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Safety of L-tryptophan technically pure, produced by *Escherichia coli* CGMCC 3667, for all animal species based on a dossier submitted by GBT Europe GmbH

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Abstract

L-Tryptophan, technically pure, is a feed additive produced by fermentation with a genetically modified strain of *Escherichia coli*. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of EFSA, issued two opinions on the safety and efficacy of the product, in which it could not conclude on the safety of this additive for target animals, consumer, user and the environment, due to the insufficient characterisation of the genetic modification. The European Commission asked the Authority to deliver an opinion on the safety of L-tryptophan, technically pure, as a nutritional additive for all animal species based on additional data submitted by the applicant. Based on new information provided on the genetic modification, including the presence/absence of antibiotic resistance genes in the production strain, the FEEDAP Panel concludes that the L-tryptophan is safe for target animals, consumers, users and the environment.

Keywords: nutritional additive, amino acids and their salts and analogues, L-tryptophan, safety, efficacy, genetically modified microorganisms

Requestor: European Commission

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Summary

Following a request from the European Commission (EC), the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of L-tryptophan, technically pure, produced by fermentation using the genetically modified strain *Escherichia coli* CGMCC 3667 for all animal species.

In 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the EFSA adopted an opinion on the safety and efficacy of L-tryptophan produced by fermentation using *E. coli* CGMCC 3667. The FEEDAP Panel could not conclude on the safety of that product for target animals, consumer, user and the environment because the genetic modification, including the presence/absence of recombinant DNA and of antibiotic resistance genes in the product, was insufficiently characterised.

The applicant provided additional information in relation to the characterisation of the production microorganism, the characterisation of the additive and its stability. The new information provided on the genetic modification was contradictory. Consequently, in another opinion of 2016, the FEEDAP Panel could not conclude on the safety of the L-tryptophan produced by fermentation with this recombinant strain of *E. coli* for target animals, consumers, users and the environment.

The applicant provided again additional information in relation to the characterisation of the production microorganism. 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.

The genetic modification is sufficiently characterised including the presence/absence of antibiotic resistance genes in the production strain. Neither the production strain nor its recombinant DNA was found in the final product. Therefore, the FEEDAP Panel concludes that the product L-tryptophan, produced by fermentation with *E. coli* CGMCC 3667, does not give raise to safety concerns for target animals, consumers, users and the environment with respect to the genetic modification of the production strain.

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1. Introduction

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

Regulation (EC) No 1831/2003[1] establishes 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, GBT Europe GmbH, is seeking a Community authorisation of L-tryptophan, technically pure, produced by fermentation with *E. 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-Tryptophan, technically pure, produced by fermentation with *Escherichia coli*
Target animal category	All animal species
Applicant	GBT Europe GmbH
Type of request	New opinion

On 10 April 2014, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product (EFSA FEEDAP Panel, 2014) could not conclude on the safety of the L-tryptophan produced by fermentation with *E. coli* CGMCC 3667 for target animals, consumer, user and the environment. Regardless of the assessment of the genetic modification, the FEEDAP Panel had concerns on the use of unprotected forms of L-tryptophan in ruminants, and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment on the safety and to allow a revision of Authority's opinion. The new information provided on the genetic modification was contradictory (EFSA FEEDAP Panel, 2016). Consequently, the FEEDAP Panel could not conclude on the safety of the L-tryptophan produced by fermentation with this recombinant strain of *E. coli* for target animals, consumers, users and the environment. The Panel reiterated its concerns on the use of unprotected L-tryptophan to ruminants and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking.

The Commission gave another possibility to the applicant to submit complementary information in order 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-tryptophan, technically pure, produced by fermentation with *E. coli* CGMCC 3667.

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

1.2. Additional information

L-Tryptophan, technically pure (minimum content of L-tryptophan 98%, 'as is' basis), was first authorised for use in animal nutrition by Directive 88/485/EEC. It is currently included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 concerning additives in feedingstuffs.

The applicant has submitted additional information on the characterisation of the production microorganism.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

2. Data and methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in August 2016 in the form of additional information[2] to a previous application on the same product.[3]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the characterisation and safety of L-tryptophan is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the 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-tryptophan, technically pure, produced by a genetically modified (GM) strain of *E. coli* (CGMCC 3667). It is intended to be used in all animal species as a nutritional additive to feed and water for drinking. The characterisation of the additive and the description of the genetic modification were assessed in previous opinions (EFSA FEEDAP Panel, 2014, 2016). The genetic modification, including the presence/absence of antibiotic resistance genes in the production strain, was insufficiently characterised. Therefore, the present opinion focuses on the new data provided by the applicant addressing this uncertainty.

3.1. Characterisation

3.1.1. Characterisation of the production organism

In the former assessment of the production strain (EFSA FEEDAP Panel, 2016), inconsistent information was found regarding the presence of a gene conferring ampicillin resistance in plasmid pEtrp, which is present in the production strain.

The applicant provided new Southern experiments[5] investigating the presence or absence in the production strain of all the antibiotic resistance genes used in the genetic modification (ampicillin and tetracycline remaining in plasmid pEtrp, chloramphenicol and apramycin used transiently). According to the results, a positive signal was observed when genomic DNA from the production strain was hybridised against a probe corresponding to the ampicillin resistance gene corresponding to the one described in the genetic modification. Furthermore, and in line with previous reports, the gene for tetracycline resistance was also found present and the genes for chloramphenicol and apramycin resistance were found absent. This is consistent with the description of the genetic modification.

According to the new data provided, the production strain *E. coli* CGMCC 3667 carries genes for ampicillin and tetracycline resistance located on the multicopy plasmid pEtrp. The absence in the final product of the production strain and of the whole ampicillin (861 bp) and tetracycline (1,191 bp) resistance genes, which was previously shown (EFSA FEEDAP Panel, 2016), was confirmed with new experiments.[6,7]

3.2. Safety

The traits introduced in the production strain *E. coli* CGMCC 3667 include tetracycline and ampicillin resistance, conferred by recombinant genes located together on a multicopy plasmid. Neither the production strain nor its recombinant DNA were found in the final product. Therefore, the product L-tryptophan, produced by fermentation with *E. coli* CGMCC 3667, does not give raise to safety

[2] Dossier reference: FAD-2016-0047.
[3] Dossier reference: FAD-2010-0290.
[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/Annex 3.
[6] Technical dossier/Annex 2.
[7] Technical dossier/Annex 1.

concerns for target animals, consumers, users and the environment with respect to the genetic modification of the production strain.

4. Conclusions

Neither the production strain nor its recombinant DNA was found in the final product. Therefore, the product L-tryptophan, produced by fermentation with *E. coli* CGMCC 3667, does not give raise to safety concerns for target animals, consumers, users and the environment with respect to the genetic modification of the production strain.

Documentation provided to EFSA

1) L-Tryptophan technically pure produced by fermentation with *E. coli*. Supplementary information. August 2016. Submitted by Global Bio-Chem (GBT Europe GmbH).

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of L-tryptophan technically pure produced by fermentation with Escherichia coli for all animal species, based on a dossier submitted by HELM AG on behalf of Global Bio-Chem Technology. EFSA Journal 2014;12(5):3673, 2 pp. doi:10.2903/j.efsa.2014.3673

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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

Abbreviations

CGMCC	China General Microbiological Culture Collection Center
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GM	genetically modified
GMO	genetically modified organism