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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Papiliotrema terrestris* strain AE-BLC

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Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the non-genetically modified *Papiliotrema terrestris* strain AE-BLC by Amano Enzyme Inc. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in the production of galacto-oligosaccharides (GOS) from lactose. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.441 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate 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 1800 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 4082. 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 a risk of allergic reactions upon dietary exposure to this food enzyme 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 under the intended conditions of use.

KEYWORDS

EC 3.2.1.23, food enzyme, lactase, *Papiliotrema terrestris*, β-D-galactoside galactohydrolase, β-Galactosidase

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

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

1.1.1 | Background as provided by the European Commission

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

On 21 December 2021, a new application has been introduced by the applicant "Amano Enzyme Inc." for the authorisation of the food enzyme Beta-galactosidase from a non-genetically modified strain of *Papiliotrema terrestris* (strain AE-BLC).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: Beta-galactosidase from a non-genetically modified strain of *Papiliotrema terrestris* (strain AE-BLC), in accordance with the Regulation (EC) No 1331/2008 stablishing a common authorization procedure for food additives, food enzymes and food flavourings.[3]

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β-galactosidase from a non-genetically modified strain of *P. terrestris* strain AE-BLC.

Additional information was requested from the applicant during the assessment process on 20 January 2023 and received on 30 September 2023 (see 'Documentation provided to EFSA').

[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.

2.2 | Methodologies

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

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

2.3 | Public consultation

According to Article 32c (2) of Regulation (EC) No 178/2002[3] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 27 June to 18 July 2023.[4] No comments were received.

3 | ASSESSMENT

IUBMB nomenclature	β-Galactosidase
Systematic name	β-D-galactoside galactohydrolase
Synonyms	Lactase; β-D-lactosidase
IUBMB No	3.2.1.23
CAS No	9031-11-2
EINECS No	232-864-1

β-Galactosidases typically catalyse the hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides. The enzyme under application also has *trans*-galactosylation activity. When lactose is used as a substrate, one lactose molecule is hydrolysed to galactose and glucose, while a second lactose molecule acts as recipient for *trans*-galactosylation, resulting in the formation of a trisaccharide. Higher molecular mass galacto-oligosaccharides are produced as the reaction proceeds. The food enzyme under application is intended to be used in the production of galacto-oligosaccharides (GOS) from lactose.

3.1 | Source of the food enzyme

The β-galactosidase is produced with the non-genetically modified yeast *P. terrestris* (homotypic synonym *Cryptococcus terrestris*) strain AE-BLC, which is deposited at the [redacted] with the deposit number [redacted].[5] The production strain was derived from a soil isolate following chemical mutagenesis[6] and was identified as *P. terrestris* [redacted].[7]

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[8] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[9]

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 mass material passes the filtration membrane and is discarded.[10] 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.[11]

[3]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[4]Accessible at https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l0900000AIpid/pc0555.

[5]Technical dossier/Source of the food enzyme/Annex 5.

[6]Technical dossier/Source of the food enzyme/Additional data September 2023/Source of the food enzyme/Annex 1.

[7]Technical dossier/Risk assessment/Source of the food enzyme/Annex 6.

[8]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.

[9]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Annex 7.

[10]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/pp. 1-7/Annex 8.

[11]Technical dossier/Risk assessment/Manufacturing process of the food enzyme/Annex 9; Additional data September 2023/Risk assessment/Manufacturing process.

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 β-galactosidase is a single polypeptide chain of 566 amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed two major bands migrating between the marker proteins of [redacted] and [redacted] kDa in all batches assigned to the target enzyme.[14] No other enzyme activities were reported.[15]

The in-house determination of β-galactosidase activity [redacted].[16]

The food enzyme has a temperature optimum around 70°C (pH 6.0) and a pH optimum around pH 5.0 (40°C). The thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 6.0). The enzyme activity decreased above 60°C, showing no residual activity after pre-incubation above 70°C.[17]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and two batches produced for the toxicological tests (Table 1).[18] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 92.4% and the mean enzyme activity/TOS ratio was 6.3 U/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches				
Parameters	**Unit**	**1**	**2**	**3**	**4**[a]	**5**[b]
β-Galactosidase activity	U/g[c]	5870	5720	5740	2550	7950
Protein	%	53.6	53.4	54.5	NA[d]	62.4
Ash	%	2.0	2.2	2.2	5.1	1.9
Water	%	5.6	6.0	4.8	4.9	5.2
Total organic solids (TOS)[e]	%	92.4	91.8	93.0	90.0	92.9
β-Galactosidase activity/TOS ratio	U/mg TOS	6.4	6.2	6.2	2.8	8.6

[a]Batch used for Ames test, *in vitro* chromosomal aberration assay and 90-day repeated oral toxicity study.
[b]Batch used for *in vitro* micronucleus test.
[c]Unit (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 and in the batches used for toxicological studies was below 5 mg/kg,[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, arsenic, cadmium and mercury contents were below the limits of detection (LoDs) of the employed methods.[20,21]

[12]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3/Allergenicity/Annex 14.
[13]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3.
[14]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/pp. 2–3.
[15]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 4.
[16]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 4/Annex 2.
[17]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/pp. 5–6.
[18]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 1/Toxicological data/Subchronic toxicity/p. 1/Annexes: 3, 11, 12, 13.1; Additional data September 2023/ Risk assessment/Chemical composition, properties and purity of the food enzyme/Genotoxicity/Annex 2/Annex 3.
[19]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 3/Toxicological data/ Annexes: 11, 12, 13.1.
[20]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 3.
[21]LoDs: Pb = 0.01 mg/kg; As, Hg = 0.005 mg/kg each; Cd = 0.001 mg/kg.

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).[22] No antimicrobial activity was detected in any of the tested batches.[23]

The presence of aflatoxins (B1, B2, G1, G2), ochratoxin A, HT-2 toxin, T-2 toxin, zearalenone, sterigmatocystin and fumonisins (B1, B2) was examined in three food enzyme batches. All were below the LoD of the applied analytical methods.[24,25] The possible presence of other secondary metabolites of concern was addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme was 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 [redacted].[26]

3.4 | Toxicological data

A battery of toxicological tests was provided, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test, an *in vitro* mammalian micronucleus test and a repeated dose 90-day oral toxicity study in rats.

The batches 4 and 5 (Table 1) used in these studies have chemical compositions and activity/TOS values similar to the batches intended for commercialisation and were considered suitable as test items.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[27]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA (pKM101) were used with or without metabolic activation (S9-mix), applying the pre-incubation method. Based on a dose-finding study, where growth inhibition was observed at the highest concentration tested without S9-mix, two main experiments were carried out in triplicate. Five concentrations of the food enzyme were used, ranging from 313 to 5000 µg/plate, corresponding to 282, 562, 1125, 2250 and 4500 µg TOS/plate in the presence of S9-mix and from 156 to 5000 µg/plate, corresponding to 140, 282, 562, 1125, 2250 and 4500 µg TOS/plate in the absence of S9-mix.

Cytotoxicity was observed as growth inhibition of the tester strains at the two highest concentrations tested without S9-mix. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme β-galactosidase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | In vitro *mammalian chromosomal aberration test*

The *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 2016) and following GLP.[28]

An experiment was performed with duplicate cultures of Chinese hamster lung fibroblast cells. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix). In a cell-growth inhibition test, no cytotoxicity above 50% was seen at any concentration tested up to 5000 µg/mL (corresponding to 4500 µg TOS/mL) in a short-term treatment (6 h exposure and 18 h recovery period) either with or without S9-mix. In a long-term treatment (24 h without recovery) without S9-mix, cytotoxicity of 50% was estimated to be at 1420 µg/mL (corresponding to 1280 µg TOS/mL)

[22]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 3; Additional data September 2023/Genotoxicity/Annex 2/Annex 3.

[23]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 3; Additional data September 2023/Genotoxicity/Annex 2/Annex 3.

[24]Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 3.

[25]LoDs: aflatoxins (B1, B2, G1, G2) = 0.2 µg/kg each; ochratoxin A = 0.5 µg/kg; T-2, HT-2 = 10 µg/kg each; zearalenone = 2 µg/kg; fumonisins (B1, B2) = 5 µg/kg.

[26]Technical dossier/Risk assessment/Source of the food enzyme/Additional data September 2023/Source of the food enzyme/Annex 1 and Supplement 2 and 3.

[27]Technical dossier/Risk assessment/Toxicological data/Genotoxicity/Annex 11 Report of Bacterial reverse mutation test.

[28]Technical dossier/Risk assessment/Toxicological data/Annex 12 Report of chromosomal aberration test.

and above. In a long-term treatment (48 h), cytotoxicity of more than 60% was observed at all concentrations tested above 75 µg/mL (corresponding to 67 µg TOS/mL).

In the chromosomal aberration test, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 625, 1250, 2500 and 5000 µg/mL (corresponding to 562, 1125, 2250 and 4500 µg TOS/mL), in the short-term treatment without S9-mix, and at 1250, 2500 and 5000 µg/mL (corresponding to 1125, 2250 and 4500 µg TOS/mL) in the short-term treatment with S9-mix. In the long-term treatment (24 h), exposed cells were scored for chromosomal aberrations at concentrations of 400, 800, 1200, 1600 and 2000 µg/mL (corresponding to 360, 720, 1080, 1440 and 1800 µg TOS/mL). In the 48 h treatment, exposed cells were scored for chromosomal aberrations at concentrations of 14.8, 22, 33 and 50 µg/mL (corresponding to 13, 20, 30 and 45 µg TOS/mL).

The frequency of chromosomal aberrations was not statistically significantly different from the negative controls at any of the concentrations tested, neither in the short-term treatments nor in the long-term treatments.

The Panel concluded that the food enzyme β-galactosidase did not induce an increase in the frequency of structural and numerical aberrations under the test conditions applied in this study.

3.4.1.3 | *In vitro mammalian micronucleus test*

The *in vitro* mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2016) and following GLP. A range-finding test and a main experiment were carried out with duplicate cultures of human lymphoblastoid (TK6) cells. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

A range-finding test was performed with the highest concentration set at 5000 µg TOS/mL in a short-term treatment (3 hours exposure and 21 h recovery period) with and without S9-mix and in a long-term treatment (24 h exposure without recovery period). In the short-term treatment, no cytotoxicity, evaluated as relative population doubling, of more than 50% was observed. In the long-term treatment, a cytotoxicity higher than 50% was noted at 8 µg TOS/mL and above. On the basis of these results, cells were exposed to the food enzyme and scored for micronuclei frequency at concentrations of 3000, 4000 and 5000 µg TOS/mL in the short-term treatment with and without S9-mix, and of 3, 6 and 8 µg TOS/mL in the long-term treatment.

The frequency of micronucleated binucleated cells (MNBNs) was not statistically significantly different to the negative controls at any concentration tested.

The Panel concluded that the food enzyme β-galactosidase 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 following GLP and in accordance with guidelines of the Japanese Ministry of Health and Welfare (1989, 1996 and 1999).[29] The study is in accordance with the OECD Test Guideline 408 (OECD, 1998) with the following deviations: detailed clinical observations and functional observations were not performed, urea was not determined in the clinical chemistry investigation, epididymides were not weighed, only two of the three regions of the brain and only one of the three levels of the spinal cord were examined microscopically. The Panel considered that these deviations are minor and do not impact on the evaluation of the study.

Groups of 12 male and 12 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 500, 1000 or 2000 mg/kg body weight (bw) per day, corresponding to 450, 900 or 1800 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

Haematological investigations revealed a statistically significant decrease in haematocrit in mid-dose females (−4%) and in the red blood cell count in low- and mid-dose females (−5% and −4%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (both parameters), there was no dose–response relationship (both parameters), the changes were small and there were no changes in other relevant parameters (other red blood cell parameters).

Clinical chemistry investigations revealed a statistically significant increase in inorganic phosphorus in high-dose males (+8%) and a decrease in chloride in high-dose males (−2%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (both parameters), the changes were small, there were no histopathological changes in kidneys and the changes were within the historical control values.

The urinalysis revealed a statistically significant increase in potassium in low- and high-dose males (+18% and +22%, respectively) and in chloride in high-dose males (+28%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (both parameters), there was no dose–response relationship (potassium) and there were no histopathological changes in the kidneys.

A statistically significant increase in the relative liver weight was detected in high-dose females (+8%). The Panel considered the change as not toxicologically relevant, as it was only observed in one sex, the change was small and there were no histopathological changes in the liver.

[29]Technical dossier/Risk assessment/Toxicological data/Subchronic toxicity/Annex 13 Report of systemic toxicity study.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1800 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity

The allergenicity assessment considered only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the β-galactosidase produced with the *Papiliotrema terrestris* strain AE-BLC 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.[30]

No information was available on oral and respiratory sensitisation or elicitation reactions of this β-galactosidase.

Occupational exposures to β-galactosidases have been reported to lead to respiratory and skin sensitization (Berstein et al., 1999; Green & Beezhold, 2011; Laukkanen et al., 2007; Mapp, 2001; Muir et al., 1997; Stöcker et al., 2016). Several studies have shown that occupationally sensitised adults may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Poulsen, 2004). Only one case of an anaphylactic reaction due to lactase, where the lactase was ingested as a tablet, has been reported (Voisin & Borici-Mazi, 2016).

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, it 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 from this source are present in the food enzyme.

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

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in the processing of lactose for the production of GOS at a recommended use level of [redacted] mg TOS/kg lactose.[31]

The food enzyme is added to lactose and held at 40–75°C (pH 4–7) to allow the reaction to occur.[32] In the presence of a high concentration of lactose, β-galactosidase transglycosylates lactose, resulting in the production of a mixture of GOS. Afterwards, the reaction mixture is subject to ion-exchange, which is expected to remove the food enzyme–TOS from the GOS products. However, no information was available in the technical dossier to indicate such removal. Therefore, the Panel considered that the food enzyme–TOS remains in the GOS products.

Based on the data provided on thermostability (see Section 3.3.1) and the heat treatment applied after the synthesis of GOS,[33] it was expected that the food enzyme is inactivated in the GOS products.

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, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 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 2 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out

[30]Technical dossier/Risk assessment/Allergenicity/pp. 1-2/Annex 14.

[31]Additional data November 2023.

[32]Technical dossier/Risk assessment/4–11 proposed conditions of use.

[33]Technical dossier/Risk assessment/4–11 proposed conditions of use.

in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.441 mg TOS/kg bw per day in infants at the 95th percentile.

TABLE 2 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥65 years
Min–max mean (number of surveys)	0.058–0.193 (12)	0.008–0.084 (15)	0.002–0.006 (19)	0.001–0.002 (21)	0–0.002 (22)	0–0.002 (23)
Min–max 95th percentile (number of surveys)	0.197–0.441 (11)	0.04–0.194 (14)	0.005–0.03 (19)	0.002–0.007 (20)	0.001–0.006 (22)	0.001–0.009 (22)

Abbreviation: 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 3.

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/ underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
The food enzyme under application is intended for the production of GOS, however, the calculation included also other indigestible oligosaccharides (e.g. fructooligosacharides)	+
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.

Abbreviation: TOS, total organic solids.

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

3.6 | Margin of exposure

A comparison of the NOAEL (1800 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.193 mg TOS/kg bw per day at the mean and of 0.001–0.441 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 4082.

4 | CONCLUSION

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme β-galactosidase produced with the non-genetically modified *P. terrestris* strain AE-BLC does not give rise to safety concerns under the intended conditions of use.

DOCUMENTATION AS PROVIDED TO EFSA

Dossier for beta-galactosidase from *Papiliotrema terrestris* AE-BLC. May 2021. Submitted by Amano Enzyme Inc.
Additional information. September 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
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
GOS	galacto-oligosaccharides
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNBN	micronucleated binucleated cells
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
WHO	World Health Organization

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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SUPPORTING INFORMATION

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

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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

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

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

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, 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).