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SCIENTIFIC OPINION

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Assessment of the feed additive consisting of L-cystine for all animal species for the renewal of its authorisation (Bretagne Chimie Fine [BCF Life Sciences])

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for the renewal of the authorisation of L-cystine as nutritional feed additive. The additive is authorised for use in all animal species (3c391). The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation. The EFSA Panel on Additives and Products or Substances used in Animal Feed concluded that the use of the feed additive in animal nutrition remains safe for the target species, the consumers and the environment. As regards the safety for the user, L-cystine is not an irritant to skin or eyes and is not a skin sensitiser. Exposure by inhalation of persons handling the additive cannot be excluded. The present application for the renewal of the authorisation does not include any modification proposal that would have an impact on the efficacy of the additive and therefore there is no need for reassessing the efficacy.

KEYWORDS

amino acid, feed additive, L-cystine, nutritional additive, safety

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Bretagne Chimie Fine (BCF Life Sciences)[2] for the renewal of the authorisation of the additive consisting of L-cystine, when used as a feed additive for all animal species (category: nutritional additives; functional group: amino acids, their salts and analogues).

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 14(1) (renewal of the authorisation). The dossier was received on 11 November 2022 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00791. The particulars and documents in support of the application were considered valid by EFSA as of 15 May 2023.

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 L-cystine, when used under the proposed conditions of use (see Section 3.1.2).

1.2 | Additional information

The additive consists of L-cystine produced by the hydrolysis of natural keratin from poultry feathers. It is currently authorised for use in feed for all animal species (3c391).[3] EFSA issued an opinion on the safety and efficacy of this product when used in feed for all animal species (EFSA FEEDAP Panel, 2013).

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[4] in support of the request of the renewal of the authorisation for the use of L-cystine as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 15 May to 15 August 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on presubmission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 7 August to 28 August 2023 for which no comments were received.

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the L-cystine in animal feed are valid and applicable for the current application.[7]

[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]Bretagne Chimie Fine (BCF Life Sciences), Boisel 56140, Pleucadeuc, France.
[3]Commission Implementing Regulation (EU) No 1006/2013 of 18 October 2013, OJ L 279, 19.10.2013, p. 59.
[4]Dossier reference: FEED-2022-3990.
[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.
[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[7]Evaluation report received on 08 May 2011 and available on the EU Science Hub: FAD-2010-0261 - European Commission (europa.eu)

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-cystine is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The subject of the assessment is the additive consisting of L-cystine. The additive is currently authorised as a nutritional feed additive (functional group: amino acids, their salts and analogues) for all animal species (3c391).[9] The present assessment is for the renewal of the authorisation.

3.1 | Characterisation

3.1.1 | Characterisation of the active substance/additive

L-Cystine has International Union of Pure and Applied Chemistry name (2R)-2-amino-3-[(2R)-2-amino-3-hydroxy-3-oxopropyl] disulfanyl-propanoic acid; Chemical Abstracts Service number 56–89-3 and the molecular formula $C_6H_{12}N_2O_4S_2$; its molecular weight is 240.3 g/mol.

The additive is authorised with a minimum content of 98.5% L-cystine. The applicant stated that some modifications to the manufacturing process had been introduced since the authorisation of the additive. These changes relate in some cases to a slight increase in the time spent in some processes, and/or to an increase of the concentration in the hydrochloric acid (HCl) used. The FEEDAP Panel considers that these changes are not expected to impact the characteristics of the final product.

No modifications are requested for the authorised specification of the L-cystine content (minimum 98.5%). The applicant lists the following specifications: ≤ 0.5% water and ≤ 0.5% components other than L-cystine.

The specifications for some impurities in the previous opinion were based on limits established in the European Pharmacopoeia for cystine (monograph 01/2008:0998). The European Pharmacopoeia issued a new monograph in 2019 (monograph 1/2019:0998) in which the limits for some impurities have changed (European Pharmacopoeia, 2023). The applicant proposes to update the specification of some impurities in accordance with the new limits established in the current European Pharmacopoeia monograph: Total ninhydrin-positive substances are specified to be now ≤ 0.5%; for any ninhydrin-positive substance, maximum of 0.2% for each.

Analytical data to confirm the specifications were provided for five batches of the additive,[10] showing the following average values (range): 99.3% cystine (99.1%–99.7%), 0.03% loss on drying (0.01%–0.07%) and 0.02% sulfated ash (0.01%–0.03%). Besides, the applicant reported analytical data of 668 batches produced in 2021 resulting in an average value of 100.1% L-cystine (range 98.5%–101.0%).

Specific optical rotation was analysed in three batches (European Pharmacopoeia method) and ranged from −222.8° to −221.5°. This range falls within the reference range specified in the EurPh (−224° to −218°) and confirms the L-enantiomer of cystine.

Ten batches of the additive were analysed for cadmium, lead, mercury and arsenic. All concentrations were below the limit of quantification (LOQ) of the analytical method, except for one batch that showed a level of mercury of 0.007 mg/kg.[11] Levels of iron, chloride, sulfates, ammonium, total ninhydrin-positive substances and tyrosine levels (three batches) were compliant with the specifications of the European Pharmacopoeia and were below the respective LOQ.[12] Three batches were analysed for amino acids other than L-cystine and the levels were below the respective LOQ.[13]

Three batches were analysed for microbiological contamination. Total aerobic counts were below the LOQ in one batch and below the limit of detection (LOD) of the analytical method in two batches. Coliforms, coagulase-positive staphylococci and *Pseudomonas aeruginosa* were not detected in one-gram samples. Yeasts were below the LOD, and filamentous fungi were below the LOQ in one batch and below the LOD in the other two batches. *Salmonella* spp. was not detected in

[8]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.

[9]Commission Implementing Regulation (EU) No 1006/2013 of 18 October 2013, OJ L 279, 19.10.2013, p. 59.

[10]Annex 2.1.3–1 Certificate of analysis. Method of analysis according to the European Pharmacopoeia.

[11]Annex 2.1.4–4 Heavy Metals Analysis and ADR1 report file to EFSA. LOQ in mg/kg was 0.01 for cadmium, 0.02 for lead, 0.05 for arsenic and 0.005 for mercury.

[12]Annex 2.1.4.1 L-cystine-positive ninhydrin substance test and ADR1 report file to EFSA. LOQ in mg/kg were 10 for iron, 200 for chloride, and 100 for sulfates. LOQ in % was 0.004 for ammonium, and 0.05 for tyrosin and for total ninhydrin positive substances.

[13]Annex 2.1.4–1 L-cystine-positive ninhydrin substance test and ADR1 report file to EFSA. LOQ for amino acids other than cystine in mg/kg ranged from 100 to 500 depending on the amino acid considered.

300-g samples.[14] Additional data reported from 200 to 800 batches (depending on the parameter analysed) tested in 2021 showed average values in accordance with the ones mentioned above.

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The additive appears as a white crystalline powder. The applicant provided some new information in relation to the physico-chemical properties of the additive which are reported below.

The bulk density analysed in three batches ranged from 840 to 890 kg/m^3.[15] The dusting potential of three batches of the additive was determined (Stauber–Heubach method) and showed values ranging from 133 to 300 mg/m^3.[16] The particle size distribution of three batches of the additive measured by laser diffraction showed that the fraction below 98 μm of diameter ranged from 0.6% to 1.8%, and no particles below 50 μm diameter.[17] In addition, the applicant provided descriptive scanning electron microscopy (SEM) analysis.[18] The presented electron micrographs were taken at relatively low magnification (μm-size scale bars) making it impossible to evaluate the presence of small particles. The quantitative analysis on particle size was not possible based on the SEM data. The volume-specific surface area (VSSA) was determined by nitrogen absorption sorptometry based on the Brunauer, Emmett and Teller (BET) method, resulting in a value of 0.06 m^2/g, corresponding to about 0.1 m^2/cm^3.

Solubility in water at 25°C is 0.11 g/L. In aqueous solutions with a pH < 2 or > 8, its solubility increases. The Panel considers that a further characterisation of the potential presence of nanoparticles is not needed.

No relevant changes in the manufacturing or composition of the additive have been introduced since the former application. Therefore, the data on shelf-life, stability and homogeneity described in the previous opinion (EFSA FEEDAP Panel, 2013) are still considered valid. However, a new study has been submitted investigating the shelf life (9 batches) of the additive when stored at 25°C in plastic bags for 48 months. No losses at the end of the storage period were observed.[19]

3.1.2 | Conditions of use

The additive is currently authorised for all animal species without minimum or maximum content. Under 'Other provisions' it is stated:

- For user safety: breathing protection, safety glasses and gloves should be worn during the handling.
- Indicate in the directions of use of the additive and premixtures:
  - Processing stability and storage conditions;
  - Supplementation with L-cystine shall depend on the requirements of the target animals for sulfur-containing amino acids and the level of other sulfur-containing amino acids in the ration.

The applicant did not request any change in the current conditions of authorisation.

3.2 | Safety

3.2.1 | Safety for the target species, consumers, and the environment

The FEEDAP Panel concluded in its previous assessment (EFSA FEEDAP Panel, 2013) that L-cystine does not raise concerns for the safety of target species, consumers and the environment.

The applicant claims that since the authorisation of the additive, no adverse effects have been reported for target animals, consumers and/or the environment.[20]

Considering that the composition of the additive and the conditions of use are the same as those previously assessed, and in the absence of reported adverse effects from the use of the additive in animal nutrition, the Panel considers that the additive remains safe for the target animals, consumers and environment.

3.2.2 | Safety for the user

In its previous assessment (EFSA FEEDAP Panel, 2013), the FEEDAP Panel concluded that "Since the product is a powder with < 1% particles < 50 μm in diameter, the possibility of exposure of the lower respiratory tract is considered to be low. Since

[14]Annex 2.1.4–5 microbiological analysis and ADR report file to EFSA. LOQ in colony forming unit (CFU)/g was 40 for total aerobic counts and 4 for filamentous fungi. LOD in CFU/g was 1 for yeasts and filamentous fungi, and 10 for aerobic plate counts.

[15]Annex 2.1.5–3 Bulk density.

[16]Annex 2.1.5–1 Dusting potential.

[17]Annex 3.3.1.1 – Particle size distribution.

[18]Annex 2.1.5–2 Nanomaterial test report.

[19]Annex 2.4.1-2(bis) Permanent stability testing report.

[20]Section of the additive, safety for the target animals, safety for consumers, safety for the environment.

no studies on the irritant or sensitising effects were provided, this product has to be considered as potentially irritating to skin, eyes and mucous membranes and as a potential dermal sensitiser. Therefore, it would be prudent to assume that exposure of skin, eyes and respiratory tract is hazardous."

The applicant states that, since 1986, no case of irritation or sensitisation has been reported from workers.

3.2.2.1 | *Effect on the respiratory tract*

No studies were submitted by the applicant. The additive has a dusting potential up to 300 mg/m^3. Therefore, exposure by inhalation is likely.

3.2.2.2 | *Effect on eyes and skin*

The applicant provided three studies in support of the safety for the users that were done using a L-cystine of different origin compared with the additive under assessment. However, considering the purity of the test item used (99% or > 98.5%), the FEEDAP Panel considers the outcome of these studies relevant for the L-cystine under assessment.

The dermal irritation potential of 99% L-cystine in rabbits was investigated in a study performed according to Organisation for Economic Co-operation and Development (OECD) Testing Guideline (TG) 404.[21] Based on the outcome of this study, the test item is not considered a dermal irritant.

The eye irritation potential of 99% L-cystine in rabbits was investigated in a study performed according to OECD TG 405.[22] A slight conjunctival redness was the only effect observed, but this was fully reversible within 72 h. Therefore, the test item was not considered an eye irritant.

The skin sensitisation potential of a L-cystine of a purity > 98.5% was investigated performing a local lymph node assay following the OECD TG 429.[23,24] Based on the results, the test item L-cystine is not considered a skin sensitiser.

3.2.2.3 | *Conclusions on the safety for the user*

The FEEDAP Panel concludes that L-cystine is not irritant to skin or eyes, and it has no potential to be a dermal sensitiser. Exposure by inhalation is likely.

3.3 | Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the original conditions that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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 Regulation[25] and Good Manufacturing Practice.

4 | CONCLUSIONS

The applicant has provided evidence that the additive currently in the market complies with the existing conditions of authorisation.

The use of the feed additive in animal nutrition is of no safety concern for the target species, the consumers and the environment.

As regards the safety for the user, L-cystine is not considered irritant to skin or eyes and is not considered to be a skin sensitiser. Exposure by inhalation of persons handling the additive cannot be excluded.

There is no need to assess the efficacy of the additive in the context of the renewal of the authorisation.

ABBREVIATIONS

CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed

[21] Study Report – L-cystine dermal irritation 2012.

[22] Study Report – L-cystine eye irritation 2012.

[23] Study Report – L-cystine LLNA 2012.

[24] https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf

[25] 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.

LOD	limit of detection
LOQ	limit of quantification
OECD	Organisation for Economic Co-operation and Development

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CONFLICT OF INTEREST
If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR
European Commission

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