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Safety and Efficacy of L-histidine monohydrochloride monohydrate produced by fermentation using *Escherichia coli* KCCM 80212 as a feed additive for all animal species

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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 L-histidine monohydrochloride (HCl) monohydrate produced by fermentation using *Escherichia coli* KCCM 80212 when used as a nutritional additive in feed for all animal species. The production strain is genetically modified. The production strain and its recombinant DNA were not detected in the final product. L-Histidine HCl monohydrate manufactured by fermentation using *E. coli* KCCM 80212 does not give rise to any safety concern regarding the genetic modification. The use of L-histidine HCl monohydrate produced by fermentation using *E. coli* KCCM 80212 is safe for the target species when used as a nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc. L-Histidine HCl monohydrate produced using *E. coli* KCCM 80212 supplemented at levels appropriate for the requirements of the target species is considered safe for the consumer. L-Histidine HCl monohydrate produced by *E. coli* KCCM 80212 is a skin sensitiser. There is a risk for persons handling the additive from the exposure to endotoxins by inhalation. The additive under assessment is not irritant to skin or eyes. The use of L-histidine HCl monohydrate produced using *E. coli* KCCM 80212 in animal nutrition is not expected to represent a risk to the environment. L-Histidine HCl monohydrate is considered an efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

Keywords: nutritional additive, amino acid, L-histidine monohydrochloride monohydrate, *Escherichia coli* KCCM 80212, Safety, feed additive

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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 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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Daesang Europe B.V.[2] for authorisation of the product L-histidine monohydrochloride monohydrate, 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 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 16 April 2020.

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 product L-histidine monohydrochloride monohydrate produced by fermentation using *Escherichia coli* KCCM 80212, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

L-Histidine monohydrochloride (HCl) monohydrate produced by fermentation with the genetically modified *Escherichia coli* KCCM 80212 has not been previously authorised as a feed additive in the European Union (EU).

L-Histidine HCl monohydrate (minimum 98% on dry matter basis) produced by *E. coli* ATCC 9637 is currently listed in the European Union Register of Feed Additives, and thus authorised in the European Union for use in feed for salmonids.[3] L-Histidine [EU Flavour Information System (FLAVIS) numbers 17.008] produced by chemical synthesis is currently listed in the European Union Register of Feed Additives, and thus authorised in the EU as a feed flavouring.[4]

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety of L-histidine HCl monohydrate produced by *E. coli* ATCC 21318 as nutritional feed additive (amino acid) for salmonids (EFSA, 2005, 2006a; EFSA FEEDAP Panel, 2020). The same Panel assessed the safety and efficacy of L-histidine HCl monohydrate produced using *Corynebacterium glutamicum* (KCCM 80179 or KCCM 80172) or *E. coli* (NITE BP-02526) when used as nutritional additive for all animal species (EFSA FEEDAP Panel, 2019a–c). The FEEDAP Panel assessed the safety and efficacy of L-histidine as feed flavouring (EFSA FEEDAP Panel, 2014). The EFSA's Scientific Panel on Food

[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] Daesang Europe B.V., Van Heuven Goedhartlaan 935, 1181 LD, Amsterdam, The Netherlands.
[3] Commission Regulation (EC) No 244/2007 of 7 March 2007 concerning the authorisation of L-histidine monohydrochloride monohydrate as feed additive. OJ L 73/6, 13.3.2007, p. 2.
[4] Commission List of the authorised additives in feedingstuffs published in application of Article 9th of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C, 50/1, 25.2.2004.

Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) evaluated L-histidine and considered it safe for use as flavours in food (EFSA, 2006b, 2008a,b; EFSA CEF Panel 2011).

L-Histidine and its hydrochloride are authorised for use in food,[5] cosmetics[6] and as a veterinary medicinal product.[7,8]

L-Histidine HCl is described in a monograph of the European Pharmacopeia (2017, monograph 01/2017: 0910).

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[9] in support of the authorisation request for the use of L-histidine HCl monohydrate produced by fermentation using *E. coli* KCCM 80212 as a feed additive.

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 L-histidine HCl monohydrate produced by fermentation using *E. coli* KCCM 80212 in animal feed. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of L-histidine HCl monohydrate produced by fermentation using *E. coli* KCCM 80212 is in line with the principles laid down in Regulation (EC) No 429/2008[11] 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 identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019d).

3. Assessment

The current application is for the authorisation of L-histidine HCl monohydrate (minimum 98% purity) produced by fermentation using a genetically modified strain of *E. coli* (KCCM 80212). The product is intended to be used in feed for all animal species as a nutritional additive (functional group: amino acids, their salts and analogues). The active substance of the product under application is L-histidine.

[5] 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.

[6] Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, pp. 1–528.

[7] 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.

[8] 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.

[9] FEED dossier reference: FAD-2020-0016.

[10] The full report is available on the EURL website: LINK to report.

[11] 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.

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive is produced by a genetically modified strain of *Escherichia coli* K-12 which is deposited [redacted] under the accession number KCCM 80212.

A bioinformatic analysis of the whole genome sequence (WGS) of the production strain confirmed its identity as an *E. coli* K-12 derivative.[13] This was based [redacted] *E. coli* K-12 is well-characterised and its safety (non-pathogenicity) has been documented (Gorbach, 1978). The strain has been shown to be ineffective in colonising the human gut (Smith, 1975) and its genome (MG1655 and W3110) has been fully sequenced (Hayashi et al., 2006).

[redacted]

The production strain was tested for its susceptibility to all the antimicrobials listed for 'Enterobacteriaceae' in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[14] All minimum inhibitory concentration (MIC) values were below or equal to the cut off values set in the Guidance and the strain is considered phenotypically susceptible to those antibiotics.

The WGS of the production strain was interrogated for the presence of antimicrobial resistance (AMR) genes [redacted] This, and the fact that the production strain was not phenotypically resistant to any of the antibiotics tested, suggest that the production strain does not carry acquired antibiotic resistance genes of concern.

The WGS of the production strain was also interrogated for the presence of toxin and virulence factor genes [redacted]

3.1.1.1. Information related to the genetically modified microorganism

Characteristics of the recipient microorganism

[redacted]

Description of the genetic modification

[redacted]

[redacted]

[14] Technical dossier/Section II/Annexes Section II/Annex 2.2.2.2a.

[redacted]

[redacted]

3.1.2. Manufacturing process

L-Histidine is produced by fermentation of the production strain. [redacted]

The applicant stated that no antibiotics are used during the production process.[18]

3.1.3. Characterisation of the product/active substance

L-Histidine monohydrochloride monohydrate (International Union of Pure and Applied Chemistry (IUPAC) name (2*S*)-2-amino-3-(1*H*-imidazol-5-yl)propanoic acid;hydrate;hydrochloride, and synonyms L-α-Amino-β-(4-imidazolyl)propionic acid monohydrochloride, glyoxaline-5-alanine hydrochloride) has the Chemical Abstracts Service (CAS) No 5934-29-2 and European Inventory of Existing Commercial Chemical Substances (EINECS) No 211-438-9. The chemical formula is $C_6H_{12}ClN_3O_3$ and the molecular weight 209.63 g/mol. The structural formula is given in Figure 1.

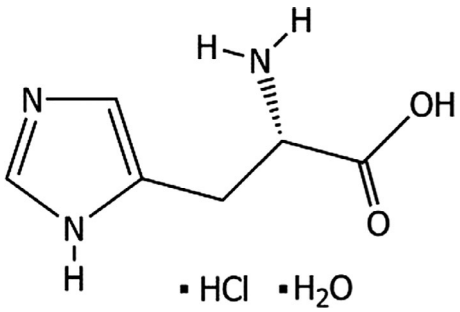

Figure 1: Molecular structure of L-histidine monohydrochloride monohydrate

According to the specification, the additive contains ≥ 98% L-histidine HCl monohydrate on a dry matter basis and ≤ 1% loss on drying.[19]

Analysis of five batches showed an average histidine HCl monohydrate content of 99.3% on a dry matter (DM) basis (range 99.2–99.4%), a loss on drying of 0.13% (range 0.02–0.32%), and an ash

[redacted]

[18] Technical dossier/Section II/Annex 2.3.1b 5.
[19] Technical dossier/Section II.1.3 and supplementary information June 2020/Sin 120620 Answers.

content of 0.09% (range 0.05–0.13%).[20] The sum of the identified material was > 99% on DM basis. Analysis of five additional batches showed an average histidine content of 74.5% (range 73.9–75.4) on DM.[21]

The specific optical rotation of the additive was measured in five batches of the final product and the average was +9.36° (range +9.34 to +9.36°).[22] The measured values were within the range of the European Pharmacopoeia (+9.2 to +10.6°) and confirmed the L-stereoisomer of histidine.

3.1.3.1. Impurities

Three batches of the additive were analysed for undesirable substances.

Heavy metals, (lead, cadmium and mercury) and arsenic were below the limits of detection (LODs).[23] In the same batches polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (Co-planar PCBs, only analysed in two batches) were found below the corresponding limit of quantification (LOQ).[24] The levels of dioxins and the sum of dioxins and dioxin-like-PCBs (upper bound) were calculated to be 0.137 ng WHO-PCDD/F-TEQ/kg and 0.269 ng WHO-PCDD/F-PCB-TEQ/kg, respectively. Non dioxin-like PCBs (ICES-6) were measured in one batch and were below 5 μg/kg. Ochratoxin A ranged from 8 to 10 μg/kg, zearalenone ranged from 25 to 33 μg/kg and citrinin ranged from 32 to 41 μg/kg, whilst aflatoxins (not described), fumonisins and deoxynivalenol were below the respective LOD.[25]

Endotoxin activity was measured in the same batches and ranged from 2,970 to 6,540 IU/g.[26]

Analysis of microbial contamination in the same three batches indicated that Enterobacteriaceae, *E. coli*, yeasts and filamentous fungi (1 g sample) were below the limit of detection. *Salmonella* spp. was absent in 25 g sample.[27]

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

The absence of DNA from the production strain was tested in three batches of the additive in triplicate. [redacted] No DNA of the production strain was detected.

3.1.3.2. Physical properties

L-Histidine HCl monohydrate is a white crystalline powder, with a bulk density of 680–700 kg/m^3,[30] and a water solubility of 168 g/L at 20°C.[31]

The dusting potential (three batches analysed by Stauber–Heubach method) ranged from 2.7 to 4.4 g/m^3.[32] The particle size distribution (three batches analysed laser diffraction) showed that the fraction of particles < 10 μm ranged from 2% to 4%, the fraction < 50 μm ranged from 6% to 18% and that < 100 μm ranged from 13% to 32% (w/v).[33]

[20] Technical dossier/Section II/Annexes II.1.3a to e. Analytical method for histidine HCl monohydrate was ion exchange chromatography with post-column ninhydrin derivatization (European Pharmacopoeia 2.2.56 method I).
[21] Technical dossier/Section II/Annex 2.1.4a and supplementary information June 2020/SIN 120620 Answers. Analytical method for L-histidine in accordance with Commission Regulation (EC) No 152/2009 III.F.
[22] Technical dossier/Section II/Annexes 2.1.3a to 3e.
[23] Technical dossier/Section II/Annex 2.1.4a. LOD (in mg/kg) was 0.01 for arsenic and lead; 0.002 for cadmium and mercury.
[24] Technical dossier/Section II/Annexes 2.1.4b1, c1 and d1. LOQ were 0.004–2 ng/kg for PCDD/F; 1–20 ng/kg for dioxin like PCBs; and 0.001–0.005 mg/kg for non dioxin like PCBs.
[25] Technical dossier/Section II/Annex 2.1.4a. LOD (in μg/kg) was 0.1 for aflatoxins, 25 for fumonisins and 134 for deoxynivalenol.
[26] Technical dossier/Section II/Annex 2.1.4e.
[27] Technical dossier/Section II/Annex 2.1.4a and supplementary information June 2020/Sin 120620 Answers. LOD was 1 CFU/g.
[redacted]
[30] Technical dossier/Section II/Annexes 2.1.3a to e.
[31] Technical dossier/Section II/Annex 2.5.2 6 His HCl feed grade MSDS.
[32] Technical dossier/Section II/Annexes 2.1.5a to 5c.
[33] Technical dossier/Section II.2.2.1/Annex II.1.5.

3.1.3.3. Stability and homogeneity

The stability of three batches was studied when stored in sealed bags protected from light at 25 or 40°C for 12 or 6 months, respectively.[34] No losses were detected at the end of the respective storage periods.

The stability of three batches of the additive was studied in a vitamin/mineral premixture (containing 23 mg choline chloride/kg) when supplemented at 3% histidine and stored in sealed plastic bags at room temperature and protected from light during six months.[35] A loss of 2.4% was detected in only one of the three batches tested.

The stability of the three batches of the additive was tested in a compound feed (mash and pelleted forms) consisting on barley and wheat (basal content of histidine was below the LOD of 0.05%) and supplemented with 0.18% histidine.[36] Mash and pelleted feed were stored in sealed plastic bags and kept at room temperature and protected from light for 3 months. Pelleting was performed at 78°C and no losses were detected attributable to the pelleting process. At the end of the storage period, losses in mash feed ranged from 6% to 23% and losses in pelleted feed from 13% to 18%.

The capacity of the additive (one batch) to distribute homogeneously in the pelleted feed described above was studied by analysing 10 subsamples.[37] The coefficient of variation was 6%.

3.1.4. Conditions of use

The additive is intended to be added directly to feed, or through complementary feed or premixtures of all animal species. No inclusion levels are proposed as these will depend on the dietary requirements of the species considered, the physiological state of the animal, the performance level and the environmental conditions, in particular on the amino acid composition of the unsupplemented diet.

3.2. Safety

3.2.1. Safety of the production organism

The genetic modifications performed to obtain the production strain KCCM 80212 [redacted] The parental strain was [redacted] K-12 which is considered to be safe. None of the introduced modifications raise a safety concern. The production strain is free of all antibiotic resistance genes used during the genetic modification process. The production strain and its DNA were not detected in the final additive. Therefore, the final product does not give raise to any safety concern with regard to the genetic modification of the production strain.

3.2.2. Safety for the target species

The essentiality of the amino acid histidine, its content in feedingstuffs, the requirements for the different target species and normal use levels were discussed in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2019a–c).

The additive is highly purified, containing > 98% L-histidine HCl monohydrate, and the amount of unidentified material is < 1% on a dry matter basis. Concerns from the use of the additive would not derive from the amino acid L-histidine, which is considered safe, but may arise from residues of the fermentation process/production strain remaining in the final product. The genetically modified production strain is an *E. coli* K-12 derivative that is considered safe (see Section 3.2.1). No cells or DNA of the production strain were found in the final product. Endotoxin activity of the product (up to 6,540 IU/g) is considered low and of no concern for the target species (Wallace et al., 2016).[38] The FEEDAP Panel considers that no safety concerns would derive from the fermentation process.

L-Histidine chelates divalent metal ions and it is necessary for the regulation and catabolism of trace elements such as zinc, copper, iron, manganese and molybdenum. High levels of histidine could therefore theoretically cause deficiencies of the free forms of these metal ions due to increased

[34] Technical dossier/Section II.4.1 and Annex 2.1.4a.
[35] Technical dossier/Section II/Annex 2.4.1a and b and supplementary information June 2020/Sin 120620 Answers.
[36] Technical dossier/Section II/Annex 2.4.1c and d and supplementary information June 2020/Sin 120620 Answers.
[37] Technical dossier/Section II/Annex 2.4.1d.
[38] Normal feedstuffs may be contaminated with varying concentrations of endotoxins, with values of 1,000 IU/mg feed not being unusual.

excretion (Aoyama et al., 1992; Aoyama and Cato, 2000; EFSA, 2005; VKM, 2016). This interaction of histidine with trace elements should be considered when formulating the animal diets.

3.2.2.1. Conclusions on safety for the target species

The use of L-histidine monohydrochloride monohydrate produced by fermentation using *E. coli* KCCM 80212 is safe for the target species when used as nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc.

3.2.3. Safety for the consumer

The product under assessment is produced by fermentation using a strain of *E. coli* (KCCM 80212) which has been demonstrated to be a K-12 derivative. The genetic modification is considered safe and no viable cells of the production strain or their DNA were detected in the final product. The additive contains minimum 98% L-histidine HCl monohydrate and the amount of unidentified material is < 1% on a dry matter basis. Therefore, the FEEDAP Panel considers that no safety concerns would derive from the fermentation process.

Histamine is a biogenic amine that can be synthesised endogenously from histidine by a L-histidine decarboxylase. Histamine can be metabolised either extracellularly by a diamino oxidase (DAO) present in the gut mucosa, or intracellularly by a histamine-*N*-methyltransferase (HNMT) (EFSA BIOHAZ Panel, 2011).The intake of histamine through fish flesh following microbial spoilage is a serious concern for consumers (EFSA BIOHAZ Panel, 2011). Histamine poisoning from fish flesh has been called 'scombroid' poisoning because of the edible fish species (e.g. tuna, mackerel) more liable to histamine formation due to the high content of histidine in their flesh. Commission Regulation (EC) No 2073/2005 sets a maximum limit of 200 mg histamine/kg flesh for sea fishery products (raw fish at the point of the first sale) of fish species associated with a high amount of histidine, in particular fish species of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae and Scombresosidae.[39]

In some fish species (e.g. Atlantic salmon), increasing levels of dietary histidine have resulted in increasing histidine deposition in fish flesh (Breck et al., 2005), and it is well known that histidine is a precursor of histamine. The main factors influencing histamine formation in fish, however, are storage time, temperature, pH, hygienic conditions (e.g. bacterial contamination, time to evisceration) or starter cultures of fermented foods, which have been reviewed in previous publications (EFSA BIOHAZ Panel, 2011; FAO, 2013, 2018; Technical report of EFSA, 2017). The FEEDAP Panel considers that supplementing the diets of the target species with histidine to cover the requirements is unlikely to result in the increase of histamine formation provided that appropriate handling and storage of fish are ensured.

To the knowledge of the FEEDAP Panel, there are no records of histamine poisoning associated with raw mammal or poultry edible tissues and products. Therefore, the FEEDAP Panel considers it unlikely that supplementation of feed with histidine to cover animal requirements will increase the risk of histamine poisoning upon consumption of such raw edible tissues and products from mammals and birds provided that appropriate handling and storage are ensured.

3.2.3.1. Conclusions on safety for the consumer

L-Histidine HCl monohydrate produced using *E. coli* KCCM 80212 supplemented at levels appropriate for the requirements of the target species is considered safe for the consumer.

3.2.4. Safety for the user

The applicant provided an inhalation toxicity study, a dermal irritation study, an eye irritation study and a skin sensitisation study testing the additive under assessment.

3.2.4.1. Effects in the respiratory system

The highest dusting potential analysed was 4.4 g/m^3 and the product has particles of inhalable, thoracic and respirable sizes (see Section 3.1.3.2). The user may be exposed by inhalation.

[39] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L, 22.12.2005, 338/1, pp. 26.

In a valid acute inhalation toxicity study according to Organisation for Economic Co-operation and Development (OECD) guideline 436, the additive showed an inhalation LC_{50} greater than 5.39 mg/l air and the test item required no classification.[40]

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 the 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) (HCN, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust, the inhalation exposure could be up to 15,987 endotoxin IU per working day, indicating thus an inhalation risk to endotoxins for persons handling the additive.

3.2.4.2. Effects in skin and eyes

The skin irritation potential of the additive was tested in a valid study performed according to OECD guideline 404, which showed that it is not a skin irritant and has no corrosive effect on skin.[41]

The eye irritation potential of the additive was tested in a valid study performed according to OECD guideline 405, which showed that it is not an eye irritant.[42]

In a valid skin sensitisation study following local lymph-node assay (LLNA) performed according OECD guideline 429,[43] the additive was found to be a skin sensitiser and has to be classified as a skin sensitiser in Category 1 (sub-category 1-B), in accordance with the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.

3.2.4.3. Conclusions on safety for the user

The endotoxin activity present in the final product represents an inhalation risk for persons handling the additive. The additive is a skin sensitiser. It is not irritant to skin or eyes.

3.2.5. Safety for the environment

The production organism and its DNA were not detected in the final product. The final product does not pose any environmental safety concern associated with the genetic modification of the production strain.

The amino acid L-histidine is a physiological and natural component of animal and plant proteins. When consumed, it will be absorbed, and the non-absorbed fraction will be incorporated into the intestinal microbial mass and excreted as such. Its use in animal nutrition would not lead to any localised increase in its concentration in the environment. The use of L-histidine HCl monohydrate produced using *E. coli* KCCM 80212 in animal nutrition is not expected to represent a risk to the environment.

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-histidine monohydrochloride monohydrate is well established in the scientific literature (NRC 1994, 1998, 2011, 2012).

In general, the product L-histidine monohydrochloride monohydrate is considered as efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

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[44] and Good Manufacturing Practice.

[40] Technical dossier/Section III/Annex 3.3.1.1 and supplementary information June 2020/Annex 1 Amendment to Final report No 1 B-02700-FR-END.
[41] Technical dossier/Section III/Annex 3.3.1.2a.
[42] Technical dossier/Section III/Annex 3.3.1.2b.g.
[43] Technical dossier/Section III/Annex 3.3.1.2c.
[44] 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.

4. Conclusions

The production strain and its recombinant DNA were not detected in the final products. L-Histidine HCl monohydrate manufactured by fermentation using *E. coli* KCCM 80212 does not give rise to any safety concern regarding the production strain and its genetic modification.

The use of L-histidine HCl monohydrate produced by fermentation using *E. coli* KCCM 80212 is safe for the target species when used as a nutritional additive to supplement the diet in appropriate amounts to cover the requirements, depending on the species, the physiological state of the animal, the performance level, the environmental conditions, the background amino acid composition of the unsupplemented diet and the status of some essential trace elements such as copper and zinc.

L-Histidine HCl monohydrate produced using *E. coli* KCCM 80212 supplemented at levels appropriate for the requirements of target species is considered safe for the consumer.

L-Histidine HCl monohydrate produced by *E. coli* KCCM 80212 is a skin sensitiser. There is a risk for persons handling the additive from the exposure to endotoxins by inhalation. The additive under assessment is not irritant to skin or eyes.

The use of L-histidine HCl monohydrate produced by *E. coli* KCCM 80212 in animal nutrition is not expected to represent a risk to the environment.

L-Histidine HCl monohydrate is considered an efficacious source of the essential amino acid L-histidine for non-ruminant animal species. For the supplemental L-histidine to be as efficacious in ruminants as in non-ruminant species, it would require protection against degradation in the rumen.

5. Documentation as provided to EFSA/Chronology

Date	Event
18/02/2020	Dossier received by EFSA. L-Histidine monohydrochloride monohydrate produced by fermentation with *Escherichia coli* KCCM 80212. Submitted by Daesang Europe B.V
02/03/2020	Reception mandate from the European Commission
16/04/2020	Application validated by EFSA – Start of the scientific assessment
12/06/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of the additive, conditions of use, safety for the user*
18/06/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
16/07/2020	Comments received from Member States
17/07/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
30/09/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation
DECOS	Dutch Expert Committee on Occupational Safety
DM	dry matter
EINECS	European Inventory of Existing Commercial Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	EU Flavour Information System
HNMT	histamine-*N*-methyltransferase
HSE	UK Health and Safety Executive
IEC-VIS/FLD	ion-exchange chromatography coupled to visible or fluorescence detection
IUPAC	International Union of Pure and Applied Chemistry
LLNA	local lymph-node assay
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
RSD_r	relative standard deviation for repeatability
RSD_R	relative standard deviation for reproducibility
WHO	World Health Organization
VKM	Norwegian Scientific Committee for Food Safety

Appendix A – Safety for the user

The effects of the 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 likely 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, equal to = 800 s per day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s (0.444 h per day). Again, assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m^3/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/m^3.

Calculation of endotoxin content of dust

Two key measurements are required to evaluate the potential respiratory hazard associated with endotoxin content of the product (the dusting potential of the product, expressed in g/m^3; 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 used instead. If the content of endotoxins of the relevant additive is a IU/g and the dusting potential is b g/m^3, then the content of endotoxins of the dust, c IU/m^3, is obtained by the simple multiplication a × b. This resulting value is further used for calculation of potential inhalation exposure by users to endotoxin 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-histidine HCl monohydrate produced by *Escherichia coli* KCCM 80212 including consideration of using filter half mask (FF P2 or FF P3)[(a)] as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	6,540	Technical dossier
	b	Dusting potential (g/m^3)	4.4	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	28,776	
	d	No of premixture batches made/working day	40	EFSA FEEDAP Panel (2012)
	e	Time of exposure (s)/production of one batch	20	EFSA FEEDAP Panel (2012)
d × e	f	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA FEEDAP Panel (2012)
f × g	h	Refined total duration of daily exposure (s)	1,600	
h/3 600	i	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3)/8-h working day	10	EFSA FEEDAP Panel (2012)
j/8 × i	k	Inhaled air during exposure (m^3)	0.56	
c × k	l	**Endotoxin inhaled (IU) during exposure/ 8-h working day**	**15,987**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3)/8-h working day	90	HCN (2010)
m × j	n	**Health-based recommended exposure limit of total endotoxin exposure (IU)/8-h working day**	**900**	
l/10		Endotoxins inhaled (IU)/8-h working day reduced by filter half mask FF P2 (reduction factor 10)	1,599	
l/20		Endotoxins inhaled (IU)/8-h working day reduced by filter half mask FF P3 (reduction factor 20)	799	

(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.

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for L-Histidine monohydrochloride monohydrate produced by fermentation using *Escherichia coli* KCCM 80212

In the current application an authorisation is sought under Article 4(1) for L-histidine monohydrochloride monohydrate produced by fermentation with the strain *Escherichia coli* KCCM 80212 (H010), under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species.

According to the Applicant, L-histidine monohydrochloride monohydrate has a minimum purity (mass fraction) of 98%. The feed additive is intended to be added directly into feedingstuffs or through premixtures. However, the Applicant did not propose any minimum or maximum content of L-histidine monohydrochloride monohydrate in feedingstuffs.

For the quantification of histidine in the feed additive and premixtures the Applicant proposed and submitted the ring-trial validated method EN ISO 17180 originally dedicated for the determination of lysine, methionine and threonine in commercial amino acid products and premixtures. This standard method is based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). It does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. The method is applicable for products and premixtures containing more than 10% in mass fraction of the amino acid.

The extension of scope of the EN ISO 17180 method to another almost identical feed additive and premixtures containing histidine has been demonstrated by the Applicant in the frame of a recent histidine dossier.

For the quantification of histidine in feedingstuffs the Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled to photometric detection (VIS). The method is designed for the analysis of amino acids in premixtures and feedingstuffs and does not distinguish between the salts and the amino acid enantiomers. This method was further ring-trial validated by 23 laboratories, resulting in the EN ISO 13903 method. The following performance characteristics were reported for the quantification of total histidine: a relative standard deviation for repeatability (RSD_r) ranging from 2.4 to 7.0% and a relative standard deviation for reproducibility (RSD_R) ranging from 13 to 23%.

Based on the performance characteristics available, the EURL recommends for official control: (i) the ring-trial validated method EN ISO 17180 based on IEC-VIS/FLD for the quantification of histidine in the feed additive and premixtures; and (ii) the ring-trial validated EU method, based on IEC-VIS for the quantification of histidine in premixtures and feedingstuffs.

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.