SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 24 January 2023

doi: 10.2903/j.efsa.2023.7828

Safety evaluation of the food enzyme endo-1,3(4)-β-glucanase from the non-genetically modified *Cellulosimicrobium funkei* strain AE-TN

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

Abstract

The food enzyme endo-1,3(4)-β-glucanase (3-(1–3;1–4)-β-D-glucan 3(4)-glucanohydrolase; EC 3.2.1.6) is produced with the non-genetically modified *Cellulosimicrobium funkei* strain AE-TN by Amano Enzyme Inc. The food enzyme was shown to contain viable cells of the production strain, which belongs to a species that has been implicated in opportunistic infections in humans. The food enzyme is intended to be used in baking processes and yeast processing. Dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 1.75 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 1,788 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 1,022. A search for the 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 reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is low. However, due to the presence of viable cells of the production strain in the food enzyme, the Panel concluded that the food enzyme cannot be considered safe.

Keywords: food enzyme, endo-1,3(4)-β-glucanase, 3-(1→3;1→4)-β-D-glucan 3(4)-glucanohydrolase, EC 3.2.1.6, β-1,3-glucanase, *Cellulosimicrobium funkei*

Requestor: European Commission

Question number: EFSA-Q-2015-00693

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, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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 IL, Tlustos C, Van Loveren H, Vernis L, Zorn H, Herman L, Roos Y, Andryszkiewicz M, Liu Y, Lunardi S, Nielsen E, Nørby K and Chesson A, 2023. Scientifc Opinion on the safety evaluation of the food enzyme endo-1,3(4)-β-glucanase from the non-genetically modified *Cellulosimicrobium funkei* strain AE-TN. EFSA Journal 2023;21(2):7828, 15 pp. https://doi.org/10.2903/j.efsa.2023.7828

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 company "Amano Enzyme Inc." for the authorisation of the food enzymes Beta-amylase from *Bacillus flexus* (strain AE-BAF), Triacylglycerol lipase from *Mucor javanicus* (strain AE-LM), Beta-glucanase from *Cellulosimicrobium cellulans* (strain AE-TN), Laccase from *Trametes hirusta* (strain AE-OR) and Protein-glutaminase from *Chrysobacterium proteolyticum* (strain AE-PG).

Following the requirements of Article 12.1 of Commission Regulation (EU) 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Beta-amylase from *Bacillus flexus* (strain AE-BAF), Triacylglycerol

[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.

lipase from *Mucor javanicus* (strain AE-LM), Beta-glucanase from *Cellulosimicrobium cellulans* (strain AE-TN), Laccase from *Trametes hirusta* (strain AE-OR) and Protein-glutaminase from *Chrysobacterium proteolyticum* (strain AE-PG) 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 the food enzyme endo-1,3(4)-β-glucanase from a non-genetically modified *C. cellulans* strain AE-TN.

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

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,3(4)-β-glucanase from a non-genetically modified *C. funkei* strain AE-TN.

Additional information was requested from the applicant during the assessment process on 13 December 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	Endo-1,3(4)-β-glucanase
Systematic name	3-(1–3;1–4)-β-D-glucan 3(4)-glucanohydrolase
Synonyms	Endo-1,3-β-D-glucanase; β-1,3-glucanase
IUBMB No	EC 3.2.1.6
CAS No	62213-14-3
EINECS No	263-462-4

Endo-1,3(4)-β-glucanases catalyse the hydrolysis of 1,3- and 1–4-β-glycosidic linkages in mixed-linked β-D-glucans resulting in the generation of partially hydrolysed β-D-glucans. The enzyme under this assessment is intended to be used in baking processes and yeast processing.

3.1. Source of the food enzyme

The endo-1,3(4)-β-glucanase is produced with the non-genetically modified bacterium *Cellulosimicrobium funkei* strain AE-TN, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan), with the deposit number [redacted].[4] The production strain was identified as *C. funkei* by [redacted].[5]

The production strain AE-TN was derived from a soil isolate [redacted]. The absence of acquired genes coding for resistance to antimicrobials was demonstrated [redacted].[6]

[4] Technical dossier/Additional information August 2022/Annex 3.
[5] Technical dossier/Additional information August 2022/Annexes 1_1 and 1_2.
[6] Technical dossier/Additional information August 2022/Annex 2.

Case study reports have implicated *C. funkei* in opportunistic infections in humans (Brown et al., 2006; Petkar et al., 2011; Rivero et al., 2019).

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]

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, 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 weight 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 endo-1,3(4)-β-glucanase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted]. 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 single major peak, accompanied by some minor peaks.[11] No other enzymatic activities were reported.[12]

The in-house determination of endo-1,3(4)-β-glucanase activity is based on the hydrolysis of freeze-dried yeast (reaction conditions: pH 7.5, 35°C, 30 min) and measurement of the change in absorbance at 660 nm. The enzyme activity is expressed in yeast lysis activity units (U)/g measured relative to an internal standard.

The food enzyme has a temperature optimum around 35°C (pH 7.5) and a pH optimum around pH 7.0 (35°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 7.5). Enzyme activity decreased by more than 90% after pre-incubation at 45°C, the highest temperature tested.[13]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (batches 1–3) and two batches produced for the toxicological tests (batches 4 and 5) (Table 1).[14] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 91.7% and the mean enzyme activity/TOS ratio was 53.6 U/mg TOS.

[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/pg. 34/Annexes: 4_1 and 4_2.
[9] Technical dossier/pg. 34-41/Annex 5.
[10] Technical dossier/Annex 6.
[11] Technical dossier/pg. 27.
[12] Technical dossier/pg. 29.
[13] Technical dossier/pg. 30–31.
[14] Technical dossier/pg. 26 and 51/Annexes: 1, 3, 9 and 10 and Additional information August 2022/ Annex 6.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches				
		1	2	3	4[(a)]	5[(b)]
Endo-1,3(4)-β-glucanase activity	U/g batch[(c)]	57,500	56,000	34,200	67,600	34,200
Protein	%	17.9	19.0	8.0	18.8	NA[(d)]
Ash	%	2.7	4.1	4.6	9.9	4.7
Water	%	4.4	4.4	4.6	4.4	5.9
Total organic solids (TOS)[(e)]	%	92.9	91.5	90.8	85.7	89.4
Activity/TOS	U/mg TOS	61.9	61.2	37.7	78.9	38.2

(a): Batch used for the genotoxicity tests.
(b): Batch used for the repeated dose 90-day oral toxicity study in rats.
(c): UNIT: U/g (see Section 3.3.1).
(d): NA: not analysed.
(e): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches was below 0.04 mg/kg,[15,16] 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).[15] No antimicrobial activity was detected in any of the tested batches.[15]

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 tested in three independent batches analysed in triplicate. [redacted] Colonies from the production strain were detected [redacted] A positive control was included.[17]

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.

Despite its slightly higher activity per unit TOS, batch 4 (Table 1) used in Ames test and in *in vitro* mammalian chromosomal aberration test was considered sufficiently representative of the batches used for commercialisation.

The batch 5 (Table 1) used in the repeated dose 90-day oral toxicity study in rats was considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[18]

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 a concentration range finding experiment, two separate experiments were carried out using triplicate plating. For strains TA98, TA100, TA1535 and WP2uvrA, cells were

[15] Technical dossier/pg. 28/Annexes: 1 and 3.
[16] Limit of detection (LoD): Pb = 0.01 mg/kg.
[17] Technical dossier/Additional information August 2022/Annexes 4, 4_2 and 4_3.
[18] Technical dossier/p. 1, annex 8.

exposed to five concentrations of the food enzyme ranging from 313 to 5,000 μg/plate (corresponding to 268, 536, 1,072, 2,141 and 4,284 μg TOS/plate), with and without S9-mix. For strain TA1537, cells were exposed to six concentrations of the food enzyme ranging from 39 to 1,250 μg/plate (corresponding to 33.4, 67, 134, 268, 536 and 1,072 μg TOS/plate) without S9-mix and from 10 to 313 μg/plate (corresponding to 8.5, 17, 33.4, 67, 134 and 268 μg TOS/plate) with S9-mix.

Cytotoxicity was observed for strain TA98 at 5,000 μg/plate and for strain TA1537 at 1,250 μg/plate without S9-mix, and at 131 μg/plate with S9-mix. 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 Chinese hamster lung cells according to 'Regulations of testing facilities for studies on new chemical substances' (2003) and following GLP.[19]

Based on the results of a cell growth inhibition study, duplicate cell cultures were exposed to the food enzyme at 625, 1,250, 2,500 and 5,000 μg/mL (corresponding to 535.5, 1,071, 2,142 and 4,284 μg TOS/mL), in a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix), and in a continuous treatment (24 h) in the absence of S9-mix. In a second continuous treatment (48 h) without S9-mix, cells were exposed to concentrations of 313, 625, 1,250 and 2,500 μg/mL (corresponding to 268, 535.5, 1,071 and 2,142 μg TOS/mL).

Cytotoxic effects, expressed as the cell growth inhibition, only exceeded 50% in the continuous treatment (48 h), without S9-mix at 5,000 μg/mL. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative control.

The Panel concluded that 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. 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, epididymides were not weighed, and only two regions of the brain and one level of the spinal cord were examined in the microscopy. The Panel considered that these deviations are not major and do not hamper 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 447, 894 or 1,788 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

The feed consumption was statistically significantly decreased (10%–13%) in high-dose males from day 70 and until the end of the dosing period. The Panel considered these changes as not toxicologically relevant because the final body weights were not affected in the treated groups.

The haematological investigation revealed a statistically significant decrease in the red blood cell count (−4%), a decrease in the haemoglobin concentration (−3%), an increase in the reticulocyte ratio (+25%) in mid-dose females, and a decrease in the haemoglobin concentration (−4%) and haematocrit (−3%) in low-dose females. The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship (all parameter), they were only observed in one sex (all parameters) and the changes were small (except for reticulocytes).

The clinical chemistry investigation revealed a statistically significant decrease in the albumin/globulin ratio (−8%) in mid-dose males. The Panel considered the change as not toxicologically relevant as there was no dose–response relationship, it was only observed in one sex, the change was small and there were no changes in other relevant parameters (in total protein or albumin levels).

[19] Technical dossier/p. 1, Annex 9.

The urinalysis revealed a statistically significant increase in osmotic pressure (+34%) in low-dose males. The Panel considered the change as not toxicologically relevant as there was no dose–response relationship and it was only observed in one sex.

A statistically significant decreased absolute kidney weight (−10%) was reported in high-dose males. The Panel considered the change as not toxicologically relevant in the absence of a change in the relative kidney weight because there were no histopathological changes in the kidneys and the change was only observed in one sex.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 1,788 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 that may be used in the final formulation.

The potential allergenicity of the endo-1,3(4)-β-glucanase produced with the non-genetically modified *C. funkei* strain AE-TN 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.[20]

No information was available on oral and respiratory sensitisation or elicitation reactions of this endo-1,3(4)-β-glucanase.

Some reports indicated that β-glucanases in pollen from olive trees may cause allergic reactions (Huecas et al., 2001; Palomares et al., 2003; Treviño et al., 2008; Callero et al., 2012). However, these allergens do not have a homology match with the food enzyme endo-1,3(4)-β-glucanase.

[redacted], a known allergen, is present in the media fed to the microorganisms. However, during the fermentation process, this 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 [redacted] are not expected to be present.

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 of such reactions to occur is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

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

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[21]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	15–**147**
Yeast processing	Dried yeast	31.8–**318.1**

(a): The description provided by the applicant has been harmonised by EFSA 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 numbers in bold were used for calculation.

[20] Technical dossier/Additional information August 2022/Annex 5.

[21] Technical dossier/p. 46; Additional data August/Answer to points 10, 11 and 12.

In baking, the food enzyme is added to flour during the preparation of dough or batter.[22] The hydrolytic action of the endo-1,3(4)-β-glucanase reduces the stickiness of the dough or batter, thus improving their handling. The food enzyme–TOS remains in bakery foods.

In yeast processing, the food enzyme is added to the dried yeast cells.[23] The endo-1,3(4)-β-glucanase hydrolyses β-glucans in the yeast cell wall. The enzymatic reaction improves the production yield of the yeast extract. The food enzyme–TOS remains in the yeast extract.

Based on data provided on thermostability (see Section 3.3.1), it was expected that the endo-1,3(4)-β-glucanase is inactivated by heat during baking or ultra-high temperature treatment in yeast processing.

3.5.2. Dietary exposure estimation

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 1.75 mg TOS/kg bw per day in infants at the 95th percentile.

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)	0.030–0.411 (11)	0.316–0.881 (15)	0.353–0.851 (19)	0.193–0.522 (21)	0.145–0.320 (22)	0.144–0.322 (22)
Min–max 95th percentile (number of surveys)	0.167–1.750 (9)	0.781–1.500 (13)	0.700–1.594 (19)	0.431–1.103 (20)	0.317–0.671 (22)	0.287–0.549 (21)

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.

[22] Technical dossier/p. 61.
[23] Technical dossier/p. 62.

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 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	+/–

+: 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 an overestimation of the exposure.

3.6. Margin of exposure

The comparison of the NOAEL (1,788 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.03–0.881 mg TOS/kg bw per day at the mean and from 0.167–1.75 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 1,022.

4. Conclusions

Based on the data provided, the Panel did not identify issues of concern arising from the toxicological examination of the food enzyme or from the assessment of its allergenicity.

However, the food enzyme contains viable cells of the production strain, which belongs to a species that has been implicated in opportunistic infections in humans. Consequently, the Panel concluded that the food enzyme endo-1,3(4)-β-glucanase produced with the non-genetically modified *Cellulosimicrobium funkei* strain AE-TN cannot be considered safe.

5. Documentation as provided to EFSA

Application for authorisation of Beta-glucanase from *Cellulosimicrobium cellulans* AE-TN in accordance with Regulation (EC) No 1331/2008. February 2015. Submitted by Amano Enzyme Inc.

Additional information. August 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
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton

LoD	limit of detection
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
WGS	whole genome sequence
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.2023.7828#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).