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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Aspergillus* sp. strain GD-FAL

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Abstract

The food enzyme β-galactosidase (EC 3.2.1.23) is produced with the non-genetically modified *Aspergillus* sp. strain GD-FAL by Godo Shusei Co., Ltd. The food enzyme is intended to be used in milk processing for the hydrolysis of lactose. The absence of viable cells of the production organism in the food enzyme was not demonstrated. Based on the assumption that all milk/dairy products are enzymatically treated, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.301 mg TOS/kg body weight per day in European populations. The data provided did not allow concerns of genotoxicity of the food enzyme to be excluded. The systemic toxicity could not be assessed in the absence of an appropriate repeated dose 90-day oral toxicity study. Consequently, a margin of exposure was not calculated. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is low. Based on the remaining concerns on genotoxicity, the inadequacies of the 90-day repeated dose oral toxicity study in rats and the missing data regarding the absence of viable cells of the production strain in the food enzyme, the Panel could not conclude on the safety of this food enzyme.

Keywords: 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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and terms of reference as provided by the requestor

1.1.1. Background as provided by the European Commission

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

An application has been introduced by the applicant "Godo Shusei Co., Ltd." for the authorisation of the food enzyme beta-galactosidase from a non-genetically modified strain *Aspergillus oryzae* (strain GD-FAL).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008[3], the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: beta-galactosidase from a non-genetically modified strain *Aspergillus oryzae* (strain GD-FAL) in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

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 *Aspergillus oryzae* (strain GD-FAL). The dossier was updated on 9 June 2021.

Additional information was requested from the applicant during the assessment process on 16 September 2021. However, some of the data requested were not provided. Consequently, the Panel concluded this assessment on the basis of the available data set.

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 existing guidance documents of EFSA Scientific Committees.

The current '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-lactosidase; exo-(1→4)-β-D-galactanase
IUBMB No	3.2.1.23
CAS No	9031-11-2
EINECS No	232-864-1

β-Galactosidases catalyse the hydrolysis of lactose to its monosaccharide units, D-glucose and D-galactose. The enzyme under this assessment is intended to be used in milk processing for the hydrolysis of lactose.

3.1. Source of the food enzyme

The β-galactosidase is produced with the non-genetically modified filamentous fungus *Aspergillus* sp. strain GD-FAL, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan), with the deposit number NITE SD 00458.[4] The production strain was identified as *Aspergillus* sp. by [redacted][5] On the basis of the data provided by the applicant, the production strain could not be identified at the species level.

Aspergillus sp. GD-FAL lacks the gene cluster responsible for the biosynthesis of aflatoxins, as shown by polymerase chain reaction (PCR).[6]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the food hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[8]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or 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

[4] Technical dossier/Appendix J.
[5] Technical dossier/Appendix C.
[6] Technical dossier/p. 20–21 and Figure 5.
[7] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[8] Technical dossier/p. 22.

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 mass material passes the filtration membrane and is discarded. Finally, the enzyme is stabilised with glycerol.[9] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[10]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The β-galactosidase is a single polypeptide chain of 1,005 amino acids.[11] The molecular mass of the mature protein, calculated from the amino acid sequence, was 110 kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about 140 kDa, accompanied by several other bands of different staining intensity.[12] No other enzymatic activities were reported.

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 and is expressed in lactase units (ALU). One ALU is defined as the amount of enzyme that will release 1 μmol of *o*-nitrophenol per minute under the conditions of the assay.[13]

The food enzyme has a temperature optimum around 60°C and a pH optimum around pH 5.0. Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. The β-galactosidase activity decreased above 50°C, showing no residual activity at 70°C.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation, one of which was used as test item in the toxicological tests (Table 1).[15] The mean total organic solids (TOS) of the three batches is 9.5% and the mean enzyme activity/TOS ratio is 121 ALU/mg TOS.

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches		
		1	2[(a)]	3
β-Galactosidase activity	ALU/g batch[(b)]	10,800	12,800	11,000
Protein	%	6.7	8.3	8.0
Ash	%	0.1	0.1	0.1
Water	%	47.9	46.3	47.3
Glycerol (excipient)	%	43.6	43.4	42.8
Total organic solids (TOS)[(c)]	%	8.4	10.2	9.8
Activity/mg TOS	ALU/mg TOS	127	124	111

(a): Batch used for the toxicological studies.
(b): ALU: lactase units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash – % excipient.

[9] Technical dossier/p. 22–24 and Figures 6–8.
[10] Technical dossier/p. 22 and Additional information September 22/Appendix E.
[11] Technical dossier/p. 15 and Appendix A.
[12] Technical dossier/Figure 1.
[13] Technical dossier/Appendix B.
[14] Technical dossier/Additional information September 22/ Figure 2.
[15] Technical dossier/Table 1 and Additional information September 22/Appendix K.

3.3.3. Purity

The lead content in the three commercial batches 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 level of arsenic was below the limit of quantification (LoQ) of the employed method.[16,17]

The food enzyme preparation 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). The food enzyme was also tested for *Pseudomonas aeruginosa* (negative in 1 g), *Staphylococcus aureus* (negative in 0.01 g), fungi (negative in 1 g), yeasts (negative in 1 g), *Listeria monocytogenes* (negative in 25 g) and *Bacillus cereus* (negative in 0.01 g). No antimicrobial activity was detected in any of the tested batches.[17]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2017). The presence of aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A, sterigmatocystin, T-2 toxin, zearalenone, kojic acid, cyclopiazonic acid and 3-nitropropionic acid was examined in two or three food enzyme batches. All were below the limit of detection (LoD) of the applied methods.[17,18] Adverse effects caused by the possible presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells of the production strain

Although requested, no information was provided on the absence of viable cells of the production strain in the food enzyme according to the Scientific Guidance for the submission of dossiers on food enzymes (EFSA CEP Panel, 2021a). The applicant argued that the routine microbiological test for yeast and fungal contaminants is sufficient to demonstrate the absence of viable cells of the production strain in food enzymes. However, this test is not adequate for this purpose.

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, an *in vitro* micronucleus test, an acute oral toxicity study in rats, a repeated dose 28-day oral toxicity study in rats and a repeated dose 90-day oral toxicity study in rats, has been provided. The batch 2 (Table 1) used in these studies is used for commercialisation, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[19]

Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* strain WP2 *uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the pre-incubation method. The experiments were carried out with triplicate plating. Six concentrations of the food enzyme in the range of 20.6–5,000 μg/plate (corresponding to 2.1–515 μg TOS/plate) were tested in a first range-finding experiment. As no cytotoxicity or precipitation at any concentration were seen in the first experiment, the highest concentration tested in the main experiment was also 5,000 μg food enzyme plate, but with a narrower range of concentrations (313–5,000 μg/plate) (corresponding to 32.2–515 μg TOS/plate).

Upon treatment with the food enzyme, there was no relevant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

[16] LoQs: Pb = 0.05 mg/kg; As = 0.1 mg/kg.
[17] Technical dossier/Additional information September 22/Appendix N.
[18] LoDs: aflatoxins = 1 μg/kg; ochratoxin A = 5 μg/kg; sterigmatocystin = 0.05 mg/kg; T-2 toxin = 0.01 mg/kg; zearalenone = 0.05 mg/kg; Kojic acid = 5 μg/kg; cyclopiazonic acid = 0.05 mg/kg; 3-nitropropionic acid = 1 mg/kg.
[19] Technical dossier/Appendix D1.

The food enzyme did not induce gene mutations under the test conditions employed in this study. However, the Panel noted that the maximum recommended concentration, expressed as TOS, was not reached, and therefore, the test was considered inconclusive.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in Chinese hamster lung cells according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[20]

A cell growth inhibition test, based on the relative population doubling (RPD), was performed where cells were exposed to the food enzyme at five concentrations ranging from 125 to 2,000 μg/mL, with and without metabolic activation (S9-mix). No inhibition of cell growth by 50% or more was observed. Based on these results, the cell cultures were exposed to the food enzyme at 250, 500, 1,000 and 2,000 μg/mL, corresponding to 26, 51.5, 103 and 206 μg TOS/mL, in a short-term treatment (6 + 18 h) with and without S9-mix, and in a continuous treatment (24 + 0 h) in the absence of S9-mix.

Cytotoxic effects were only observed in the continuous treatment (19% decrease in RPD at 2,000 μg/mL). The frequency of structural chromosomal aberrations in treated cultures were three times higher than the values detected in the solvent control and above the historical control range at the lowest concentration scored (500 μg/mL) in the 6 + 18 h treatment with S9-mix and at the highest concentration scored (2,000 μg/mL) in the 24 + 0 h treatment without S9-mix. The frequency of numerical chromosomal aberrations in the treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the results of the *in vitro* chromosomal aberration assay indicate a potential clastogenic activity, which needs to be further investigated.

3.4.1.3. *In vivo* micronucleus test

The *in vivo* mammalian erythrocyte micronucleus test was carried out in Sprague–Dawley rats according to the OECD Test Guideline 474 (OECD, 1997c) and following GLP.[21]

The food enzyme was tested for its ability to induce micronuclei in the polychromatic erythrocytes (PCE) in bone marrow of treated rats. Based on a range finding study, where no clinical signs of toxicity and no difference in findings between male and female animals were observed, the enzyme concentrate was administered for two consecutive days, at 500, 1,000 and 2,000 mg/kg body weight (bw) per day (corresponding to 10.5, 103 and 206 mg TOS/kg bw per day), to groups of six male Crl:CD(SD) rats. Negative (water) and positive (cyclophosphamide 20 mg/kg bw) control groups were included.

The absence of clinical signs of toxicity up to the highest dose tested did not provide evidence of systemic exposure to the test item. Rats treated with food enzyme exhibited %PCE values and mean frequencies of MNPCE that were not statistically different from those seen in concurrent vehicle controls for all dose groups. The group mean MNPCE frequencies observed were similar to concurrent vehicle controls for all dose groups and were also within the laboratory's historical control data set. The study was considered inconclusive, because it was not carried out at the maximum tolerated dose, expressed as TOS, and no evidence of bone marrow exposure was provided.

3.4.1.4. Conclusions on the genotoxicity assessment

The Panel concluded that the *in vitro* clastogenic potential of the food enzyme could not be ruled out by the *in vivo* micronucleus test. The applicant was requested to provide an *in vitro* micronucleus test according the OECD test guideline 487 in order to rule out the concern for clastogenic damage and to investigate the potential for aneugenicity, but such a study was not provided.

Overall, considering the limitation of the bacterial gene mutation test, the lack of the requested *in vitro* micronucleus assay and the inconclusive result of the available *in vivo* micronucleus test, the Panel concluded that the data available were insufficient to evaluate the genotoxicity of the food enzyme.

3.4.2. Repeated dose 28-day oral toxicity study in rats

The repeated dose 28-day oral toxicity study was performed in accordance with the OECD Test Guideline 407 (OECD, 2008) and following GLP.[22] Groups of five male and five female Sprague–Dawley

[20] Technical dossier/Appendix D3.
[21] Technical dossier/Appendix D2.
[22] Technical dossier/Appendix G.

(Crl:CD(SD)) rats received by gavage the food enzyme in doses of 500, 1,000 and 2,000 mg/kg bw per day, corresponding to 10.5, 103 and 206 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

A statistically significant decrease in feed consumption was observed in mid-dose males on days 27–28 (−18%).

The haematological investigation revealed a statistically significant decrease in the percentage of neutrophils (−48%) and an increase in the percentage of lymphocytes (+11%) in mid-dose females.

The clinical chemistry investigation revealed a statistically significant increase in γ-glutamyl transpeptidase (+67%) and chloride (+2%) in high-dose males.

In the urinalysis, the total excretion was statistically significantly decreased in males for sodium (low and high dose, −23%, −29%), potassium (all dose groups, −21%, 24%, 24%) and chloride (all dose groups, −19%, 21%, 23%).

There was a statistically significant increase in the absolute (+51%) and relative (+54%) thymus weight in low-dose females.

The Panel considered these changes as not toxicologically relevant due to the absence of a dose–response relationship (feed consumption, neutrophils, lymphocytes, sodium, potassium, thymus weight) and since the changes were only observed in one sex (all changes).

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

The Panel identified the no observed adverse effect level (NOAEL) of 206 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Repeated dose 90-day oral toxicity study in rats

The repeated dose 90-day oral toxicity study was performed following GLP.[23] One group of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in a dose of 2,000 mg/kg bw per day, corresponding to 206 mg TOS/kg bw per day. Controls received the vehicle (water for injection). Only one dose group was included in this study, which is a major deviation from OECD TG 408 (OECD, 1998) that requires at least three dose levels and a concurrent control, except when a limit test is conducted. The study provided did not comply with the requirement of a limit test to be performed with a dose of 1,000 mg/kg bw per day expressed as TOS. Despite requested in line with the EFSA CEF Panel 'Guidance on the Submission of a Dossier on Food Enzymes' from 2009, the applicant did not provide a repeated dose 90-day oral toxicity study performed with the minimum of three doses.

3.4.4. Allergenicity

The allergenicity assessment considers 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 *Aspergillus* strain GD-FAL 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.[24]

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 (Muir et al., 1997; Bernstein et al., 1999; 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). Two case reports describing allergic reactions (swollen throat, shortness of breath and difficulty in swallowing) following ingestion of lactase pills, and confirmation by antigen challenge, have been reported (Binkley, 1996; Voisin and Borici-Mazi, 2016).

[23] Technical dossier/Appendix I.
[24] Technical dossier/Additional information September 2022/Appendix L.

[redacted] a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[25]), is used as a raw material 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 are present in the food enzyme.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is added to milk and intended to be used for lactose degradation in milk processing at an intended use level up to 7,500 ALU/kg lactose.[26] Considering that cow's milk contains about 5% lactose, this corresponds to 3.1 mg TOS/kg milk.

The hydrolysis of lactose in milk releases D-galactose and D-glucose. No separation step is applied to remove the enzyme from the final foods: the lactose-reduced milk and milk products.[27]

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 provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEF 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).

In the EU, milk and dairy products are widely consumed by consumers of all age groups. The prevalence of lactose malabsorption has been reported to range from 19% to 37% in western, southern and northern Europe (Stöcker et al., 2016). Symptoms of lactose malabsorption (i.e. lactose intolerance) are generally avoided by limiting or avoiding foods and drinks that contain lactose. Consequently, lactose-intolerant individuals, who opt to consume dairy products will chose products with very low lactose content (either naturally low or with reduced content). Lactose-reduced milk is readily available in the EU, however, other lactose-reduced products, such as cheese, quark and yoghurt, are available to a much lesser degree. The Comprehensive Database currently does not provide sufficient detail to estimate food intake specifically for lactose-intolerant population groups. Exposure was therefore estimated based on the assumption that lactose-intolerant people may exert similar consumption patterns of dairy products as non-lactose-intolerant population groups. However, to reflect on the relative low availability of lactose-reduced dairy products other than milk, two scenarios were calculated.

The first scenario (A) assumes that all milk and dairy products are lactose-reduced due to enzyme treatment. The second scenario (B) considers all milk as lactose-reduced, but assumes that only a fraction of other dairy products are lactose-reduced due to the enzyme treatment. Scenario A is more

[25] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[26] Technical dossier/p. 27 and Additional information September 2022.

[27] Technical dossier/p. 26.

conservative than scenario B. The selection of the relevant milk and milk products and the technical factors applied were subject to a public consultation.[28]

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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). Under scenario A, the highest dietary exposure to the food enzyme–TOS was estimated to be about 0.301 mg TOS/kg bw per day for the 95th percentile in young children below 1 year of age. Under scenario B, the highest dietary exposure to the food enzyme–TOS was estimated to be about 0.22 mg TOS/kg bw per day for the 95th percentile in toddlers. The shift of age group seen under these two scenarios reflects the expansion of food items by age in young children.

Table 2: 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
Scenario A – all milk and dairy products consumed are enzymatically lactose-reduced products						
Min–max mean (number of surveys)	0.005–0.091 (11)	0.049–0.123 (15)	0.049–0.104 (19)	0.015–0.046 (21)	0.011–0.025 (22)	0.008–0.022 (22)
Min–max 95th (number of surveys)	0.029–0.301 (9)	0.146–0.264 (13)	0.095–0.181 (19)	0.037–0.096 (20)	0.027–0.056 (22)	0.025–0.049 (21)
Scenario B – all milk and a fraction of dairy products consumed are enzymatically lactose-reduced products						
Min–max mean (number of surveys)	0.001–0.046 (11)	0.002–0.088 (15)	0.017–0.081 (19)	0.001–0.030 (21)	0.001–0.011 (22)	0.001–0.010 (22)
Min–max 95th (number of surveys)	0.004–0.146 (9)	0.059–0.220 (13)	0.048–0.143 (19)	0.002–0.063 (20)	0.003–0.036 (22)	0.010–0.025 (21)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
	Exposure to food enzyme–TOS
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+

[28] https://www.efsa.europa.eu/en/call/call-input-data-exposure-assessment-food-enzymes-7th-call

Sources of uncertainties	Direction of impact
	Exposure to food enzyme–TOS
Inclusion of semi-soft and soft cheeses that are ripened normally less than 3 months	+
Use of recipe fractions in disaggregating FoodEx categories likely to contain the food enzyme	+/–
Use of technical factors in the exposure model	+/–

+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.

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

3.6. Margin of exposure

As the concern for genotoxicity could not be excluded and in the absence of an appropriate test for systemic toxicity, no margin of exposure was calculated.

4. Conclusions

Based on the results of the genotoxicity tests, the inadequacies of the 90-day repeated dose oral toxicity study in rats, and the missing data regarding the absence of viable cells of the production strain in the food enzyme, the Panel could not conclude on the safety of the food enzyme β-galactosidase produced with the *Aspergillus* strain GD-FAL.

5. Documentation as provided to EFSA

Application of authorisation of β-galactosidase (Lactase) preparation produced by *Aspergillus oryzae*. January 2021. Submitted by Godo Shusei Co., Ltd.

Additional information. September 2022. Submitted by Godo Shusei Co., Ltd.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNPCE	micronucleated bone marrow polychromatic erythrocytes

OECD	Organisation for Economic Cooperation and Development
PCE	polychromatic erythrocytes
PCR	polymerase chain reaction
RPD	relative population doubling
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organisation

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.2022.7677#support-information-section).

The file contains two sheets, corresponding to two tables

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 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).