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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Aspergillus oryzae* strain AE-LA

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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 *Aspergillus oryzae* strain AE-LA by Amano Enzyme Inc. The food enzyme was considered free from viable cells of the production organism. The food enzyme is intended to be used for lactose hydrolysis in milk processing, production of fermented milk products, whey processing and the manufacture of enzyme-modified dairy ingredients. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 1.651 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 1,656 mg TOS/kg bw per day, the highest dose tested. This results in a margin of exposure of at least 1,003. 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 is considered to be low. Based on the data provided, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: Food enzyme, β-galactosidase, β-D-galactoside galactohydrolase, EC 3.2.1.23, lactase, *Aspergillus oryzae*

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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:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) 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.

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

Five applications have been introduced by the companies "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Trichoderma reesei* (DP-Nzb48) and Thermolysin from *Geobacillus caldoproteolyticus* (DP-Fzj32), and "Amano Enzyme Inc." for the authorisation of the food enzymes AMP deaminase from *Streptomyces murinus* (strain AE-DNTS), Beta-galactosidase from *Aspergillus oryzae* (strain AE-LA) and Dextranase from *Chaetomium erraticum* (strain AE-DX).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and 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, pp. 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 Alpha-amylase from a genetically modified strain of *Trichoderma reesei* (DP-Nzb48), Thermolysin from *Geobacillus caldoproteolyticus* (DP-Fzj32), AMP deaminase from *Streptomyces murinus* (strain AE-DNTS), Beta-galactosidase from *Aspergillus oryzae* (strain AE-LA) and Dextranase from *Chaetomium erraticum* (strain AE-DX) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme β-galactosidase from the non-genetically modified *A. oryzae* strain AE-LA.

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 the *A. oryzae* strain AE-LA.

Additional information was requested from the applicant during the assessment process on 27 October 2021 and received on 17 May 2022 (see 'Documentation provided to EFSA').

Following the reception of additional data, EFSA requested a clarification teleconference on 11 July 2022, after which the applicant provided additional data on 15 July 2022.

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	β-galactosidase
Systematic name	β-D-galactoside galactohydrolase
Synonyms	lactase; β-D-lactosidase
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 lactose (β-D-galactosyl-1,4-D-glucoside) resulting in the release of D-galactose and D-glucose. The enzyme under this assessment is intended to be used for the hydrolysis of lactose in milk processing, production of fermented milk products, whey processing and the manufacture of enzyme-modified dairy ingredients.

3.1. Source of the food enzyme

The β-galactosidase is produced with the filamentous fungus *Aspergillus oryzae* strain AE-LA, which is deposited at the [redacted], with deposit number [redacted].[4] The production strain is not genetically modified.

The parental strain [redacted] was isolated from [redacted]. The production strain was obtained from the parental strain by classical mutagenesis and identified as *A. oryzae* by

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

phylogenetic analysis based on the sequence of the nuclear ribosomal internal transcribed spacer (ITS) region and of the calmodulin gene.[5]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[6], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[7]

The production strain is grown as a pure culture using an industrial medium in a [redacted] fermentation system or in a solid-phase culture system, with conventional process controls in place. After completion of the fermentation, in the case of the solid-phase culture, the substrate is dispersed with water. In both fermentation systems, the solid biomass is removed by filtration, leaving a filtrate 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 mass material passes the filtration membrane and is discarded.[8] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme, which were the same for both fermentation systems.[9]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The β-galactosidase is a single polypeptide chain of [redacted] amino acids.[10] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[10] The food enzyme was analysed by size exclusion chromatography. The chromatograms of three food enzyme batches for commercialisation showed a consistent pattern containing a single peak.[11] No other enzymatic activities were reported.[12]

The determination of β-galactosidase activity is based on hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (reaction conditions: pH 4.5, 37°C, 15 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenol spectrophotometrically at 420 nm. The enzyme activity is expressed in lactase units (ALU)/g. One ALU is defined as the quantity of enzyme that will liberate *o*-nitrophenol at a rate of 1 μmol/min under the conditions of the assay.[13]

The food enzyme has a temperature optimum around 60°C (pH 4.5) and a pH optimum around pH 4.5 (30°C).[14] Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.5). β-Galactosidase activity was stable up to 50°C and decreased sharply at higher temperatures. No residual activity was detected at 70°C.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation produced by submerged fermentation is 61.5% and the mean enzyme activity/TOS ratio is 249 ALU/mg TOS. The mean TOS of the three food enzyme batches for commercialisation produced by solid-phase fermentation is 93.1% and the mean enzyme activity/TOS ratio is 80 ALU/mg TOS.[16]

[5] Technical dossier/Additional information May 2022/Annex 1.
[6] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[7] Technical dossier/p. 36.
[8] Technical dossier/p. 36–42 and Annex 5.
[9] Technical dossier/p. 37, 39 and Annex 6 and Additional information May 2022/Information about β-galactosidase from *A. oryzae*.
[10] Technical dossier/p. 29 and Annex 11.
[11] Technical dossier/p. 28.
[12] Technical dossier/p. 7, 30.
[13] Technical dossier/p. 30 and Annex 2.
[14] Technical dossier/p. 31,32.
[15] Technical dossier/p. 32.
[16] Technical dossier/p. 27 and Additional information May 2022/Information about β-galactosidase from *A. oryzae*.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches						
		Submerged fermentation				Solid-phase fermentation		
		1	2	3	4[(a)]	5	6	7
β-galactosidase activity	ALU/g batch[(b)]	154,000	154,000	151,000	112,000	72,000	71,000	80,300
Protein	%	58.3	58.1	57.2	NA	44.9	54.6	54.2
Ash	%	33.2	33.2	33.5	10.4	1.3	1.7	1.9
Water	%	5.0	5.3	5.3	6.8	5.0	5.4	5.3
Total organic solids (TOS)[(c)]	%	61.8	61.5	61.2	82.8	93.7	92.9	92.8
Activity/mg TOS	ALU/mg TOS	249	250	247	135	77	76	87

NA: not analysed.
(a): Batch used for the toxicological studies.
(b): ALU: lactase unit (see Section 3.3.1).
(c): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three commercial batches[17,18] and in the batch used for toxicological studies[19] was below 5 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 cadmium and mercury in the batch used for toxicological studies were below the limits of detection (LODs) of the employed methodologies. For arsenic, the average concentration determined in the toxicological batch was 0.3 mg/kg.[19,21] The Panel considered this concentration as not of concern.

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*)[22] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[20] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[23]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, ochratoxin A, sterigmatocystin, HT-2 toxin, T-2 toxin, zearalenone and sterigmatocysteine was examined in the three food enzyme batches obtained from submerged culture and in three additional batches produced by solid-phase culture. All were below the LOD of the applied methods.[24,25] Adverse effects caused by the potential presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. Ten grams of product were suspended in 90 mL of saline solution and 10 mL of the suspension were filtered. Filters were placed onto agar plates of non-selective medium and incubated at [redacted]°C for [redacted] days. No colonies were produced. A positive control was included.[26]

[17] LOD: Pb = 0.05 mg/kg.
[18] Technical dossier/Annex 3.
[19] Technical dossier/Annex 8/p. 13.
[20] Technical dossier/p. 7.
[21] LOD: Pb = 0.05 mg/kg; Cd = 0.01 mg/kg; Hg = 0.01 mg/kg.
[22] Technical dossier/Annex 1, Annex 3 and Annex 8/p. 14.
[23] Technical dossier/Annex 1, Annex 3 and Annex 8/p. 14.
[24] Technical dossier/Annexes 1 and 3 and Additional information May 2022/Information about β-galactosidase from *A. oryzae*.
[25] LOD: Aflatoxins = 0.2 μg/kg, Ochratoxin A = 0.05 μg/kg; HT-2 toxin = 10.0 μg/kg; T-2 toxin = 10 μg/kg; Zearalenone = 10 μg/kg; Sterigmatocystein = 10 μg/kg.
[26] Technical dossier/Additional information May 2022/Annex 3.

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats has been provided. The tests were performed following Japanese good laboratory practice (GLP) that are considered equivalent to the Organisation for Economic Co-operation and Development (OECD) GLP.[27] Batch 4 (Table 1) used in these studies has a lower chemical purity compared to the commercial batches produced by submerged fermentation and, thus, is considered suitable as a test item for the food enzyme manufactured under submerged fermentation conditions. Batch 4 is also considered suitable as a test item for solid-phase fermentation, because the composition of the fermentation media is identical for both processes except for the water content. The downstream processing is the same, apart from the addition of dextrin as a drying aid for the solid-phase batches. None of the mycotoxins tested were present in any of the batches from both fermentation systems analysed.[28]

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Japanese GLP.[29]

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. A dose-finding test was carried out using five concentrations: 50, 150, 500, 1,500 and 5,000 μg food enzyme/mL, corresponding to 41.4, 124.2, 414, 1,242 and 4,140 μg TOS/mL. No cytotoxicity was observed at any concentration tested. On the basis of these results, two separate experiments were carried out using five concentrations of the food enzyme (from 313 to 5,000 μg food enzyme/mL, corresponding to 259, 517.5, 1,035, 2,070 and 4,140 μg TOS/mL).

No cytotoxicity was observed at any concentration tested. 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 fibroblast cells (CHL/IU) according to OECD Test Guideline 473 (OECD, 1997b) and following Japanese GLP.[30]

The dose-finding study was performed at concentrations ranging from 78 to 5,000 μg food enzyme/mL (corresponding to 64.5 and 4,414 μg TOS/mL). No inhibition of cell growth by 50% or more was observed in a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix), whereas around 50% cell growth inhibition was observed in the continuous treatment (24 h). Based on these results, the cells were exposed to the food enzyme at 625, 1,250, 2,500 and 5,000 μg food enzyme/mL (corresponding to 517.5, 1,035, 2,070 and 4,140 μg TOS/mL), in a short-term treatment with and without S9-mix, and at 313, 625, 1,250, 2,500 and 5,000 μg food enzyme/mL (corresponding to 259, 517.5, 1,035, 2,070 and 4,140 μg TOS/mL) in a continuous treatment (24 h) in the absence of S9-mix.

Cytotoxic effects were observed at higher concentrations only for the continuous treatment (relative cell growth 57% and 51% at 2,500 and 5,000 μg/mL, respectively). The frequency of structural and numerical chromosomal aberrations in treated cultures was evaluated at 1,250, 2,500 and 5,000 μg food enzyme/mL and it was comparable to the values detected in negative controls.

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

[27] Technical dossier p. 53.
[28] Technical dossier/Additional information May 2022/Information about β-galactosidase from *A. oryzae*.
[29] Technical dossier/Annex 8.
[30] Technical dossier/Annex 9.

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 the Japanese guidelines (JMHW: Ordinance 21, 1997 and JMHW Pharmaceutical Affairs Bureau, Notification 424, 1997) and following Japanese GLP.[31] 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 areas of the brain and one level of the spinal cord were examined in the microscopy. The Panel considered that these deviations were minor and did not impact the evaluation of the study. Groups of 12 male and 12 female Sprague–Dawley (Crj:CD(SD)) rats received by gavage the food enzyme at 500, 1,000 and 2,000 mg/kg body weight (bw) per day corresponding to 424, 828 and 1,656 mg TOS/kg bw per day. Controls received the vehicle (water for injection). Furthermore, a recovery control and a high-dose group were included in the study each comprising six males and six females and terminated 4 weeks after the end of treatment.

One high-dose male died on day 88. No abnormalities were observed for this animal at necropsy and by histopathological examination. The death was considered by the Panel as accidental.

The overall body weight gain was statistically significantly decreased in low-dose females (−13%). The Panel considered the change as not toxicologically relevant as the body weight of this group was not significantly different compared to the control group, there was no dose–response relationship and the change was only observed in one sex.

The feed consumption was statistically significantly increased on day 35, day 42 and day 91 in mid-dose males (+10%, +10% and +11%, respectively) and on day 42 in high-dose males (+7%), and it was decreased on days 7 and 17 in high-dose females (−6% and −10%, respectively). The Panel considered the changes as not toxicologically relevant as they were only recorded sporadically, there was no dose–response relationship (for males), the changes were small, and there were no statistically significant changes in body weight and body weight gain.

Haematological investigation revealed a statistically significant prolongation of prothrombin time (PT) in the recovery high-dose males (+5%) and an increase in platelets count in the recovery high-dose females (+14%). The Panel considered the changes as not toxicologically relevant as they were not present at the end of the treatment (both parameters), the changes were small (both parameters), the changes were only observed in one sex (both parameters) and there were no changes in other parameters for blood clotting potential.

Clinical chemistry investigation revealed a statistically significant decrease in triglycerides (−48%), lactate dehydrogenase (LDH) (−20%), calcium (−4%), albumin (−5%), albumin to globulin ratio (A/G) (−8%) and an increase in γ-globulin (+26%) in mid-dose females, a decrease in glucose (−11%) and A/G (−9%) in low-dose females. After the recovery period, a statistically significant increase in α_2-globulin was recorded in the high-dose recovery females (+12%). The Panel considered the changes as not toxicologically relevant as the changes were small (except for triglycerides), there was no dose–response relationship (all parameters), the changes were only observed in one sex (all parameters) or they were only recorded after the recovery period (α_2-globulin).

The urinalysis in week 5 revealed a statistically significant increase in the incidence of higher urinary pH and presence of protein, ketones and phosphate crystals in high-dose males, an increase in the incidence of urine with protein and phosphate crystals in high-dose females, an increase in sodium excretion in high-dose males (+30%), a decrease in urine total protein concentration in mid- and high-dose males (−31% and −22%) and a decrease in activity of N-acetyl-β-D-glucosaminidase (NAG) in high-dose females (−48%).

The urinalysis in week 13 revealed a statistically significant increase in the incidence of higher urinary pH in males and females from mid- and high-dose groups, an increase in the incidence of samples of urine with protein in low-, mid- and high-dose males and in high-dose females, an increase in the incidence of urine with ketones in mid- and high-dose males and high-dose females, and an increase in the incidence of urine with phosphate crystals in mid- and high-dose males and high-dose females, an increase in sodium excretion in high-dose males (+55%), a decrease in NAG activity in high-dose males (−48%), a decrease in potassium (−23%) and chloride (−26%) in high-dose females. The Panel considered the changes as not toxicologically relevant as they could be attributed to the test article content of phosphate, protein and sodium, which could affect urinary pH, presence

[31] Technical dossier p. 53/Annexes 10.1, 10.2 and 10.3.

of ketones, presence of phosphate crystals, and excretion of cations. In addition, there were no histopathological changes in the kidneys.

Statistically significant changes in organ weights included an increase in absolute brain weight in low- and mid-dose males (+5% and +4%, respectively) and in relative lung weights in the low- and mid-dose females (+11% and +8.3%, respectively). The Panel considered the changes as not toxicologically relevant as they were small (both parameters), they were only observed in one sex (both parameters), there was no dose–response relationship (both parameters). In addition, the changes were not accompanied by histopathological findings (both parameters).

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

The Panel identified the no observed adverse effect level (NOAEL) of 1,656 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the β-galactosidase produced with *A. oryzae* strain AE-LA 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.[32]

No information is available on oral and respiratory sensitisation or elicitation reactions of this β-galactosidase.[33]

Some studies have shown that adults with occupational asthma caused by an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). A few case reports are available that describe allergic reactions upon oral exposure to lactase or β-galactosidase in individuals, respiratorily sensitised to β-galactosidase (Stöcker et al., 2016; Voisin and Borici-Mazi, 2016).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[34]) are used as raw materials ([redacted]). In addition, [redacted] and [redacted], known sources of allergens, are also present in the media fed to the microorganisms.[35] However, during the fermentation process, these products will be degraded and utilised by the microorganism 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 potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

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 is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The applicant intended to use this food enzyme in four food manufacturing processes at the recommended use levels summarised in Table 2.

[32] Technical dossier/Annex 11.

[33] Technical dossier/Additional information May 2022/Information about beta-galactosidase from A. oryzae_20220513/Answer to point 8.

[34] 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.

[35] Technical dossier/Annex 6.

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 for general uses	6–**18**
Production of fermented milk products	Milk	6–**18**
Whey processing	Whey protein concentrate	6–**18**
Manufacture of enzyme modified dairy ingredients[(d)]	Cream and buttermilk powder[(e)]	**575**[(e)]

TOS: total organic solids.
(a): The description has been harmonised by EFSA on the basis of additional information provided by the applicant in May and July 2022 and 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/pp. 47–49, Additional information May 2022/Information about beta-galactosidase from A. oryzae_20220513/Answer to points 9, 10 and 11, Additional information July 2022.
(d): Additional information May 2022/Answer to point 9, confirmed in the Additional data July 2022.
(e): Additional information July 2022.

Two different dairy materials can be treated with this food enzyme: milk or whey. β-Galactosidase hydrolyses lactose to glucose and galactose. The treatment makes milk more suitable for lactose-intolerant individuals and sweeter. Adding β-galactosidase together with microbial cultures during fermentation would result in lactose-reduced yoghurt. Treatment of the cheese whey or whey permeate would result in lactose-reduced and sweeter whey syrups.[36] No separation step is applied to remove the enzyme from the treated milk, milk products or whey.[37]

The enzymatically treated milk or whey can be consumed directly, but can also be used as ingredient in a large variety of foods.[38] The enzymatic treatment also decreases the sandiness of lactose crystals in products like ice cream.

To produce enzyme-modified dairy ingredients (EMDI), the food enzyme is used in cream and butter milk powder manufactured together with other relevant enzymes. The β-galactosidase hydrolyses lactose to glucose and galactose, whilst other enzymes contribute to the intensified flavour of EMDI products. The food enzyme remains in the EMDI products.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the β-galactosidase is inactivated by heat during the pasteurisation step.

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

[36] Technical dossier/Additional information May 2022/Information about beta-galactosidase from A. oryzae_20220513/Answer to point 10.
[37] Technical dossier/p. 48 and Additional information May 2022/Information about beta-galactosidase from A. oryzae_20220513/Answer to point 10.
[38] Technical dossier/Additional information May 2022/Information about beta-galactosidase from A. oryzae_20220513/Answer to point 11.

European countries (Appendix B). The highest dietary exposure was estimated to be about 1.651 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)	0.035–0.446 (11)	0.072–0.666 (15)	0.140–0.573 (19)	0.028–0.213 (21)	0.028–0.095 (22)	0.011–0.087 (22)
Min–max 95th (number of surveys)	0.154–1.651 (9)	0.700–1.542 (13)	0.309–0.948 (19)	0.092–0.446 (20)	0.083–0.268 (22)	0.083–0.192 (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	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

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

3.6. Margin of exposure

A comparison of the NOAEL (1,656 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.011–0.666 mg TOS/kg bw per day at the mean and from 0.083 to 1.651 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 1,003.

The Panel considered that this margin of exposure is sufficient to cover any remaining uncertainty associated with the fact that the test item used in the 90-day study was only obtained from a food enzyme manufactured under submerged fermentation conditions.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme β-galactosidase produced with *A. oryzae* strain AE-LA under both submerged and solid-phase fermentation conditions does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1) Application for authorisation of β-galactosidase from *Aspergillus oryzae* AE-LA in accordance with Regulation (EC) No 1331/2008. November 2015. Submitted by Amano Enzyme Inc.
2) Additional information. May 2022. Submitted by Amano Enzyme Inc.
3) Additional information. July 2022. Submitted by Amano Enzyme Inc.

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Abbreviations

ALU	lactase unit
bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
EMDI	enzyme-modified dairy ingredients
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
LDH	lactate dehydrogenase
LOD	limit of detection
MOE	margin of exposure
NAG	*N*-acetyl-β-D-glucosaminidase
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PT	prothrombin time
TOS	total organic solids
WHO	World Health Organization

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

Information provided in this Appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7569#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 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).