Assessment : Safety assessment of the application for L-tryptophan produced by *Escherichia coli* KCCM 80210 as a feed additive for all animal species, from Daesang Europe B. V.

Reference Number RP 1125

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Contents

1.Executive summary

The FSA/FSS have undertaken an assessment of application RP 1125 for the use of L-tryptophan (≥ 98%[1]) produced by *Escherichia coli* KCCM 80210 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 2021;19(3):6425) 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 and therefore, the use of the L-tryptophan additive, as described in this application, is safe and is not liable to have an adverse effect on the target species, environmental safety and human health at the intended concentrations of use. The dusting potential, along with the potential endotoxin concentration, indicates an inhalation risk for workers handling the product.

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 2021; 19(3): 6425,

Question number: EFSA-Q-2020-00499

In accordance with Retained EU Regulation 1831/2003 on feed additives, the application RP 1125 for the use of L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210 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 L-tryptophan was the EN ISO 13904:2016 method based on high performance liquid chromatography with fluorescence detection

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 (**2018**), 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-tryptophan produced by fermentation with the genetically modified *Escherichia coli* KCCM 80210. The application is for the additive as a solid as L-tryptophan.

The production strain of the L-tryptophan is from the species *E. coli* K-12 which is well characterised 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.

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

[2] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2021. Scientific Opinion on the safety and efficacy of the feed additive consisting of L-tryptophan produced by Escherichia coli KCCM 80210 for all animal species (Daesang Europe BV).EFSA Journal 2021; 19(3):6425. https://doi.org/10.2903/j.efsa.2021.6425

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

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.) The impurities 1,1′-ethylidene-bis-l-tryptophan (EBT) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) were determined as < 10 mg/kg and 1 mg/kg respectively. These are below the established maximum levels[4] of 10 mg/kg and 390 mg/kg for EBT and the sum of all impurities (which includes MTCA) respectively.

Physiochemical properties, homogeneity data and stability data (as shelf life of the products) were presented for the additive. The solid additive L-tryptophan 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 2% (both forms).

Five batches of L-tryptophan were analysed for heavy metals (mercury, lead, cadmium and arsenic). Three batches were analysed for dioxins, mycotoxins (aflatoxins, citrinin, ochratoxin A, zearalenone, fumonisins and deoxynivalenol), microbiological contamination (*E.coli*, total *Enterobacteriaceae*, *Salmonella* spp, yeasts and filamentous fungi) and endotoxin activity. No safety concerns were noted with results from the analyses although the highest concentration measured of endotoxins of approximately 5000 EU/g 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-tryptophan is a natural component of any consumed animal products. The applicant is seeking to use L-tryptophan as a nutritional feed additive and the product has been assessed on this basis.

3.5 Toxicological data

L-tryptophan is currently authorised as a feed additive for all species as a nutritional additive, using a different production organism than the one used in this application. This application is for the use of L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210.

The additive has particles of inhalable, and respirable sizes and therefore the worker may be exposed through inhalation. An acute inhalation toxicity test was performed in rats under GLP and following OECD TG 403. The inhalation median lethal

[4] European Pharmacopoeia, 9th Edition, 2017. European Directorate for the Quality of Medicines and Health, Monograph 01/2017:1272.

concentration LC_{50} was estimated to be > 5.13 mg/L air, therefore the additive was not given a classification for inhalation toxicity.

The skin irritancy potential of the additive was tested in rabbits 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 mice in a GLP study performed according to OECD TG 429 which found the additive is not a skin sensitiser.

The potential eye irritancy of the additive was tested in rabbits in a GLP study performed according to OECD TG 405. The results showed the additive to be a mild eye irritant.

4. EFSA assessment and conclusions

L-tryptophan produced by fermentation with *Escherichia coli* KCCM 80210 (K-12 strain) 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.

The additive is not a skin irritant or deemed a skin sensitiser. It is a mild eye irritant. Due to the products dusting potential and potential endotoxin concentration in the dust, the additive does represent an inhalation risk for workers handling the product. This conclusion was reached by comparing the calculated inhalation exposure of endotoxins per day of 2,300 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-tryptophan is excreted as urea/uric acid, indole-related compounds and carbon dioxide. The use of this additive in animal nutrition would not lead to any increases in the concentration of L-tryptophan in the environment. Furthermore, the application does not raise safety concerns for the environment regarding the genetic modification of the production strain *Escherichia coli* KCCM 80210.

Regarding safety for the target species, there are no safety concerns noted relating to the inserted genes of *Escherichia coli* KCCM 80210 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. Bacterial endotoxin concentrations of approximately 5,000 EU/g in the additive are far below the benchmark concentration of 1,000,000 EU/g usually found in feed.

The use of the L-tryptophan additive does not raise safety concerns for non-ruminants. Impurity concentrations for EBT and MTCA of < 10 mg/kg and 1 mg/kg do not raise safety concerns. For unprotected tryptophan additives (those which are not administered with a product to by-pass rumen digestion), ruminant metabolism can

results in increasing concentrations of 3-methylindole (skatole) which has been linked to pulmonary disease. Therefore, there may be a risk for an increased concentration of potentially toxic metabolites if unprotected tryptophan is administered to ruminants.

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[5]. 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-tryptophan additive, as described in this application, is not liable to have an adverse effect on the target species in non-ruminants. There may be a risk for an increased concentration of potentially toxic metabolites if unprotected tryptophan is administered to ruminants. The dusting potential, along with the potential endotoxin concentration and as a mild irritant to eyes, indicates a risk for workers handling the product. There are no safety concerns regarding environmental safety and human health at the intended concentrations of use.

[5] https://joint-research-centre.ec.europa.eu/system/files/2020-12/finrep-fad-2020-0038-tryptophan.pdf

8. References

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