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Safety evaluation of the food enzyme α-glucosidase from the *Aspergillus niger* strain AE-TGU

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Lieve Herman, Jaime Aguilera, Magdalena Andryszkiewicz, Davide Arcella, Yi Liu, Elsa Nielsen, Karin Norby and Andrew Chesson

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. The food enzyme is free from viable cells of the production organism. The food enzyme is intended to be used in baking processes, cereal-based processes, brewing processes and starch processing for the production of glucose syrups and other starch hydrolysates. Since residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of glucose syrups, dietary exposure was only calculated for the remaining three food processes. Based on the maximum use levels recommended, dietary exposure was estimated to be up to 0.64 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,062 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 1,650. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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† Deceased.

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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 micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

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 European Union (EU) Community 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.

The following three applications have been submitted for the authorization of food enzymes: from "Amano Enzyme Inc." for Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU); from the "Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" for Endo-1,3(4)-beta-glucanase, Endo-1,4-beta-xylanase and Cellulase from *Talaromyces emersonii*; from "AMFEP" for Cellulase, Endo-1,3(4)-beta glucanase and Endo-1,4-beta xylanase obtained from *Trichoderma reesei*.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU), Endo-1,3 (4)-beta-glucanase, Endo-1,4-beta-xylanase and Cellulase from *Talaromyces emersonii*, and Cellulase,

[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] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

Endo-1,3(4)-beta glucanase and Endo-1,4-beta xylanase obtained from *Trichoderma reesei* in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme alpha-glucosidase from *A. niger* (strain AE-TGU).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme alpha-glucosidase from *A. niger* (strain AE-TGU). The dossier was updated on 28 April 2016.

Additional information was requested from the applicant during the assessment process on 7 June 2021 and was consequently provided (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, 2009b) and the relevant guidances of the EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

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. The food enzyme is intended to be used in baking processes, cereal-based processes, brewing processes, and starch processing for the production of glucose syrups and other starch hydrolysates.

3.1. Source of the food enzyme

The α-glucosidase is produced with the filamentous fungus *A. niger* strain AE-TGU, which is deposited at the Biological Resource Center (NBRC, Japan), with deposit number [redacted][4] The production strain was derived from the parental strain [redacted][5]

The production strain was identified as *A. niger* [redacted][6]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.[8]

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[4] Additional data November 2021/Annex 2.
[5] Additional data November 2021/Annex 3.
[6] Additional data November 2021/Annex 1.
[7] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[8] Technical dossier/2nd submission/Annex 3.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After completion of the fermentation and secretion of the enzyme into the culture medium, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[9] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[10]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The mature α-glucosidase is a single polypeptide chain of ███ amino acids.[11] The molecular mass of the mature protein was calculated from the amino acid sequence is ████ kDa. The food enzyme was analysed by size exclusion chromatography. The chromatograms of the three food enzyme batches for commercialisation showed a consistent pattern containing a major peak, accompanied by some minor peaks.[12] No other activities were reported.[13]

The determination of α-glucosidase activity is based on hydrolysis of 4-nitrophenyl α-D-glucopyranoside (reaction conditions: pH 5.0, 40°C, 20 min). The enzymatic activity is determined by measuring the release of *p*-nitrophenol spectrophotometrically at 420 nm. One unit is defined as the quantity of enzyme that liberates 1 μmol of *p*-nitrophenol per minute under the conditions of assay.[14]

The in-house determination of transglucosidase activity is based on hydrolysis of 1-*O*-methyl-α-D-glucose (reaction conditions: pH 5.0, 40°C, 60 min). The enzymatic activity is determined by measuring the release of glucose spectrophotometrically at 660 nm. One unit is defined as the quantity of enzyme that liberates 1 μg of glucose in 60 min under the conditions of assay.[15]

The food enzyme has a temperature optimum around 60°C (pH 5.0) and a pH optimum around pH 5.0 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.0). α-Glucosidase activity decreased above 60°C showing no residual activity above 75°C.[13]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and three batches produced for the toxicological tests (Table 1).[16] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 9.1% and the mean enzyme activity/TOS ratio is 4,419 U/mg TOS for its transglycosylation activity and 0.85 U/mg TOS for its hydrolytic activity.

[9] Technical dossier/2nd submission/pp. 41-49/Annexes: 4 and 10.
[10] Technical dossier/2nd submission/Annex 5/Additional data November 2021.
[11] Technical dossier/2nd submission/pp. 31.
[12] Technical dossier/2nd submission/pp. 28.
[13] Technical dossier/2nd submission/pp. 33.
[14] Technical dossier/2nd submission/Annex 2.1.
[15] Technical dossier/2nd submission/Annex 2.2.
[16] Technical dossier/2nd submission/pp. 28, 67-68/Annexes: 1, 9.2 and 10/Additional data November 2021.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches					
		1	2	3	4[(a)]	5[(b)]	6[(c)]
α-Glucosidase activity	U/g batch[(d)]	62	79	90	–	–	–
Transglucosylase activity	U/g batch[(d)]	325,000	410,000	472,000	371,771	580,750	3,016,000
Protein	%	2.9	3.6	4.1	7.2	7.3	29.7
Ash	%	9.9	10.3	9.7	0.1	0.2	4.8
Water	%	82.1	80.0	80.8	84.7	87.7	3.1
Dextrin	%	–	–	–	–	–	39.0
Total organic solids (TOS)[(e)]	%	8.0	9.7	9.5	15.2	12.1	53.1
Activity/mg TOS (hydrolysis)	U/mg TOS	0.78	0.81	0.95	–	–	–
Activity/mg TOS (transglycosylation)	U/mg TOS	4,063	4,227	4,968	2,446	4,800	5,680

(a): Batch used for the Ames test.
(b): Batch used for chromosome aberration assay.
(c): Batch used for the repeated dose 90-day oral toxicity study in rats.
(d): UNIT: U/g (see Section 3.3.1).
(e): TOS calculated as 100% – % water – % ash (and for batch 6% dextrin).

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for the repeated dose 90-day oral toxicity study was below 5 mg/kg[17,18] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[19] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[17]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, ochratoxin A, sterigmatocystin, HT-2 toxin, T-2 toxin and zearalenone was examined in the three food enzyme batches and were all below the limit of detection (LOD) of the applied method.[20,21] Adverse effects due to the potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated No colonies were produced.[22]

[17] Technical dossier/2nd submission/Annexes: 1 and 9.2.
[18] LoQ: Pb = 0.05 mg/kg.
[19] Technical dossier/2nd submission/Annexes: 1 and 9.2/Additional data November 2021/Annex 6.
[20] Technical dossier/2nd submission/Annex 3.1.
[21] LoDs: aflatoxins (B1, B2, G1 and G2) = 0.5 μg/kg each; HT-2 toxin = 250 μg/kg, T-2 toxin = 100 μg/kg, sterigmatocystin = 20 μg/kg, DON = 100 μg/kg; ochratoxin A = 0.5 μg/kg.
[22] Additional data November 2021/Annex 4.

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats has been provided. The batches 4, 5 and 6 (Table 1) used in these studies have similar chemical purity, and thus are considered suitable as test items.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to 'Standards for mutagenicity studies using bacteria and other related matters' (Japan, 1985) and 'Guidebook of mutagenicity study using bacteria' (Japan, 1986).[23] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the pre-incubation method. In a preliminary experiment, four concentrations of the food enzyme (from 1.6 to 100 μL/plate, corresponding to 237.5, 950, 3,800 and 15,200 μg TOS/plate) were tested in duplicate. In the main experiment, five concentrations (from 6.3 to 100 μL/plate, corresponding to 950, 1,900, 3,800, 7,600 and 15,200 μg TOS/plate) were tested in triplicate. No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997) and following GLP.[24] Two separate experiments in duplicate cultures were performed. In the first experiment, eight concentrations of the food enzyme ranging from 78 to 5,000 μg/mL were tested in a short-term treatment, 3 h followed by 17 h recovery period (3 + 17 h), with and without metabolic activation (S9-mix). No inhibition of cell growth by 50% or more was observed. At concentrations of 2,500, 3,750 and 5,000 μg/mL (corresponding to 304, 456 and 607 μg TOS/mL) cells were scored for chromosomal aberrations. In a second experiment, the cells were exposed to the food enzyme at six concentrations ranging from 156 to 5,000 μg/mL (corresponding to 19–607 μg TOS/mL) in short-term treatments (3 + 17 h and 3 + 41 h) in the presence of S9-mix. In addition, cells were treated continuously for 20 h and for 44 h in the absence of S9-mix. Cells treated in the (20 + 0 h) and (3 + 17 h) treatments were scored for chromosomal aberrations at concentrations of 1,250, 2,500 and 5,000 μg/mL (corresponding to 152, 304 and 607 μg TOS/mL). Cells treated in the (44 + 0 h) and (3 + 41 h) treatments were scored for chromosomal aberrations only at the highest concentration tested corresponding to 607 μg TOS/mL. Cytotoxic effects were only observed in experiments with S9-mix: 48% mitotic inhibition at the lowest concentration (78 μg/mL) and 45% at 5,000 μg/mL in the (3 + 17 h) treatment; and up to 47% mitotic inhibition at 5,000 μg/mL in the (3 + 41 h) treatment. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the historical control data.

The Panel concluded that the food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with guidelines of the Japanese Ministry of Health and Welfare (1996 and 1999) and following GLP.[25] The study is in accordance with OECD Test Guideline 408 (OECD, 1998) with the following deviations: detailed clinical observations and functional observations were not performed, urea was not determined and epididymides were not weighed. The Panel considered that these deviations are minor and do not impact the evaluation of the study.

[23] Technical dossier/2nd submission/Annex 7.
[24] Technical dossier/2nd submission/Annex 8.
[25] Technical dossier/2nd submission/Annexes: 9.1 and 9.2.

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 500, 1,000 or 2,000 mg/kg body weight (bw) per day corresponding to 265.5, 531 or 1,062 mg TOS/kg bw per day, respectively. Controls received the vehicle (distilled water for injection).

No mortality was observed.

The clinical chemistry investigation revealed a statistically significant decrease in aspartate aminotransferase (AST) in low-dose males (−23%) and in inorganic phosphorus in mid-dose males (−7%), and an increase in alanine aminotransferase (ALT) in high-dose females (+182%). The Panel considered these changes as not toxicologically relevant as only observed in one sex (all parameters), in the absence of a dose-response relationship (AST, inorganic phosphorus), a low magnitude of the changes (AST, inorganic phosphorus), and in the absence of changes in other relevant parameters (ALT) such as in other liver enzymes, in liver weights and no histopathological changes in the liver.

The urinalysis revealed a statistically significant decrease in total potassium excretion in low-dose males (−23%). The Panel considered this change as not toxicologically relevant as only observed in one sex and in the absence of a dose-response relationship.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 1,062 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient, which may be used in the final formulation.

The potential allergenicity of the α-glucosidase produced with the *A. niger* strain AE-TGU was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[26]

No information is available on oral and respiratory sensitisation or elicitation reactions of this α-glucosidase.

Alpha-glucosidase has been associated with allergic reactions to yellow fever mosquito bites. No allergic reactions upon dietary exposure to any α-glucosidase have been reported in the literature.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[27]) are used as raw materials ( ). In addition, known sources of allergens, are also present in the media fed to the microorganisms However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in five food processes at the recommended use levels summarised in Table 2.

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[26] Technical dossier/2nd submission/pp. 69-70/Additional data November 2021/Annex 5.

[27] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[28]

Food manufacturing process[(a)]	Raw material (RM)	Recommended dosage of the food enzyme (mg TOS/kg RM)[(b)]
Baking processes	Flour	5.4–**53.8**
Cereal-based processes	Rice for cooked rice	8.5–**38.4**
	Flour for pasta and noodles	0.3–**3.1**
	Starch for batter	0.4–3.8
	Starch for steamed fish paste	2.5–**25.4**
Starch processing for the production of glucose syrups and other starch hydrolysates	Starch	38.4–154
Brewing processes	Cereals	7.7–**38.4**

TOS: total organic solids.
(a): The description provided by the applicant has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): Numbers in bold were used for calculation.

In baking processes, the food enzyme is added to the flour to make dough.[28] The α-glucosidase releases glucose from starch which is available for fermentation. The food enzyme–TOS remains in the dough.

In cereal-based processes, the food enzyme is added to a variety of raw materials (rice, flour, starch) to produce, for example, cooked rice, pasta, noodles, batter for deep fried foods.[28] The food enzyme–TOS remains in the final foods.

In starch processing for glucose syrups production and other starch hydrolysates, the food enzyme is added during the saccharification step.[28] The food enzyme–TOS is removed during the downstream purification process (EFSA CEP Panel, 2021a).

In brewing processes, the food enzyme is added during the mashing step in order to obtain a more uniform and predictable production process and yield. It is also added during fermentation, providing the possibility to control the desired level of fermentable sugars.[28] The food enzyme–TOS remains in beers.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme is inactivated during baking processes.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was not calculated for starch processing for glucose syrups production and other starch hydrolysates. Dietary exposure was calculated only for the remaining three food processes.

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEF Panel, 2021b). 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 one day 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 3 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 41 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure at the 95th percentile was estimated to be about 0.6 mg TOS/kg bw per day in infants, toddlers and children below 10 years of age.

[28] Additional data November 2021/Annex 7.

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	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)	0.013–0.153 (11)	0.119–0.331 (15)	0.134–0.318 (19)	0.075–0.195 (21)	0.061–0.157 (22)	0.059–0.120 (22)
Min–max 95th percentile (number of surveys)	0.063–0.640 (9)	0.293–0.559 (13)	0.260–0.584 (19)	0.158–0.411 (20)	0.131–0.353 (22)	0.116–0.209 (21)

TOS: total organic solids.

3.5.3. 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 4.

Table 4: 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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure assessment – starch processing for glucose syrups production and other starch hydrolysates	–

TOS: total organic solids.
+: uncertainty with potential to cause overestimation of exposure.
–: uncertainty with potential to cause underestimation of exposure.

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

The exclusion of one food manufacturing process (starch processing for glucose syrups production and other starch hydrolysates) from the exposure assessment was based on > 99% of TOS removal during this process and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (1,062 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.013–0.331 mg TOS/kg bw per day at the mean and from 0.063–0.640 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 1,659.

4. Conclusions

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

5. Documentation as provided to EFSA

Application for authorisation of Alpha-glucosidase from *Aspergillus niger* AE-TGU. April 2016. Submitted by Amano Enzyme Inc.

Additional information. November 2021. Submitted by Amano Enzyme Inc.

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Abbreviations

AST	aspartate aminotransferase
ALT	alanine aminotransferase
Bw	body weight
CAS	Chemical Abstracts Service

CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DRF	dose-range finding
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
MOE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.7240#support-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
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, 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, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(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).