivative of *E. coli* K-12. It is intended to be used in all animal species as a nutritional additive (functional group amino acids, their salts and analogues) in feed.

3.1. Characterisation

This product has been characterised in a previous scientific opinion (EFSA FEEDAP Panel, 2015) with the exception of the genetic modification, for which the absence of antibiotic resistance genes in the production strain (and therefore in the product) was insufficiently proven.

Additional data have been submitted on the genetic modification process of the production strain, the physico-chemical properties of the additive and on its manufacturing process.

3.1.1. Impurities

The endotoxin activity in the former product (three batches analysed) ranged from 6.3 to 6.6 IU/mg.⁵ An improvement in the manufacturing has been introduced in the downstream process to reduce the amount of bacterial endotoxins in the final product. The filtration by ceramic membrane has been modified (from a former pore size of 300 kDa to the current smaller size of 20 kDa) and reverse osmosis has been applied to reduce the amount of endotoxins in the process water.⁶ New data have been submitted on the bacterial endotoxin activity of the product, measured in three batches. The values ranged from 0.12 to 0.28 IU/mg (European Pharmacopoeia 2.6.14 methods).⁷

3.1.2. Physical properties

The applicant stated that the production process had been modified to increase the crystal size of the final product and consequently decrease its dustiness. The evaporation temperature has been lowered (75–72°C), the evaporation rate reduced (2.5–2.0 m³/h), the evaporation time prolonged (12–15 h) and the cooling time extended from 3 to 5 h.⁸

New analytical data have been provided to characterise the particle size and dustiness of the new final product. The particle size distribution (three batches analysed by laser diffraction) had a fraction of particles < 50 µm of diameter ranging from 0.2% to 1.2% (v/v) (mean particle diameter is about 600 µm).⁹ In the former product (five batches analysed, method unknown), about 2.3% (w/w) of particles had a diameter < 50 µm.¹⁰ The dusting potential (three batches analysed by the Stauber–Heubach method) ranged from 1.3 to 1.6 g/m³.¹¹ The dusting potential of the product assessed in 2015 (three batches) ranged from 1.8 to 2.1 g/m³.¹²

⁴ 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.

⁵ Technical dossier FAD-2010-0281/Supplementary information January 2013/Attachment 19.

⁶ Technical dossier/Supplementary information March 2016/Attachment 2.

⁷ Technical dossier/Technology improvement on production process, Supplementary information on endotoxin levels and Attachments 1–3.

⁸ Technical dossier/Technology improvement on production process.

⁹ Technical dossier/Supplementary information March 2016/Attachments 5 and 6.

¹⁰ Technical dossier FAD-2010-0281/Section II.5 and Annex II.1.2.

¹¹ Technical dossier/Supplementary information March 2016/Attachment 5.

¹² Technical dossier FAD-2010-0281/Supplementary information May 2014/Reply 4/Attachment 7.

3.1.3. Characterisation of the production strain *E. coli* CGMCC 7.57¹³

In the former assessment of the production strain (EFSA FEEDAP Panel, 2015), no conclusive evidence was provided regarding the absence of antibiotic resistance genes (transiently used in the genetic modification) in the genome of the production strain or in the product.

The applicant has now provided analyses indicating that those genes are not present in the production strain.¹⁴

3.2. Safety

In its previous assessment (EFSA FEEDAP Panel, 2015), the FEEDAP Panel could not conclude on the safety of the product L-lysine HCl produced by fermentation with this recombinant *E. coli* strain for target animals, consumers, users and the environment because the genetic modification, including the presence or absence of antibiotic resistance genes in the production strain (and therefore in the product), was insufficiently characterised.

The applicant has provided evidence that the production strain does not carry the antibiotic resistance genes transiently used during the genetic modification. Absence in the product of recombinant DNA corresponding to the inserted sequences was previously shown (EFSA FEEDAP Panel, 2015). Therefore, L-lysine monohydrochloride manufactured by fermentation with *E. coli* CGMCC 7.57 does not raise any safety concern for the target animals, consumers, users and the environment with regard to the genetic modification of the production strain.

3.2.1. Safety for the user

In the previous opinion (EFSA FEEDAP Panel, 2015), it was concluded that there was no concern for users with respect to skin or eye irritancy and dermal sensitisation. Nevertheless, the level of endotoxins present in the product and its dusting potential indicated an inhalation risk for the user. The concerns on the safety of the genetic modification may also have implications on safety for the user.

3.2.1.1. Effects on the respiratory system

Although a previous assessment was performed in March 2015 (EFSA FEEDAP Panel, 2015), technological improvement in the manufacturing process makes it necessary to reassess the safety for the user when exposed by inhalation to the bacterial endotoxin activities found in the additive.

The bacterial endotoxin activity (the three new batches) ranges from 0.12 to 0.28 IU/mg.¹⁵ The dusting potential ranges from 1.3 to 1.6 g/m³.¹⁶

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 (2012b) is described in Appendix A. The health-based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based on the calculation of the potential endotoxin content in dust (Wallace et al., 2016), the inhalation exposure could be up to 246 endotoxin IU per 8-h working day, indicating no risk from the exposure to endotoxins for people handling the additive.

3.2.1.2. Conclusions on safety for the user

The additive L-lysine monohydrochloride is not a skin or eye irritant nor a skin sensitiser. The levels of endotoxins present in the product and its dusting potential indicate no health risk for the user.

4. Conclusions

No recombinant antibiotic resistance genes are present in the production strain and therefore in the final product. The L-lysine monohydrochloride manufactured by fermentation using *E. coli* CGMCC 7.57

¹³ This section has been amended following the applicable provisions on confidentiality.

¹⁴ Technical dossier/Supplementary information March 2016/Attachment 1 Lysine HCl detection of antibiotic-resistant genes.

¹⁵ Technical dossier/Supplementary information on endotoxin levels and Attachments 1–3.

¹⁶ Technical dossier/Supplementary information March 2016/Attachment 5.

does not raise safety concerns for the target species, consumers, users and the environment with regard to the genetic modification of the production strain.

The levels of endotoxins present in the product and its dusting potential indicate no health risk for the user.

Documentation provided to EFSA

- 1) Dossier L-lysine in the form of L-lysine monohydrochloride, technically pure. October 2015. Submitted by Feedway Europe NV/Meihua Holdings Group Co., Ltd.
- 2) Dossier L-lysine in the form of L-lysine monohydrochloride, technically pure. Supplementary information. November 2015. Submitted by Feedway Europe NV.
- 3) Dossier L-lysine in the form of L-lysine monohydrochloride, technically pure. Supplementary information. March 2016. Submitted by Feedway Europe NV.

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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of L-lysine monohydrochloride produced by fermentation with *Escherichia coli* for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Group Co. Ltd. EFSA Journal 2015;13(3):4052, 16 pp. doi:10.2903/j.efsa.2015.4052
- EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. doi:10.2903/j.efsa.2011.2193
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- Wallace RJ, Gropp J, Dierick N, Costa LG, Martelli G, Brantom PG, Bampidis V, Renshaw DW and Leng L, 2016. Risk associated with endotoxins in feed additives produced by fermentation. Environmental Health, 15, 1–7.

Abbreviations

| | |
|--------|---|
| CGMCC | China General Microbiological Culture Collection Centre |
| DECOS | Dutch Expert Committee on Occupational Safety |
| DNA | deoxyribonucleic acid |
| EC | European Commission |
| FEEDAP | EFSA Panel on Additives and Products or Substances Used in Animal Feed |
| GM | genetically modified |
| GMO | EFSA Panel on Genetically Modified Organisms |
| HCl | monohydrochloride |
| HSE | British Health Safety Executive |
| IU | International unit of endotoxin activity. One IU corresponds to one endotoxin unit (EU) |

Appendix A – Safety for the user

The effects of endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

Calculation of maximum acceptable levels of exposure from feed additives

The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012b) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s, equal to 800 s/day. With an uncertainty factor of 2, the maximum inhalation exposure would occur for $2 \times 800 \text{ s} = 1,600 \text{ s}$ (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 $0.444 \times 1.25 = 0.556 \text{ m}^3/\text{day}$. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than $900/0.556 = 1,619 \text{ IU/m}^3$.

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)).¹⁷ 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 $a \text{ IU/g}$ and the dusting potential is $b \text{ g/m}^3$, then the content of endotoxins of the dust in the air, $c \text{ IU/m}^3$, is obtained by simple multiplication, $a \times b$. This resulting value is further used for calculation of the potential inhalatory exposure of users to endotoxins from the additive under assessment (Table A.1) (EFSA FEEDAP Panel, 2012b).

Table A.1: Estimation of user exposure to endotoxins from the additive L-lysine HCl produced by *Escherichia coli* CGMCC 7.57, including consideration of using filter half mask (FFP2 or FFP3)^(a) as a preventive measure

| Calculation | Identifier | Description | Amount | Source |
|-------------------|------------|--|--------|--|
| | <i>a</i> | Endotoxin content IU/g product | 280 | Technical dossier |
| | <i>b</i> | Dusting potential (g/m ³) | 1.58 | Technical dossier |
| $a \times b$ | <i>c</i> | Endotoxin content in the air (IU/m ³) | 442 | |
| | <i>d</i> | No. premixture batches made/working day | 40 | EFSA FEEDAP Panel (2012b) |
| | <i>e</i> | Time of exposure (s) per production of one batch | 20 | EFSA FEEDAP Panel (2012b) |
| $d \times e$ | <i>f</i> | Total duration of daily exposure/worker (s) | 800 | |
| | <i>g</i> | Uncertainty factor | 2 | EFSA FEEDAP Panel (2012b) |
| $f \times g$ | <i>h</i> | Refined total duration of daily exposure/worker (s) | 1,600 | |
| $h/3 \text{ 600}$ | <i>i</i> | Refined total duration of daily exposure (h) | 0.44 | |
| | <i>j</i> | Inhaled air (m ³) per 8-h working day | 10 | EFSA FEEDAP Panel (2012b) |
| $j/8 \times i$ | <i>k</i> | Inhaled air during exposure (m ³) | 0.56 | |
| $c \times k$ | <i>l</i> | Endotoxin inhaled during exposure per 8-h working day | 246 | |
| | <i>m</i> | Health-based recommended exposure limit of endotoxin (IU/m ³) per 8-h working day | 90 | Health Council of the Netherlands (2010) |
| $m \times j$ | <i>n</i> | Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day | 900 | |
| $l/10$ | | Endotoxins inhaled (IU) per 8-h working day reduced by filter half mask FFP2 (reduction factor 10) | 25 | |
| $l/20$ | | Endotoxins inhaled (IU) per 8-h working day reduced by filter half mask FFP3 (reduction factor 20) | 12 | |

(a): Filtering face piece or filtering half mask according to European standard EN 149. They are graded from 1 to 3 depending on their filtering capacity.

¹⁷ The *Limulus amoebocyte lysate* is an aqueous extract of circulating amoebocytes from the horseshoe crab (*Limulus polyphemus*). This extract reacts with minute quantities of bacterial endotoxin (lipopolysaccharide from the walls of Gram-negative bacteria) and this reaction is the basis of the assay used for the detection and quantification of bacterial endotoxins.