Assessment : Safety assessment of the application for L-histidine monohydrochloride monohydrate produced by *Escherichia coli* K-12 KCCM 80212 as a feed additive for all animal species, from Daesang Europe B. V.

Reference Number RP 1105

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1.Executive summary

The FSA/FSS have undertaken an assessment of application RP 1105 for the use of L-histidine monohydrochloride (≥ 98%[1]) produced by fermentation with *Escherichia coli* KCCM 80212 as a feed additive for all animal species, from Daesang Europe B. V. Van Heuven Goedhartlaan 935, 1181 LD Amstelveen, Netherlands (category: nutritional additives; functional group: amino acids, their salts and analogues, to satisfy the nutritional needs of the target species with this essential amino acid).

A number of feed additive applications have been received by Great Britain (GB) where EFSA, prior to the end of the transition period, evaluated an application for the product. FSA/FSS have reviewed the EFSA opinion (EFSA Journal 2020;18(11):6287) and confirm that they are adequate and relevant for GB risk analysis and used this to form the basis of the GB opinion. A full risk assessment of this application was not considered necessary by FSA and FSS.

The FSA/FSS risk assessors concluded that the EFSA opinions are adequate and relevant for GB risk analysis. Therefore, the use of the L-histidine additive, as described in this application, is safe and is not liable to have an adverse effect on the target species, worker safety, environmental safety and human health at the intended concentrations of use.

There are no specific conditions or restrictions in relation to handling, labelling, post-market monitoring requirements and use of this additive as described in this application. Maximum Residue Limits (MRLs) are not required for this additive.

2.Background and purpose of review

EFSA Journal 2020; 18(11):6287,

Question number: EFSA-Q-2020-00189

In accordance with Retained EU Regulation 1831/2003 on feed additives, the application RP 1105 for the use of L-histidine monohydrochloride produced by fermentation with *Escherichia coli* KCCM 80212 as a feed additive for all animal species from Daesang Europe B. V. has been submitted for authorisation in each nation of Great Britain (GB).

Whilst it was a Member State of the EU, the UK accepted the assessments of EFSA in respect of authorisations for regulated food and feed products. Since the end of the transition period, FSA/FSS has adopted equivalent technical guidance and quality assurance processes to be able to undertake GB risk assessments for regulated product applications.

[1] The analytical method for histidine hydrochloride monohydrate was ion exchange chromatography with post-column ninhydrin derivatisation (European Pharmacopoeia 2.2.56 method I).

However, a number of applications have been received by GB where EFSA, prior to the end of the transition period, evaluated an application for the product for which an application is now made to GB. FSA/FSS has decided to make use of the EFSA risk assessment, where this is appropriate, in forming its opinion. Therefore, FSA/FSS risk assessors have reviewed the EFSA opinion[2] for the application below in the context of intended GB use and have concluded that the intended uses are safe.

In reviewing the EFSA risk assessment opinion the reviewers have verified that the standard approach as outlined in the relevant guidance[3] has been followed and the arguments made are consistent with the data summarised in the opinion. Consideration has been given to the processes undertaken to ensure the opinions are robust and whether there are any aspects that would require further review such as specific issues for the countries of the GB. The result of the assessment is that the EFSA scientific opinions are adequate also for GB risk analysis. Therefore, a full risk assessment has not been performed by FSA/ FSS.

3.Details of the EFSA assessment

Methodology applied in the EFSA opinions

EFSA FEEDAP guidance: Guidance on the assessment of the safety of feed additives for the environment (**2019**), Guidance on the assessment of the safety of feed additives for the target species (**2017a**), Guidance on the identity, characterisation and conditions of use of feed additives (**2017b**), Guidance on the assessment of the safety of feed additives for the consumer (**2017c**), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (**2018a**), Guidance on studies concerning the safety of use of the additive for users/workers (**2012**) and principles in Regulation (EC) No 429/2008.

3.1 Source/organism

The additive contains L-histidine produced by fermentation with the genetically modified *Escherichia coli* KCCM 80212. The application is for the additive as a solid as L-histidine monohydrochloride monohydrate.

The production strain of the L-histidine is from the species *E. coli* K-12 which is well characterised (genome MG1655, W3110 sequenced) and its safety is well established in the supporting literature.

For this application, the production strain was sequenced using a whole genome approach and evaluated for susceptibility to antibiotics and the presence of virulence factor and toxin genes. From this it was concluded that the strain is not phenotypically resistant to any of the antibiotics tested.

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[2] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2020. Safety and efficacy of L-histidine monohydrochloride monohydrate produced by fermentation with *Escherichia coli* KCCM 80212 as a feed additives for all animal species. EFSA Journal 2020; 18(11):6287. https://doi.org/10.2903/j.efsa.2020.6287

[3] See reference list for the full set of guidance applied.

3.2 Genetic modification step

The assessment of the genetic modification of the production strain was performed. There are no safety concerns noted relating to the inserted genes and the production strain does not contain acquired (by modification) additional antimicrobial resistance genes.

3.3 Specification

Information provided on the identity, composition and specifications of the production species does not raise safety concerns (the published EFSA opinions are redacted whilst confidentiality claims are determined.)

Physiochemical properties, homogeneity data and stability data (as shelf life of the products) were presented for the additive. The solid additive L-histidine is highly purified and homogenous within a pelleted final feed ration. Stability data for the additive within six batches of exemplar feed rations (three batches for mash and three for pelleted forms) and three batches of feed pre-mix were provided. Maximum losses in mash and pellet forms after 3 months were 23% and 18% respectively.

Three batches of L-histidine were analysed for heavy metals (mercury, lead, cadmium and arsenic), dioxins, mycotoxins (aflatoxins, citrinin, ochratoxin A, zearalenone, fumonisins and deoxynivalenol), endotoxins and microbiological contamination (*E.coli*, total *Enterobacteriaceae*, *Salmonella* spp, yeasts and filamentous fungi). No safety concerns were noted with results from the analyses. The highest endotoxin activity measured of 6,540 EU/g is considered low and of no safety concern to the target species (although it may represent a risk to workers given the product's dusting potential).

This application included the request for use within all species. No recommended inclusion rates were discussed in the application due to the varying concentrations that may be required due to the animal species, nutritional specification of the feed ration, environmental conditions and the current health state of the animal.

3.4 Exposure assessment

Not relevant as L-histidine is a natural component of any consumed animal products. The applicant is seeking to use L-histidine as a nutritional feed additive and the product has been assessed on this basis.

3.5 Toxicological data

L-histidine is currently authorised as a feed additive for all species as a nutritional additive, using different production species than the one used in this application. This application is for the use of L-histidine produced by fermentation with *Escherichia coli* KCCM 80212.

The additive has particles of inhalable, thoracic, and respirable sizes and therefore the worker may be exposed through inhalation. An acute inhalation toxicity test was performed under GLP and following OECD TG 436. The inhalation lethal concentration LC_{50} was estimated to be > 5.39 mg/L air, therefore the additive was not given a classification for inhalation toxicity.

The skin irritancy potential of the additive was tested in a GLP study performed according to OECD TG 404. The additive was concluded to be non-irritant to skin. A

skin sensitisation test was undertaken in a GLP study performed according to OECD TG 429 which found the additive to be a skin sensitiser.

The potential eye irritancy of the additive was tested in a GLP study performed according to OECD TG 405. The results showed the additive to not be an eye irritant.

4. EFSA assessment and conclusions

L-Histidine produced by fermentation with *Escherichia coli* KCCM 80212 raises no concerns for the consumer of the products obtained from animals fed the additive. *Escherichia. coli* K-12 is well characterised, and its safety is well established in the supporting literature.

Histamine is a biogenic amine that can be formed from L-histidine and its intake has been classified by EFSA as 'a serious concern for consumers' (EFSA BIOHAZ Panel, 2011). It is commonly formed through microbial degradation of fish tissue. EFSA's assessment in this case is that to their knowledge there are no known occurrences of histamine poisoning associated with edible mammal or poultry products. Therefore, EFSA conclude that as long as appropriate handling and storage practices are maintained that there should be no increase in risk in histamine poisoning of edible mammal poultry products from this feed additive.
Regarding its use as a feed additive in fish, EFSA conclude that the use of L-histidine for nutritional supplementation should not result in the increase of histamine within fish tissues provided that appropriate handling and storage of fish are ensured.

The additive is not a skin or eye irritant but is deemed a skin sensitiser. The additive does represent an inhalation risk for workers handling the product. This conclusion was reached by comparing the calculated potential inhalation exposure of endotoxins per day of 15,987 EU against the provisional inhaled endotoxin exposure limit of 900 EU, set by the UK Health and Safety Executive (HSE, 2013).

No risks to the environment are expected and no further environmental risk assessment is required. The additive is present as a natural component of both plants and animals. If given to animals, L-histidine is absorbed and the non-absorbed fraction excreted as such. The use of this additive in animal nutrition would not lead to any increases in the concentration of L-histidine in the environment. Furthermore, the application does not raise safety concerns for the environment regarding the production strain *Escherichia coli* KCCM 80212.

Regarding safety for the target species, there are no safety concerns noted relating to the inserted genes of *Escherichia coli* KCCM 80212 and the strain does not

contain acquired (by modification) additional antimicrobial resistance genes. The production strain and its DNA were not detected in the final additive product.

The use of the L-histidine additive does not raise safety concerns for the target animal.

5. Caveats and uncertainties

There are no further caveats or uncertainties to highlight.

6. FSA Conclusion on reliability and applicability

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the EFSA Panel, whilst this is only briefly summarised this description is consistent with the conclusions.

6.1 Analytical Method Review

FSA/FSS accepts the EURL analytical method evaluation report[4]. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

7. Outcome of assessment

FSA/FSS has reviewed the EFSA opinions and consider them adequate and relevant for GB risk analysis. Therefore, the opinions were used to form the basis of the GB opinion. A full risk assessment of this application was not considered necessary by FSA and FSS.

FSA/FSS had access to all supporting documentation that was provided to the EFSA Panel by the applicant, and subsequently used to form the EFSA opinion. FSA/FSS agree with the safety conclusions outlined in the EFSA opinions.

Following the principles outlined in the background for making use of the EFSA opinions, the FSA/FSS opinion is that the L-histidine additive, as described in this application, is safe and is not liable to have an adverse effect on the target species, worker safety, environmental safety and human health at the intended concentrations of use.

[4] https://joint-research-centre.ec.europa.eu/system/files/2020-10/finrep-fad-2020-0016-histidine.pdf

8. References

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017a. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

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