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## Safety of the feed additives consisting of L-lysine monohydrochloride and L-lysine sulfate produced by *Corynebacterium glutamicum* CCTCC M 2015595 for all animal species (Kempex Holland B. V.)

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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 the safety of L-lysine monohydrochloride and L-lysine sulfate produced using *Corynebacterium glutamicum* CCTCC M 2015595 for all animal species. In 2019, the FEEDAP Panel issued an opinion on the safety and efficacy of the products. In that assessment, the Panel could not conclude on the safety of the additives for the target species, the consumers and the environment due to the uncertainties regarding the possible genetic modification of the strain used to obtain the production strain *C. glutamicum* CCTCC M 2015595 and on the possible presence of viable cells and DNA of the production strain in the final products. Moreover, in the absence of data, the FEEDAP Panel could not conclude on the safety of the additives for the users. The applicant provided supplementary data to characterise the strain from which the production strain under assessment was obtained. However, uncertainties remain on its origin and history of modification, including whether it has been genetically modified. The production strain *C. glutamicum* CCTCC M 2015595 did not show the presence of acquired antimicrobial resistance genes nor of toxin and virulence factors genes. Moreover, as viable cells and DNA of the production strain were not detected in both final formulations, L-lysine HCl and L-lysine sulfate do not raise safety concerns as regards the production strain. The FEEDAP Panel concluded that L-lysine HCl and L-lysine sulfate produced by *C. glutamicum* CCTCC M 2015595 are safe for the target species, consumers and for the environment. No additional data have been provided on the safety of the additives for users. Therefore, the conclusions from the Panel remained that in the absence of data, no conclusions on the safety of the additives for the user can be drawn.

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## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>1</sup> establishes the 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, Kempex Holland B. V., is seeking a Community authorisation of L-lysine monohydrochloride, L-lysine sulfate from *Corynebacterium glutamicum* CCTCC M 2015595 as a feed additive to be used as an amino acid for all animal species (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Nutritional additive
<b>Functional group of additive</b>	Amino acid
<b>Description</b>	L-lysine monohydrochloride, L-lysine sulfate from <i>Corynebacterium glutamicum</i> CCTCC M 2015595
<b>Target animal category</b>	All animal species
<b>Applicant</b>	Kempex Holland B. V.
<b>Type of request</b>	New opinion

In its opinion of 26 February 2019 the Panel on Additives and Products used in Animal Feed of the European Food Safety Authority ("Authority"), owing to the uncertainties regarding the possible genetic modification of the strain used to obtain the production strain *C. glutamicum* CCTCC M 2015595 and on the possible presence of viable cells and DNA of the production strain in the final product, could not conclude on the safety of L-lysine HCl and L-lysine sulfate produced with *C. glutamicum* CCTCC M 2015595 for the target species, the consumers, the users and the environment. Moreover, in the absence of data, the FEEDAP Panel could not conclude on the safety of the additive for the user. The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 16 December 2019.

In view of the above, the Commission asks the Authority to deliver a new opinion on L-lysine monohydrochloride, L-lysine sulfate from *Corynebacterium glutamicum* CCTCC M 2015595 as a feed additive for all animal species based on the additional data submitted by the applicant.

### 1.2. Additional information

L-Lysine monohydrochloride and L-lysine sulfate produced by *C. glutamicum* CCTCC M 2015595 are not authorised in the European Union. The FEEDAP Panel adopted an opinion on these additives produced by *C. glutamicum* CCTCC M 2015595 in 2019 (EFSA FEEDAP Panel, 2019). In that assessment, owing to the uncertainties regarding the possible genetic modification of the strain used to obtain the production strain *C. glutamicum* CCTCC M 2015595 and on the possible presence of viable cells and DNA of the production strain in the final products, the FEEDAP Panel could not conclude on the safety of the additives for the target species, the consumers and the environment. Moreover, in the absence of data, the FEEDAP Panel could not conclude on the safety of the additives for the users.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>2</sup> to a previous application on the same product.<sup>3</sup>

<sup>1</sup> 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.

<sup>2</sup> FEED dossier reference: FAD-2019-0059.

<sup>3</sup> FEED dossier reference: FAD-2016-0052.

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of L-lysine monohydrochloride and L-lysine sulfate produced by *C. glutamicum* CCTCC M 2015595 is in line with the principles laid down in Regulation (EC) No 429/2008<sup>4</sup> 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 characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

## 3. Assessment

L-Lysine monohydrochloride (HCl) and L-lysine sulfate are produced by fermentation by a strain of *Corynebacterium glutamicum* (CCTCC M 2015595) and are intended to be used as nutritional additives (functional group: amino acids, their salts and analogues) in feed or water for drinking for all animal species.

L-Lysine HCl contains by specification  $\geq 78.8\%$  L-lysine on dry matter basis;  $\leq 1\%$  moisture;  $\leq 0.5\%$  other amino acids;  $\leq 0.5\%$  total sugars;  $\leq 0.3\%$  ash and  $\leq 0.05\%$  ammonium salt. L-Lysine sulfate contains by specification  $\geq 73\%$  lysine sulfate ( $\geq 55\%$  lysine on dry matter basis),  $\leq 5\%$  other amino acids,  $\leq 7\%$  total sugars,  $\leq 4\%$  loss on drying,  $\leq 4\%$  residues on ignition,  $\leq 3\%$  ammonium salt and  $\geq 6\%$  protein.

These products were assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2019), but the information regarding the production strain and the final additives did not allow the Panel to characterise them in full. In particular, no information was provided on the origin and history of modification of the strain used to obtain the production strain *C. glutamicum* CCTCC M 2015595 and it was not possible to assess whether it was genetically modified and sequences of concern eventually introduced that would remain in the production strain. Moreover, the data provided did not allow the Panel to conclude on the presence/absence of viable cells and DNA of the production strain in the final products. Owing to this lack of information, the Panel could not conclude on the safety aspects regarding the use of these additives for target animals, consumers and the environment. In that assessment the Panel also concluded that in the absence of data, no conclusions can be drawn on the safety of the additives for the users.

The applicant has now provided supplementary information to address the limitations regarding the characterisation of the production strain and the additives. No additional information has been submitted regarding user safety.

### 3.1. Characterisation of the production strain

L-Lysine present in the additives under assessment is produced by a strain of *C. glutamicum* which is deposited in the China Center for Type Culture Collection (CCTCC) with accession number CCTCC M 2015595.<sup>5</sup>

The applicant provided new data based on the whole genome sequence (WGS) that confirmed the taxonomic identification of the production strain as *C. glutamicum*.<sup>6</sup> This was based on

The production strain was obtained from *C. glutamicum* CCTCC 1.0563 by conventional mutagenesis (UV and nitrosoguanidine (NTG) were used as mutagenic agents). The mutants were screened for high lysine production capacity and the production strain CCTCC M 2015595 was selected (EFSA FEEDAP Panel, 2019). The strain CCTCC 1.0563, from which the production strain under assessment was obtained, was purchased from the CCTCC.<sup>7</sup> No additional information has been

<sup>4</sup> 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.

<sup>5</sup> Technical dossier FAD-2016-0052/Section II/Annex 2.2.1.2b.

<sup>6</sup> Technical dossier FAD-2019-0059/Annex\_1\_CONFID.

<sup>7</sup> Technical dossier FAD-2019-0059/Supplementary information October 2020/FAD-2019-0059\_SIn\_250520 Answers, reply to question 1.

provided on the origin and history of modifications of the strain *C. glutamicum* CCTCC 1.0563, including whether it has been genetically modified or not. The applicant provided new data based on [REDACTED] to characterise the strain CCTCC 1.0563 which confirmed its taxonomic identification as *C. glutamicum* and showed that the strain does not carry acquired antimicrobial resistance (AMR) genes.<sup>8</sup>

In order to show that the strain CCTCC 1.0563, from which the production strain under assessment was obtained, was not genetically modified the applicant performed a [REDACTED] analysis [REDACTED].

However, the Panel considers that this information (in the form of a statement) does not allow to establish whether the strain *C. glutamicum* CCTCC 1.0563 has been subject to genetic modifications. Therefore, uncertainties remain on the origin of the strain CCTCC 1.0563 and on whether it has been subject to genetic modifications.

[REDACTED] Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018) [REDACTED] was assessed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2019). [REDACTED]

[REDACTED].<sup>9</sup> The applicant has submitted new data based on the interrogation of the WGS for the presence of AMR genes.<sup>6</sup> This was done [REDACTED]

[REDACTED]. No genes of concern were identified.

The WGS of the production strain CCTCC M 2015595 was also interrogated for the presence of toxin and virulence factor genes [REDACTED]

[REDACTED].<sup>10</sup> No relevant hits were identified.

### 3.2. Absence of the production strain in the additives

#### 3.2.1. L-lysine HCl

The presence of viable cells of the production strain in the final additive was investigated in three batches of L-lysine HCl.<sup>11</sup> [REDACTED]

[REDACTED] No cells of the production strain were found in three independent batches of the final product L-lysine HCl.

The applicant also investigated the presence of DNA of the production strain in three batches of L-lysine HCl, analysed in triplicate.<sup>12</sup> [REDACTED]

[REDACTED] No DNA of the production strain was detected.

#### 3.2.2. L-lysine sulfate

The presence of viable cells of the production strain in the final additive was investigated in three batches of L-lysine sulfate,<sup>13</sup> analysed in triplicate. [REDACTED]

<sup>8</sup> Technical dossier FAD-2019-0059/Supplementary information October 2020/Annex 2.

<sup>9</sup> Technical dossier FAD-2016-0052/Supplementary information April 2018/Annex 1.

<sup>10</sup> Technical dossier FAD-2019-0059/Supplementary information December 2020/Annex 1.

<sup>11</sup> Technical dossier FAD-2019-0059/Supplementary information October 2020/Annex 10 and Supplementary information December 2020/Annex 2.

<sup>12</sup> Technical dossier FAD-2019-0059/Supplementary information October 2020/Annex 5 and Supplementary information December 2020/Annex 4a.

<sup>13</sup> Technical dossier FAD-2019-0059/Annex\_3\_CONFID.

The applicant investigated the presence of DNA of the production strain in three batches of L-lysine sulfate, analysed in triplicate.<sup>14</sup>

No DNA of the production strain was detected.

### 3.3. Safety of the additives for target species, consumers, users and environment

The safety aspects regarding the use of the additives in feed have been previously assessed (EFSA FEEDAP Panel, 2019). In the previous opinion, the FEEDAP Panel concluded that 'Owing to the uncertainties regarding the possible genetic modification of the strain used to obtain the production strain and on the possible presence of viable cells and DNA of the production strain in the final product, the FEEDAP Panel cannot conclude on the safety of the additives L-lysine HCl and L-lysine sulfate produced with *C. glutamicum* CCTCC M 2015595 for the target species, the consumers, the users and the environment.' In that assessment the Panel concluded also that 'In the absence of data, the FEEDAP Panel cannot conclude on the safety of the additive for the user'.

The applicant has provided new data based on [REDACTED] to characterise the strain *C. glutamicum* CCTCC 1.0563, from which the production strain under assessment was obtained. However, uncertainties remain on its origin and history of modification, including whether it has been genetically modified. The bioinformatic analyses of the WGS of the production strain *C. glutamicum* CCTCC M 2015595 did not show the presence of acquired AMR genes nor genes coding for toxin and virulence factors. Moreover, as viable cells and DNA of the production strain were not detected in any of the final formulations, the products L-lysine HCl and L-lysine sulfate do not raise safety concerns as regards the production strain.

Considering the above, the FEEDAP Panel concludes that the additives are safe for the target species, consumers of products derived from animals fed the additives and for the environment.

In the current application, no additional data have been provided on the safety of the additives for users. Therefore, the conclusions from the Panel remain that in the absence of data, no conclusions on the safety of the additives for the user can be drawn.

## 4. Conclusions

Uncertainties remain on the origin and history of modification of the strain *C. glutamicum* 1.0563, from which the production strain under assessment was obtained, including whether it has been genetically modified. The production strain *C. glutamicum* CCTCC M 2015595 did not show the presence of acquired AMR genes nor of toxin and virulence factor genes. Moreover, as viable cells and DNA of the production strain were not detected in both final formulations, the products L-lysine HCl and L-lysine sulfate do not raise safety concerns as regards the production strain. L-Lysine HCl and L-lysine sulfate produced by *C. glutamicum* CCTCC M 2015595 are safe for the target species, consumers of products derived from animals fed the additives and for the environment.

In the absence of data, no conclusions on the safety of the additives for the user can be drawn.

## 5. Documentation as provided to EFSA/Chronology

Date	Event
12/09/2019	Dossier received by EFSA. L-lysine monohydrochloride and L-lysine sulphate from <i>Corynebacterium glutamicum</i> CCTCC M 2015595. Submitted by Kempex Holland B.V.
13/01/2020	Reception mandate from the European Commission (updated mandate received on 09/02/2021)
03/04/2020	Application validated by EFSA – Start of the scientific assessment

<sup>14</sup> Technical dossier FAD-2019-0059/Supplementary information October 2020/Annex 6 and Supplementary information December 2020/Annex 5.

Date	Event
25/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
15/10/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
09/11/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
09/12/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## 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>
- 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, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018. 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>
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## Abbreviations

AMR	antimicrobial resistance
CTCC	China Center for Type Culture Collection
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
WGS	whole genome sequence