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SCIENTIFIC OPINION

Safety evaluation of an extension of use of the food enzyme α-glucosidase from the non-genetically modified *Aspergillus niger* strain AE-TGU

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Abstract

The food enzyme α-glucosidase (α-D-glucoside glucohydrolase; EC 3.2.1.20) is produced with the non-genetically modified *Aspergillus niger* strain AE-TGU by Amano Enzyme Inc. A safety evaluation of this food enzyme was made previously, in which EFSA concluded that this food enzyme did not give rise to safety concerns when used in four food manufacturing processes. Subsequently, the applicant requested to extend its use to include three additional processes. In this assessment, EFSA updated the safety evaluation of this food enzyme when used in a total of seven food manufacturing processes. The dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.693 mg TOS/kg body weight (bw) per day in European populations. When combined with the no observed adverse effect level previously reported (1062 mg TOS/kg bw per day, the highest dose tested), the Panel derived a margin of exposure of at least 1532. Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that this food enzyme does not give rise to safety concerns under the revised intended conditions of use.

KEYWORDS

Aspergillus niger, EC 3.2.1.20, EFSA-Q-2014-00800, EFSA-Q-2023-00309, food enzyme, non-genetically modified microorganism, α-glucosidase

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1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

Alpha-glucosidase from a non-genetically modified strain of *Aspergillus niger* (strain AE-TGU) is a food enzyme included in the Register of food enzymes[3] to be considered for inclusion in the Union list and thus subject to a risk assessment by the European Food Safety Authority (EFSA).

On 4 November 2022, a new application has been introduced by the applicant "Amano Enzyme Inc." for an extension of the conditions of the use of the food enzyme alpha-glucosidase from a non-genetically modified strain of *Aspergillus niger* (strain AE-TGU).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: alpha-glucosidase from a non-genetically modified strain of *Aspergillus niger* (strain AE-TGU), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[4]

1.1.3 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of an extension of the conditions of use for the α-amylase from the non-genetically modified *Aspergillus niger* strain AE-TGU.

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]https://food.ec.europa.eu/safety/food-improvement-agents/enzymes/eu-list-and-applications_en

[4]OJ L 354, 31.12.2008, p. 1.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for the authorisation of the extension of use of food enzyme α-glucosidase from non-genetically modified *Aspergillus niger* AE-TGU.

Additional information was requested from the applicant during the assessment process on 06 November 2023 and received on 22 November 2023 (see 'Documentation provided to EFSA').

2.2 | Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant existing guidance documents of EFSA Scientific Committee.

The current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) have been followed to evaluate this application.

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[5] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 5 to 26 December 2023.[6] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	α-Glucosidase
Systematic name	α-D-glucoside glucohydrolase
Synonyms	Maltase; α-1,4-glucosidase; glucoinvertase
IUBMB no	EC 3.2.1.20
CAS no	9001-42-7
EINECS no	232-604-7

α-Glucosidases catalyse the hydrolysis of terminal α-1,4 linkages at the non-reducing ends of starch and maltose with the release of glucose. At high substrate concentrations, the food enzyme also catalyses transglycosylation reactions to form α-1,6 linkages.

All aspects concerning the safety of this food enzyme, when used in four food manufacturing processes, were evaluated in March 2022 (EFSA CEP Panel, 2022). Following an application for use in three additional food manufacturing processes and revised use levels for those previously evaluated, EFSA revises the exposure assessment and updates the safety evaluation of this food enzyme when used in seven food manufacturing processes.

3.1 | Dietary exposure

The current dietary exposure supersedes section 3.5 of the previous evaluation (EFSA CEP Panel, 2022).

3.1.1 | Revised intended use of the food enzyme

The food enzyme is intended to be used in seven food manufacturing processes at the revised recommended use levels summarised in Table 1.

[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. 1–24.

[6]https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0lTk0000001uhh/pc0744

TABLE 1 Updated intended uses and use levels of the food enzyme.[7]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)	
		Current evaluation[b]	Previous evaluation[b,c]
Processing of cereals and other grains			
• Production of baked products	Flour	**53.8**	5.4–**53.8**
• Production of cereal-based products other than baked	Rice, cereals, potato starch	38.4 (non-infant cereal products)	8.5–**38.4** (cooked rice) 0.3–3.1 (pasta and noodles)
	Cereals	**53.3** (infant cereal products)	0.4–**3.8** (batter) 2.5–**25.4** (steam fish paste)
• Production of glucose syrups and other starch hydrolysates	Cereals	153.8	38.4–154
• Production of brewed products	Cereals, rice	**76.9**	7.7–**38.4**
• Production of distilled alcohol	Cereals	307.6	
• Production of non-wine vinegar	Cereals	**7.7**	
Processing of plant- and fungal-derived products			
• Production of plant-based analogues of milk and milk products	Cereals, legumes, oilseeds, nuts, etc.	**76.9**	

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The numbers in bold represent the maximum recommended use levels and were used for calculation.

[c]The previous evaluation is made for the food enzyme application EFSA-Q-2014-00800.

The Panel noted an increase in the use level recommended for the brewing processing in the current assessment when compared to the previous level. The applicant ascribes this change to a modification of the current food manufacturing process.[8]

The additional three uses of the food enzyme are described below.

In the production of distilled alcohol, the food enzyme is added to cereals during the slurry mixing, the liquefaction, the pre-saccharification or the fermentation steps.[9] It converts liquefied starch into a glucose-rich solution, increasing the amounts of fermentable sugars to produce alcohol.[10] The food enzyme-TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2023).

In the production of non-wine vinegars, the food enzyme is added to milled cereals during slurry mixing[11] to improve the sensory properties of the final products[12] in which the food enzyme remains.

In production of plant-based analogues of milk and milk products, the food enzyme is added to the plant-based beverage prior to pasteurisation, to improve the sensory properties of the final food.[13,14] The food enzyme-TOS remains in the final foods.

Based on the thermostability evaluated previously (EFSA CEP Panel, 2022) and the downstream processing steps applied in the food manufacturing processes, it is expected that the *α*-glucosidase is inactivated or not carried over into the final foods.

3.1.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), dietary exposure was calculated only for the five food manufacturing processes where the food enzyme-TOS remains in the final foods.

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day

[7]Additional information November 2023.

[8]Additional data November 2023.

[9]Technical dossier/Intended use(s) in food and use level(s)/4–11 Proposed conditions of use/pp.3–4.

[10]Technical dossier/Intended use(s) in food and use level(s)/Annex Flow chart of each application/p. 9.

[11]Techincal dossier/Intended use(s) in food and use level(s)/4–11 Proposed conditions of use/p. 2.

[12]Techincal dossier/Intended use(s) in food and use level(s)/Annex Flow chart of each application/p. 4.

[13]Technical dossier/Intended use(s) in food and use level(s)/4–11 Proposed conditions of use/pp. 4–5.

[14]Technical dossier/Intended use(s) in food and use level(s)/4–11 Proposed conditions of use/Annex Flow chart of each application/p. 10.

per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme-TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.693 mg TOS/kg bw per day in infants at the 95th percentile.

TABLE 2 Updated dietary exposure to the food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.030–0.269 (12)	0.128–0.372 (15)	0.067–0.308 (19)	0.020–0.211 (21)	0.075–0.188 (22)	0.068–0.137 (23)
Min–max 95th percentile (number of surveys)	0.129–0.693 (11)	0.298–0.607 (14)	0.163–0.577 (19)	0.049–0.365 (20)	0.169–0.518 (22)	0.139–0.271 (22)

3.1.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

TABLE 3 Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate.

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme-TOS was always calculated based on the recommended maximum use level	+
Production of cereal-based products other than baked: although two use levels were available, only the higher one was used in the calculation	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of two food processes from the exposure assessment – Production of glucose syrups and other starch hydrolysates – Production of distilled alcohol	–

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to estimate the exposure to the food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

The exclusion of two food manufacturing processes from the exposure estimation was based on > 99% of TOS removal. This is not expected to impact on the overall estimate derived.

3.2 | Margin of exposure

In the previous evaluation, the Panel identified a no observed adverse effect level (NOAEL) of 1,062 mg TOS/kg bw per day, the highest dose tested, resulting in a margin of exposure (MOE) of at least 1650 (EFSA CEP Panel, 2022).

A comparison of the NOAEL with the newly derived exposure estimates of 0.020–0.372 mg TOS/kg bw per day at the mean and from 0.049 to 0.693 mg TOS/kg bw per day at the 95th percentile resulted in a MOE of at least 1532.

4 | CONCLUSION

Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that the food enzyme α-glucosidase produced with the non-genetically modified *Aspergillus niger* strain AE-TGU does not give rise to safety concerns under the revised intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of α-glucosidase from *Aspergillus niger* AE-TGU in accordance with the Regulation (EC) No 1331/2008. November 2022. Submitted by Amano Enzyme Inc.

Additional information. November 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
IUBMB	International Union of Biochemistry and Molecular Biology
MoE	margin of exposure
NOAEL	no observed adverse effect level
RM	Raw Material
TOS	total organic solids

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

QUESTION NUMBER

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro* Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion, however, they are included in Appendix A for testing purpose.

[a]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).