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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Neobacillus* sp. strain AE-LT

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, Natália Kovalkovičová, Yi Liu, Giulio di Piazza, Sandra Rainieri, Rita Ferreira de Sousa and Andrew Chesson

Abstract

The food enzyme β-galactosidase (EC 3.2.1.23) is produced with the non-genetically modified *Neobacillus* sp. strain AE-LT by Amano Enzyme Inc. The strain is not cytotoxic and does not harbour any known virulence factor or antimicrobial resistance gene. The presence of viable cells of the production strain in the food enzyme could not be excluded, but the likelihood of this being a hazard is considered low. The food enzyme is intended to be used for lactose hydrolysis in milk processing and the manufacture of galacto-oligosaccharides (GOS). The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 2.971 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 1,223 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 412. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur 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: food enzyme, β-galactosidase, EC 3.2.1.23, β-D-galactoside galatohydrolase, *Bacillus circulans*, *Neobacillus* sp., non-genetically modified organism

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

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

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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[1] 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 CEF Panel, 2009) 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[1] on food enzymes.

Five applications have been introduced by the companies 'Genencor International B.V., 'Amano Enzyme Inc.' and 'DSM Food Specialties B.V.' for the authorisation of the food enzymes endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* (DP-Nzd22), acylglycerol lipase from *Penicillium camemberti* (strain AE-LG), beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), beta-galactosidase from *Bacillus circulans* (strain AE-LT) and arabinofuranosidase from *Aspergillus niger* (strain ARF).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain 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, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* (DP-Nzd22), acylglycerol lipase from *Penicillium camemberti* (strain AE-LG), beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), beta-galactosidase from *Bacillus circulans* (strain AE-LT) and arabinofuranosidase from *Aspergillus niger* (strain ARF) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme β-galactosidase from a non-genetically modified *Neobacillus* sp. strain AE-LT (formerly known as *Bacillus circulans* strain AE-LT).

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 *Bacillus circulans* strain AE-LT.

Additional information was requested from the applicant during the assessment process on 15 October 2020 and 16 November 2021, which was received on 27 August 2021 and 15 February 2022, respectively (see 'Documentation provided to EFSA').

2.2. Methodologies

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

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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[4]

IUBMB nomenclature	**β-galactosidase**
Systematic name	β-D-galactoside galatohydrolase
Synonyms	lactase; β-lactosidase; β-D-lactosidase; β-D-galactanase
IUBMB No.	EC 3.2.1.23
CAS No.	9031-11-2
EINECS No.	232-864-1

β-Galactosidases catalyse the hydrolysis of the β-(1,4)-glycosidic linkage of e.g., lactose (β-D-galactosyl-1,4-D-glucoside) and the transgalactosylation of lactose to generate galacto-oligosaccharides (GOS). The food enzyme under evaluation is intended to be used for lactose hydrolysis in milk processing and the manufacture of GOS.

3.1. Source of the food enzyme[5]

The β-galactosidase is produced with the non-genetically modified bacterium *Neobacillus* sp. strain AE-LT (formerly *Bacillus circulans* strain AE-LT), which is deposited at [redacted], with deposit number

[4] Technical dossier/1st submission/p. 23.
[5] Technical dossier/1st submission/p. 34–37; Technical dossier/Additional data, 27 August 2021/Annex 1, Annex 2; Technical dossier/Additional data, 15 February 2022/Annex 1, Annex 7.

[redacted].[6] To obtain the taxonomic identification of the production strain, [redacted] No genes of concern were identified.[7]

The strain AE-LT showed no cytotoxic activity [redacted].[8]

3.2. Production of the food enzyme[9]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-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 leaving a supernatant containing the food enzyme. 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.[11] The food enzyme was dried before analysis. 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.[12]

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[13]

The β-galactosidase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. No other enzymatic activities were reported.

The in-house determination of β-galactosidase activity is based on hydrolysis of lactose (reaction conditions: pH 6.0, 40°C). The enzymatic activity is determined by measuring the release of glucose spectrophotometrically at 505 nm with a commercial test. The enzyme activity is expressed in Lactase Unit (LU)/g. One Lactase Unit (LU) is defined as the amount of enzyme that liberates 1 μmoL of glucose per minute under the conditions described.[15]

The food enzyme has a temperature optimum around 65°C (pH 6.0) and a broad pH optimum between pH 5.5 and 9.0 (40°C).[16] Thermostability of the food enzyme was tested by pre-incubation at different temperatures for up to 60 min (pH 6.0). Pretreatment at 65°C resulted in the complete loss of activity after 20 min.

[6] Technical dossier/Additional data, 27 August 2021/Annex 3.
[7] Technical dossier/Additional data, 27 August 2021/Annex 4, Annex 5.
[8] Technical dossier/Additional data, 27 August 2021/Annex 6.
[9] Technical dossier/1st submission/p. 38–46; Technical dossier/1st submission/Annex 4, Annex 5, Annex 6; Technical dossier/Additional data, 27 August 2021.
[10] 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.
[11] Technical dossier/1st submission/p. 16, 40; Technical dossier/1st submission/Annex 5.
[12] Technical dossier/1st submission/Annex 6.
[13] Technical dossier/1st submission/p. 29.
[14] Technical dossier/Additional data, 27 August 2021/Annex 8.
[15] Technical dossier/2nd submission/Annex 1.
[16] Technical dossier/1st submission/p. 32–33.

3.3.2. Chemical parameters[17]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and a further batch produced for the toxicological tests (Table 1). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 77% and the mean enzyme activity/mg TOS ratio is 35.9 LU/mg TOS.

Table 1: Compositional data of the food enzyme[(d)]

Parameters	Unit	Batches			
		1	2	3	4[(a)]
β-galactosidase activity	LU/g batch[(b)]	29,800	29,400	23,600	3,820
Protein	%	48.6	53.7	48.4	6.4
Ash	%	18.6	18.9	19.5	0.7
Water	%	4.3	3.9	3.9	90
Total organic solids (TOS)[(c)]	%	77.1	77.2	76.6	9.3
Activity/mg TOS	LU/mg TOS	38.7	38.1	30.8	41.1

(a): Batch used for the toxicological studies.
(b): LU: Lactase Unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.
(d): Technical dossier/1st submission/p. 24; Technical dossier/1st submission/Annex 1.

3.3.3. Purity[18]

The lead content in the three commercial batches was below 0.2 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). In addition, the levels of arsenic, cadmium and mercury were below 0.35, 0.17 and 0.02 mg/kg, respectively.[19,20] The Panel considered these concentrations as not of concern.

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). No antimicrobial activity was detected in any of the tested batches.[21]

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[22]

The absence of viable cells of the production strain in the food enzyme was tested in three independent batches analysed in triplicate. [redacted]. Appropriate positive and negative controls were included. Colonies were detected in all samples tested. The morphological methods applied do not allow contaminants to be distinguished from the production strain.

Although the presence of viable cells of the production strain in the food enzyme cannot be excluded, the Panel considered that the likelihood of this being a hazard is low. The strain is not cytotoxic and does not harbour any known virulence factor or antimicrobial resistance gene.

3.4. Toxicological data[23]

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. Batch 4 (Table 1) used in these studies is considered sufficiently representative of the batches used for commercialisation.

[17] Technical dossier/1st submission/p. 24; Technical dossier/1st submission/Annex 1; Technical dossier/Additional data, 27 August 2021/Annex 9.
[18] Technical dossier/1st submission/p. 28; Technical dossier/1st submission/Annex 1, Annex 3.
[19] Technical dossier/1st submission/Annex 3.
[20] Technical dossier/1st submission/Annex 1/LOQ: Pb = 0.005 mg/kg; As = 0.002 mg/kg; Cd = 0.001 mg/kg; Hg = 0.001 mg/kg.
[21] Technical dossier/1st submission/Annex 8.
[22] Technical dossier/Additional data, 27 August 2021/Annex 7.
[23] Technical dossier/1st submission/p. 57–62.

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, 1997a) and following good laboratory practice (GLP).[21] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. Two separate experiments were carried out in triplicate. In a range-finding test, eight concentrations of the food enzyme were tested (from 0.305 to 5,000 μg/plate, corresponding to 0.03 to 465 μg TOS/plate). The main experiment was carried out using six concentrations of the food enzyme (from 156 to 5,000 μg/plate, corresponding to 15, 29, 58, 116, 233 and 465 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no increase in revertant colony numbers above the control values in any strain 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 in Chinese hamster lung fibroblasts (CHL/IU cell line) according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[24]

A dose-finding study was performed at 10 concentrations of the food enzyme ranging from 10 to 5,000 μg/mL (0.9 to 465 μg TOS/mL) and a 50% cell growth inhibition concentration was observed at 5,000 μg/mL (465 μg TOS/mL) for the short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix). Growth inhibition was similarly observed at 55 μg/mL (5.1 μg TOS/mL) for 24 h continuous treatment and at 9 μg/mL (0.8 μg TOS/mL) for 48 h continuous treatment. Based on these results, the cells were exposed in duplicate to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 116.3, 233 and 465 μg TOS/mL) in a short-term treatment with and without S9-mix, at 20, 40, 60 and 80 μg/mL (corresponding to 1.9, 3.7, 5.6 and 7.4 μg TOS/mL) in a continuous treatment (24 h) and at 5, 7.5, 10 and 12.5 μg/mL (corresponding to 0.5, 0.7, 0.9 and 1.2 μg TOS/mL) in a continuous treatment (48 h) in the absence of S9-mix. Cytotoxic effects were observed at the highest concentration tested in the continuous treatment conditions. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls.

The Panel concluded that food enzyme β-galactosidase did not induce structural and numerical aberrations 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 Japanese Guidelines (Notification No. 29, MHW, 1996) and Japanese Principles of Good Laboratory Practice (2000 and 2001).[25] The study is in accordance with OECD Test guideline 408 (OECD, 1998) with the following deviations: sensory activity examination, functional observations (motor activity and grip strength), determination of blood urea nitrogen, weighing of epididymides and a histopathological examination of medulla/pons were not performed. The Panel considered that these deviations are not sufficiently major to exclude the evaluation of the study. Groups of 12 male and 12 female Sprague–Dawley [Crj:CD(SD)IGS] rats received by gavage the food enzyme in doses of 3,228, 6,575 or 13,150 mg/kg per day, corresponding to 300, 611 or 1,223 mg TOS/kg bw per day. Controls received the vehicle (water for injections).

No mortality was observed.

The haematological investigation revealed a statistically significant decrease in haemoglobin concentration (−7%) and haematocrit (−4%) in low-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters), the magnitude of the changes was low (both parameters) and there was no dose–response relationship (both parameters).

[24] Technical dossier/1st submission/Annex 9.
[25] Technical dossier/1st submission/Annex 10.

The clinical chemistry investigation revealed a statistically significant increase in total triglyceride concentration in low-dose males (+42%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and there was no dose–response relationship.

Statistically significant changes in organ weights included an increase in the absolute salivary gland weight in the low-dose males (+13%) and in the absolute and the relative salivary gland weights in high-dose males (+15% and +13%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex and there were no histopathological changes in the salivary gland.

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

The Panel identified the no observed adverse effect level (NOAEL) of 1,223 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[26]

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

The potential allergenicity of the food enzyme β-galactosidase produced with *Neobacillus* sp. AE-LT 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.[27]

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

Cases of occupational allergy following exposure by inhalation of β-galactosidase have been reported (Stöcker et al., 2016). However, several studies have shown that adults with occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, two case reports describing allergic reactions (swollen throat, shortness of breath and difficulty in swallowing) following ingestion of β-galactosidase pills, and confirmation by antigen challenge, have been reported (Binkley, 1996; Voisin and Borici-Mazi, 2016).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[28]) are used as raw materials ([redacted]). In addition, [redacted], known sources of allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these materials are not expected to be present.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two food processes. Intended uses and the recommended use levels are summarised in Table 2.

[26] Technical dossier/1st submission/p. 62–63; Technical dossier/1st submission/Annex 11.
[27] Technical dossier/1st submission/Annex 11.
[28] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Lactose hydrolysis in milk processing	Milk	28–**42**
Manufacture of galacto-oligosaccharides	Lactose	28–**99**

TOS: total organic solids.
(a): The description 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.
(c): Technical dossier/1st submission/p. 53; Technical dossier/Additional data, 27 August 2021/Answer 10 to 13.

To hydrolyse lactose in milk, the food enzyme is added to milk, resulting in the release of galactose and glucose. No separation step is applied to remove the enzyme from the final foods (lactose-reduced milk and milk products).[29]

For the production of GOS, the food enzyme is added to lactose during the incubation step. In the presence of a high concentration of lactose, β-galactosidase will transglycosylate lactose resulting in the production of GOS. The β-galactosidase is also used to hydrolyse unreacted lactose remaining in GOS. Downstream treatment of the GOS products involves filtration and deionisation,[30] which are expected to remove residues of the food enzyme–TOS from the final GOS products. The final GOS products (six batches) contain about 60% of GOS and 0.01% nitrogen.[31] However, the data provided did not demonstrate the removal of more than 99% TOS.

Based on data provided on thermostability (see Section 3.3.1), it is expected that this ß-galactosidase may be inactivated and denatured by heat treatment depending on the pasteurisation conditions.

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 1 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 about 2.971 mg TOS/kg bw per day in toddlers at the 95th percentile.

[29] Technical dossier/1st submission/p. 50.
[30] Technical dossier/1st submission/p. 70.
[31] Technical dossier/Additional data, 27 August 2021/Annex 10, LOD = 0.01% in GOS, LOD = 0.002% in the food enzyme by Kjeldahl method.

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)	0.080–0.639 (11)	0.033–1.169 (15)	0.203–1.084 (19)	0.009–0.396 (21)	0.011–0.145 (22)	0.008–0.132 (22)
Min–max 95th (number of surveys)	0.187–1.963 (9)	0.775–2.971 (13)	0.635–1.936 (19)	0.023–0.844 (20)	0.028–0.489 (22)	0.132–0.342 (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 was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Inclusion of semi-soft and soft cheeses that are ripened normally less than 3 months	+
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	+/–

TOS: total organic solids; GOS: galacto-oligosaccharides.
+: 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 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 a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,223 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.008–1.169 mg TOS/kg bw per day at the mean and from 0.023 to 2.971 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure of at least 412.

4. Conclusions

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

5. Documentation as provided to EFSA

Technical dossier 'Application for authorisation of beta-galactosidase from *Bacillus circulans* AE-LT in accordance Regulation (EC) No 1331/2008'. 29 August 2014 (1st submission) and 30 January 2015 (2nd submission). Submitted by Amano Enzyme Inc.

Additional information. 27 August 2021. Submitted by Amano Enzyme Inc.

Additional information. 15 February 2022. Submitted by Amano Enzyme Inc.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CHL/IU	Chinese hamster lung fibroblasts
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on genetically modified organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	a standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
GOS	galacto-oligosaccharides
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
LU	Lactase Unit
MHW	Ministry of Health and Welfare
[redacted]	[redacted]
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OECD	Organisation for Economic Cooperation and Development
RM	raw material
[redacted]	[redacted]
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
[redacted]	[redacted]
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.2022.7573#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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 1 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).