Assessment : Assessment of the application for authorisation of fumonisin esterase produced from *Komagataella phaffii* DSM 32159 (Biomin GmbH) as a feed additive for all animal species

Reference Number RP1591

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1.Executive summary

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) have undertaken an assessment of application RP1591 for the authorisation of fumonisin esterase (FUMzyme, minimum of 3,000 U esterase/g[1]) as a feed additive for use in all species, from Biomin GmbH, Erber Campus 1, 3131 Getzerdorf, Austria (category: technological additives; functional group: substances for the reduction of the contamination of feed by mycotoxins (intended to degrade fumonisin mycotoxins contaminants in feed)).

A feed additive application has 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(7):6207, 12 pp.) alongside a previous EFSA opinion for this additive (EFSA Journal 2018;16(5):5269, 18 pp.) and confirmed that they are adequate and relevant for GB risk analysis and used this to form the basis of GB opinion.

The FSA/FSS risk assessors concluded that the EFSA opinion is adequate and relevant for GB risk analysis. Therefore, the use of fumonisin esterase (FUMzyme), 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(7):6207 **Question number**: EFSA-Q-2019-00624

In accordance with Retained EU Regulation 1831/2003 on feed additives, the application RP1591 for the authorisation of fumonisin esterase (FUMzyme) as a feed additive for use in all species, from Biomin GmbH has been submitted for authorisation in GB.

Whilst it was a Member State of the EU, 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.

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

[1] One unit (U) is the enzymatic activity that releases 1 µmol propane-1,2,3-tricarboxylic acid per minute from 100 IM FB1 in 20 mM Tris-Cl buffer pH 8.0 with 0.1 mg/mL bovine serum albumin at 30°C.

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 output of the EFSA risk assessments 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 GB. The result of the assessment is that the EFSA scientific opinion is adequate also for GB risk analysis. Therefore, a full risk assessment has not been performed by FSA/FSS.

3.Details of the EFSA assessment

3.1 Methodology applied in the EFSA opinion

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

3.2 Source/organism

The additive is produced by the genetically modified microorganism *Komagataella phaffii* DSM 32159. The characterisation of this production strain was described in a previous EFSA evaluation (EFSA, 2018b). The parent organism *K. phaffii* CBS 7435(NRRL Y-11430, ATCC 76273) was considered to qualify for assessment under the Qualified Presumption of Safety (QPS) approach. A gene from the α-proteobacterium *Sphingopyxis* sp. MTA144, which codes for a carboxylesterase which catalyses the hydrolysis of fumonisin B1 to aminopentol 1 (producing fully de-esterified fumonisin, HFB1), was used to transform the parent organism and produce the production organism.

3.3 Genetic modification step

The genetic modification was assessed in a previous EFSA evaluation (EFSA, 2018b). The description of the genetic modification was redacted. However, EFSA concluded that the genetic modifications gave no cause for concern.

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[2] EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2020. Scientific Opinion on the safety and efficacy of fumonisin esterase from *Komagataella phaffii* DSM 32159 as a feed additive for all animal species. EFSA Journal 2020;18(7):6207,12 pp. https://doi.org/10.2903/j.efsa.2020.6207

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

3.4 Specification

The additive contains a minimum of 3000 U/g esterase. The characterisation of the additive was previously considered by EFSA (EFSA, 2018b). Routine monitoring in three different production batches of the additive had shown that the concentrations of lead, cadmium, mercury and arsenic were in all cases below the respective limits of quantification (<0.5, <0.1, <0.01 and <0.5 mg/kg, respectively). The analysis for 11 mycotoxins associated with cereals in 3 batches also showed all were below the limits of quantification. Quality control parameters for microbial contaminants were set at a maximum of 30 colony-forming units (CFU)/g for total coliforms, a maximum of 100 CFU/g individually for yeasts and filamentous fungi and the absence of *Escherichia coli* and *Salmonella* spp. in 25 g of product. No viable production strain nor its recombinant DNA had been found in the final product. The production process of the strain and the manufacturing process of the additive are as previously evaluated by EFSA (EFSA, 2018b).

The applicant has provided new data regarding the presence of recombinant DNA from the production strain in the additive. No recombinant DNA was detected by real-time qPCR in three batches of the additive (in triplicate); the detection limit was 10 ng/g product or 10^4 cells/g of product.

The additive is applied to feed to be fermented (e.g. silage) as a spray, dissolved in water. Therefore, the applicant has submitted a stability study in water. The additive was dissolved in aqueous solutions with or without FCE buffer (FCE buffer = 20 mM TrisHCl pH 8, 0.1 mg/mL BSA) and stored at room temperature for 48 hours. Two replicates per medium and three samples per replicate were analysed. The losses reported ranged from 4.4% to 12.9%.

The additive is intended for use in fermenting feeds of all species, with a minimum inclusion rate of 40 U/kg fresh material and no specified maximum inclusion rate. There is no withdrawal period intended.

3.5 Exposure assessment

Not applicable.

3.6 Toxicological data

EFSA previously concluded that the source production strain *K. phaffi* qualifies for the QPS (qualified presumption of safety) assessment approach when used for enzyme production (EFSA, 2018b). The genetic modifications result in increased production of fumonisin esterase and the modified production strain carries several copies of the Zeocin resistance gene and the Geneticin resistance gene incorporated into its chromosome. Data from the applicant showed that neither the viable production strain nor its recombinant DNA were found in three batches of the final additive.

The submitted toxicological studies were previously evaluated by EFSA (2018b). These were a bacterial reverse mutation test conducted according to OECD guideline 471, an *in vitro* chromosome aberration test conducted according to OECD guideline 473, an *in vivo* mammalian erythrocyte micronucleus test conducted according to OECD guideline 474 and a subchronic (90-day) toxicity study in rats conducted according to OECD guideline 408. EFSA concluded that the additive was

negative in all the genotoxicity studies and produced no adverse effects in the subchronic toxicity.

Considering the nature of the genetic modification and the extensive purification undertaken to exclude DNA fragments from the final additive, the production or retention of toxic metabolites produced during fermentation was considered improbable, and the results of the genotoxicity studies and subchronic toxicity study supported this.

EFSA noted that metabolites resulting from the complete or partial de-esterification of fumonisins have been assessed previously (EFSA, 2014) and found to be less toxic than the parent fumonisins. Therefore, the use of this additive would not introduce any hazards for consumers.

The applicant provided tolerance studies in weaning piglets, chickens for fattening, turkeys for fattening and laying hens that had previously been evaluated by EFSA (EFSA, 2018b). EFSA previously extrapolated their conclusions that the additive was safe at the then-proposed maximum inclusion rate of 300 U/kg in the feed to all poultry and pig species as there was a wide margin of safety (100 fold) to concentrations which were tolerated in these studies. EFSA considers that these conclusions can be extended to the current application. In addition, the NOAEL from the subchronic toxicity study was 2000 mg/kg bw/day (highest dose tested) of the test item, which contained fumonisin esterase at a concentration of 8650 U/g. Applying the procedure to establish a maximum safe daily dose for the target species in EFSA's guidance on the assessment of the safety of feed additives for the target species (EFSA, 2017b), including the use of an uncertainty factor of 100, the lowest safe maximum concentration in feed would be 1927 U/kg, in chickens for fattening. EFSA has concluded that this supports the safety of the additive in feed for all species.

EFSA previously assessed the safety of the user for this additive (EFSA, 2018b) and concluded that it is not toxic by inhalation and the respiratory exposure is likely to be low; however, a risk of sensitisation via the respiratory route cannot be excluded. EFSA concluded that the additive is not a skin or eye irritant and is not considered as a dermal sensitiser. EFSA considers that these conclusions remain applicable to the current application.

EFSA previously evaluated the safety for the environment for this additive (EFSA, 2018b) and concluded, based its assessment on the safety of the production strain and on the likely degradation of the enzyme in the digestive tract of the animal and in soils, that no risks to the environment were expected when the additive was used under its proposed conditions of use. EFSA considers that these conclusions are also applicable to the current application.

4. EFSA assessment and conclusions

EFSA concluded that the use of the additive under the proposed conditions of use is safe for target animals, consumers and the environment.

Regarding risks to users, the additive is not toxic by inhalation and respiratory exposure is likely to be low. However, it cannot be excluded that it is a respiratory sensitiser. It is not irritant to skin and is not considered a dermal sensitiser.

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. The conclusions for this renewal are largely based on a previous evaluation (EFSA, 2018b), which assessed the safety of the additive when used in the feed of pigs and poultry.

6.1 Analytical Method Review

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application. 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 opinion and consider it adequate and relevant for GB risk analysis. Therefore, the opinion was used to form the basis of the GB opinion.

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 opinion.

The FSA/FSS opinion is that fumonisin esterase from *Komagataella phaffii* DSM 32159 (Biomin GmbH) as a feed additive for all animal species, 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.

8. References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

[4] The report is available on the EURL website: https://joint-research-centre.ec.europa.eu/system/files/2013-09/FinRep-FAD-2013-0002-FUMzyme.doc_.pdf

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the safety and efficacy of fumonisin esterase (FUMzyme®) as a technological feed additive for pigs. EFSA Journal 2014;12(5):3667, 19 pp. doi:10.2903/j.efsa.2014.3667

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