SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 6 December 2022

doi: 10.2903/j.efsa.2023.7753

Safety evaluation of the food enzyme glucan 1,4-α-glucosidase from the non-genetically modified *Rhizopus arrhizus* strain AE-G

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Abstract

The food enzyme glucan 1,4-α-glucosidase (4-α-D-glucan glucohydrolase; EC 3.2.1.3) is produced with the non-genetically modified *Rhizopus arrhizus* strain AE-G by Amano Enzyme Inc. The food enzyme is free from viable cells of the production organism. The applicant proposed the use of the food enzyme in baking processes, coffee processing and manufacture of enzymatically modified dairy ingredients (EMDI). The Panel considered only the baking processes as the relevant intended use of this food enzyme. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.94 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 a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,868 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 1,987. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and one match was found. The Panel considered that under the intended conditions of use the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns when used in baking processes under the intended conditions of use.

Keywords: food enzyme, glucan 1,4-α-glucosidase, 4-α-D-glucan glucohydrolase, EC 3.2.1.3, amyloglucosidase, glucoamylase, *Rhizopus arrhizus*

Requestor: European Commission

Question number: EFSA-Q-2015-00272

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Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

Declarations 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.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Roos Y, Andryszkiewicz M, Liu Y, Lunardi S, Nielsen E, Nørby K and Chesson A, 2023. Scientific Opinion on the safety evaluation of the food enzyme glucan 1,4-α-glucosidase from the non-genetically modified *Rhizopus arrhizus* strain AE-G. EFSA Journal 2023;21(1):7753, 15 pp. https://doi.org/10.2903/j.efsa.2023.7753

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

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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.

Five applications have been introduced by the companies "Amano Enzyme Inc.", "DSM Food Specialties B.V." and "Novozymes A/S" for the authorisation of the food enzymes Glucoamylase from *Rhizopus oryzae* (strain AE-G), Beta-glucosidase from *Penicillium multicolour* (strain AE-GLY), Peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX), Beta-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JA) and Triacylglycerol lipase from *Aspergillus niger* (strain AE-L), respectively.

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 five applications fall within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

[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, 11.03.2011, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes Glucoamylase from *Rhizopus oryzae* (strain AE-G), Beta-glucosidase from *Penicillium multicolour* (strain AE-GLY), Peroxidase from a genetically modified strain of *Aspergillus niger* (strain MOX), Beta-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-JA) and Triacylglycerol lipase from *Aspergillus niger* (strain AE-L) in accordance with Article 29 of Regulation (EC) No 178/2002 and 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 the food enzyme glucoamylase from *Rhizopus oryzae* strain AE-G.

Recent data identified the production microorganism as *Rhizopus arrhizus* (Section 3.1). Therefore, this name will be used in this opinion instead of *Rhizopus oryzae*.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glucoamylase from *R. oryzae* (strain AE-G). The dossier was updated on 15 October 2015.

Additional information was requested from the applicant during the assessment process on 08 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 following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have 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	Glucan 1,4-α-glucosidase
Systematic name	4-α-D-glucan glucohydrolase
Synonyms	Glucoamylase, amyloglucosidase, 1,4-α-D-glucan glucohydrolase
IUBMB No	EC 3.2.1.3
CAS No	9032-08-0
EINECS No	232-877-2

Glucan 1,4-α-glucosidases catalyse the hydrolysis of terminal (1–4)-linked α-D-glucose residues successively from non-reducing ends of amylopectin and amylose with the release of glucose. The applicant proposed to use this food enzyme in baking processes, coffee processing and manufacture of enzymatically modified dairy ingredients (EMDI).

3.1. Source of the food enzyme

The glucan 1,4-α-glucosidase is produced with the non-genetically modified filamentous fungus *R. arrhizus* strain AE-G, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan), with the deposit number [redacted].[4] The production

[4] Technical dossier/Additional information July 2022/Annex 2.

strain was identified as *R. arrhizus* by [redacted].[5]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[6], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.[7]

The production strain is grown as a solid-state fermentation on fermentation trays. The fermentation is run under a controlled atmosphere [redacted] After completion of the fermentation, water is added and the mix is then separated into biomass and a solution containing the enzyme protein 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.[8] 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.[9]

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 glucan 1,4-α-glucosidase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted]. The apparent molecular mass of the enzyme, estimated from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, was [redacted].[10] The food enzyme was also analysed by size exclusion chromatography and the chromatograms of the three food enzyme batches for commercialisation showed a consistent protein pattern.[11] No other enzymatic activities were reported.[12]

The in-house determination of glucan 1,4-α-glucosidase activity is based on the quantitation of reducing groups released during the hydrolysis of potato starch (reaction conditions: pH 5.0, 37°C, 10 min). The enzyme activity is expressed in starch saccharifying activity units (U)/g. One U is the amount of enzyme that produces sugars equivalent to 1 mg of glucose per minute.[13]

The food enzyme has a temperature optimum between 55°C and 60°C (pH 5.0) and a pH optimum between pH 4.0 and 5.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.0). Glucan 1,4-α-glucosidase activity decreased above 40°C, showing no residual activity above 60°C.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[15] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 79.1% and the mean enzyme activity/TOS ratio was 6.3 U/mg TOS.

[5] Technical dossier/Additional information July 2022/Annex 1.
[6] 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.
[7] Technical dossier/1st submission/Annex 4.
[8] Technical dossier/1st submission/p. 37-44/Annex 5.
[9] Technical dossier/1st submission/Annex 6.
[10] Technical dossier/1st submission/p. 29.
[11] Technical dossier/1st submission/p. 27.
[12] Technical dossier/1st submission/p. 30.
[13] Technical dossier/1st submission/Annex 2.
[14] Technical dossier/1st submission/p. 31–32.
[15] Technical dossier/1st submission/p. 26, 54/2nd submission/Annexes: 1, 2; Additional data July 2022.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Glucoamylase activity	U/g batch[(b)]	5,170	4,730	5,050	5,230
Protein	%	53.5	49.8	51.8	51.9
Ash	%	16.6	13.8	14.9	2.0
Water	%	5.6	5.9	5.9	4.6
Total organic solids (TOS)[(c)]	%	77.8	80.3	79.2	93.4
Glucoamylase activity/TOS	U/mg TOS	6.6	5.9	6.4	5.6

(a): Batch used for the toxicological studies.
(b): U: see Section 3.3.1.
(c): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three commercial batches was < 0.01 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[16,17]

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).[17] No antimicrobial activity was detected in any of the tested batches.[17]

Strains of *Rhizopus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisins (B1, B2), T2-toxin, HT2-toxin, zearalenone and sterigmatocystin was examined in the three food enzyme batches. Concentrations were all below the respective limits of detection (LoD) of the applied analytical methods.[17,18] The possible presence of other secondary metabolites of concern 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 in three independent batches analysed in triplicate. [redacted] Colonies morphologically different from the production strain were produced. A positive control was included.[19]

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 sub-chronic (180 day) toxicity study in rats, was provided with the initial application.[20] Subsequently, additional toxicological data were submitted in the form of an Ames test, an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats. The Panel decided to use the most recently conducted toxicity tests for safety evaluation, as the test item reflects better the food enzyme under application. The batch 4 (Table 1) used in these new studies has similar chemical composition and activity/TOS ratio to the commercial batches and thus is considered suitable as a test item.

[16] LoD: Pb = 0.01 mg/kg.
[17] Technical dossier/1st submission/p. 28–29/2nd submission/Annexes: 1, 2.
[18] LoDs: aflatoxins: B1, B2, G1 and G2 = 0.2 μg/kg each; ochratoxin A = 0.5 μg/kg; fumonisins B1 and B2 = 5.0 μg/kg each; T2-toxin, HT2-toxin, zearalenone, sterigmatocystin = 10.0 μg/kg each.
[19] Technical dossier/Additional information July 2022/Annex 3.
[20] Technical dossier/1st submission/Annexes 8, 9, 10.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

An Ames test was performed according to OECD Test Guideline 471 (OECD, 2020) and following Good Laboratory Practice (GLP).[21]

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. Based on the results obtained in a preliminary range finding test, five concentrations of food enzyme 313, 625, 1,250, 2,500 and 5,000 μg TOS/plate were examined in two separate experiments, performed with triplicate plating and ± S9-mix. Overall, upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain or concentration tested, either with or without S9-mix.

The Panel concluded that the food enzyme glucan 1,4-α-glucosidase did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* micronucleus test

The *in vitro* mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.14.[22]

Two separate experiments were performed with duplicate cultures of the lymphoblastoid cell line TK6. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix). In the first experiment, cells were exposed to the food enzyme in a short-term treatment (4 h exposure and 20 h recovery period) either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme in the short-term treatment with S9-mix and in a long-term treatment (24 h exposure without recovery period) without S9-mix.

In a range-finding test, no cytotoxicity, estimated by the relative population doubling, was seen in the short-term treatments, with S9-mix, when tested up to 5,000 μg TOS/mL. In the short-term and long-term treatment, without S9-mix, cytotoxicity of 50% was seen at 4,570 and 1,480 μg TOS/mL, respectively.

In short-term treatment, without S9-mix, cells were exposed to and scored for the frequency of binucleated cells with micronuclei (MNBN) at five concentrations of 3,000 to 5,000 μg TOS/mL. In the short-term treatment, with S9-mix, cells were treated and scored for MNBN at concentrations of 1,250, 2,500 and 5,000 μg TOS/mL and in the long-term treatment, without S9-mix, cells were treated and scored for MNBN at six concentrations of 500 to 1,750 μg TOS/mL.

The frequency of MNBN was not statistically significantly different compared to the negative control values under the test conditions applied in the present study. All results were within the historical control range.

The Panel concluded that the food enzyme glucan 1,4-α-glucosidase did not induce an increase in the frequency of MNBNs under the test conditions applied in 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 OECD Test Guideline 408 (OECD, 2018) with the following deviation: oestrus cycle was not determined. The Panel considered that this deviation is minor and does not impact on the evaluation of the study. 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 467, 934 or 1,868 mg TOS/kg bw per day. Controls received the vehicle (water for injection).[23]

One high-dose female was found dead immediately after dosing on day 57 of administration. The Panel considered the death as due to mis-dosing.

In the functional observations, a statistically significant increase in the motor activity was observed during the 30–40 min interval in mid-dose males (+200%). The Panel considered the change as not toxicologically relevant as it was only recorded sporadically, it was only observed in one sex and there was no dose–response relationship.

The haematological investigation revealed a statistically significant decrease in the red blood cell distribution width in low-dose females (−3.4%). The Panel considered the change as not

[21] Technical dossier/Additional information July 2022/Annex 7.
[22] Technical dossier/Additional information July 2022/Annex 8.
[23] Additional data July 2022/Annex 9.

toxicologically relevant as it was only observed in one sex, there was no dose–response relationship, the change was small and there were no changes in other relevant parameters (i.e. in other red blood cell parameters).

The clinical chemistry investigation revealed a statistically significant increase in calcium in mid-dose females (+3.9%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, there was no dose–response relationship and the change was small.

Statistically significant changes in hormone levels included an increase in serum concentration of tri-iodothyronine (T3) in low-dose males (+22%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, there was no dose–response relationship and there were no changes in other relevant parameters (i.e. in the serum concentrations of other thyroid hormones, T4 and TSH).

Statistically significant changes in organ weights included an increase in the relative liver weight in mid-dose females (+7%), and a decrease in the absolute and relative kidney weights in low-dose males (−11% and −8%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (all parameters), the changes were small (all parameters) and there were no histopathological changes in the liver or the kidneys.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1,868 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the glucan 1,4-α-glucosidase produced with *R. arrhizus* strain AE-G 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, one match was found.[24] The matching allergen was Sch c 1, a glucoamylase from *Schizophyllum commune*, an enzyme described as an occupational respiratory allergen associated with baker's asthma (Quirce et al., 2002; Toyotome et al., 2014).

No information is available on oral and respiratory sensitisation or elicitation reactions of this glucan 1,4-α-glucosidase.

Several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any glucan 1,4-α-glucosidase have been reported in the literature.

[redacted], products that may cause allergies (listed in the Regulation (EU) No 1169/2011[25]), are used as raw materials. 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 fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues are present in the food enzyme.

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

[24] Technical dossier/Additional information July 2022/Annex 4.

[25] 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.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food processes at the recommended use levels summarised in Table 2.[26]

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

Food manufacturing process[(a)]	Raw material	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	40–**79**
Coffee processing	Coffee bean	4,800
Manufacture of enzymatically modified dairy ingredients (EMDI)	Cream and butter	1,500

(a): The name 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): The number in bold was used for calculation.

In baking processes, the food enzyme is added to flour during the preparation of the dough or batter.[27] The glucan 1,4-α-glucosidase releases glucose from starch, which may be fermented by yeast. The food enzyme–TOS remains in bakery foods.

The applicant has withdrawn the use of this food enzyme in yeast processing[28] and suggested its use in coffee processing and in the manufacturing of enzyme modified dairy ingredients to enhance the flavour formation *in situ*.[29] The substrates of glucan 1,4-α-glucosidases are not present in cream and butter, consequently the Panel did not consider this use as relevant for the food enzyme. The Panel did not consider plausible the use of this food enzyme in coffee processing, given the absence of relevant amounts of α-glucan substrates in green coffee beans. Therefore, these two uses were excluded from the exposure calculation.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the glucan 1,4-α-glucosidase is inactivated by heat during baking.

3.5.2. Dietary exposure estimation

For the use of this food enzyme in baking processes, chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP 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 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.94 mg TOS/kg bw per day in infants at the 95th percentile.

[26] Technical dossier/pp. 47–50; Additional data July 2022/Answer to points 10 and 11.
[27] Technical dossier/p. 46.
[28] Technical dossier/Additional data July 2022/Answer to point 10.
[29] Technical dossier/Additional data July 2022/Answer to point 11.

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)	0.015–0.220 (11)	0.170–0.473 (15)	0.190–0.457 (19)	0.104–0.280 (21)	0.078–0.172 (22)	0.077–0.173 (22)
Min–max 95th (number of surveys)	0.086–0.940 (9)	0.419–0.804 (13)	0.372–0.857 (19)	0.232–0.592 (20)	0.171–0.357 (22)	0.155–0.295 (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	
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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 of food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,868 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.015–0.473 mg TOS/kg bw per day at the mean and from 0.086 to 0.94 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 1,987.

4. Conclusion

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme glucan 1,4-α-glucosidase produced with *R. arrhizus* strain AE-G does not give rise to safety concerns when used in baking processes under the intended conditions of use.

5. Documentation as provided to EFSA

Application for authorisation of Glucoamylase from *Rhizopus oryzae* AE-G in accordance with Regulation (EC) No 1331/2008. January 2015. Submitted by Amano Enzyme Inc.

Additional information. July 2022. Submitted by Amano Enzyme Inc.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
EMDI	enzymatically modified dairy ingredients

FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
ITS	internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNBN	binucleated cells with micronuclei
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
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.2023.7753#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, Hungary, 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, Hungary, 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, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, 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).