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Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified *Aspergillus oryzae* strain GL 470

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Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the *Aspergillus oryzae* strain GL 470 by Shin Nihon Chemical Co., Ltd. The food enzyme is free from viable cells of the production organism. It is intended to be used in five food manufacturing processes; lactose hydrolysis in milk processing, production of fermented milk products, whey processing, manufacture of enzyme-modified dairy ingredients and in the manufacture of galacto-oligosaccharides. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 1.388 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 repeated dose 90-day oral toxicity studies in rats. The Panel identified a no observed adverse effect level of 7,000 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 5,043. 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 concluded 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 considered to be low. Based on the data provided and considering the most recent complete toxicological data set, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, lactase, β-galactosidase, β-D-galactoside galactohydrolase, EC 3.2.1.23, *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:

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Five applications have been introduced by the applicant "Intertek Scientific & Regulatory Consultancy" for the authorization of the food enzymes 3-Phytase from *Aspergillus niger* (strain PHY93-08), Alpha-amylase from *Aspergillus niger* (strain A 29–286), Invertase and Exo-beta-glucosidase from *Aspergillus niger* (strain IN 319), Alpha-galactosidase from *Aspergillus niger* (strain AGS614) and Lactase from *Aspergillus oryzae* (strain GL 470).

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 Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the following food enzymes 3-Phytase from *Aspergillus niger* (strain PHY93-08), Alpha-

[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.

amylase from *Aspergillus niger* (strain A 29–286), Invertase and Exo-beta-glucosidase from *Aspergillus niger* (strain IN 319), Alpha-galactosidase from *Aspergillus niger* (strain AGS614) and Lactase from *Aspergillus oryzae* (strain GL 470) 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 lactase (β-galactosidase) from *A. oryzae* strain GL 470.

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 *Aspergillus oryzae* strain GL 470.

Additional information was requested from the applicant during the assessment process on 7 July 2020 and 19 February 2021 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the 'EFSA Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant existing guidance documents of 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 generation of D-galactose and D-glucose. The enzyme can also show *trans*-galactosylation activity. One lactose molecule is hydrolysed to galactose and glucose, while a second lactose molecule acts as recipient for *trans*-galactosylation, resulting in the formation of a trisaccharide. Higher molecular mass galacto-oligosaccharides (GOS) are also produced as the reaction proceeds. The food enzyme under application is intended to be used in five food manufacturing processes: lactose hydrolysis in milk processing, production of fermented milk products, whey processing, manufacture of enzyme modified dairy ingredients and in the manufacture of GOS.

3.1. Source of the food enzyme

The β-galactosidase is produced with the non-genetically modified filamentous fungus *Aspergillus oryzae* strain GL 470, which is deposited at the Centre for Agriculture and Biosciences International Genetic Resource Collection (CABI, United Kingdom), with the deposit number SD141.[4] The strain is a wild-type isolated from food. It was identified as *A. oryzae* by sequence analysis of the 5.8S rDNA (ITS), partial β-tubulin (*benA*) and partial calmodulin (*cam*) genes.[5]

[4] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex I.2.
[5] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex I.4.

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 in a solid state fermentation system with conventional process controls in place. After completion of the fermentation, the enzyme is extracted [redacted] and the biomass is then removed by centrifugation/filtration leaving a filtrate containing the food enzyme. The filtrate 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.[9,10]

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, derived from the amino acid sequence, was calculated to be 110 kDa.[12] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[13] A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 116 kDa, consistent with the expected mass of the enzyme.[14] The protein profile also included bands of lower staining. No other enzymatic activities were reported.[15]

The in-house 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.[16]

The food enzyme has a temperature optimum around 55°C (pH 4.5) and a pH optimum around pH 5.0 (37°C). Thermostability was tested by pre-incubation of the food enzyme for 15 min at different temperatures (pH 4.5). Enzyme activity decreased above 50°C, showing no residual activity above 65°C.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation[18] and three batches produced for the toxicological tests[19,20] (Table 1). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 11.4% and the mean enzyme activity/TOS ratio is 183 U/mg TOS.

[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/ Additional info January 2021/Attachment B.
[8] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 33–35.
[9] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex III.
[10] Technical dossier/ Additional info January 2021.
[11] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 15–16.
[12] Technical dossier/Additional information January 2021.
[13] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 17.
[14] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 15–17.
[15] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 25.
[16] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 20–21 and Annex II.1.
[17] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 21–24.
[18] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 36–38 and Annex IV and annex V.
[19] Technical dossier/spontaneous data submission March 20/CD-rom 2/ Annex IV and V.
[20] Technical dossier/Additional information January 2021/Attachment D.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches					
		1	2	3	4[(a)]	5[(b)]	6[(c)]
β-Galactosidase activity	ALU/mL batch[(d)]	20,100	20,600	21,800	21,000	59,400	21,900
Protein	%	10.5	9.5	11.0	11.0	21.8	9.4
Ash	%	0.4	0.5	0.3	0.5	0.2	0.3
Water	%	87.9	88.2	88.6	87.4	67.1	88.7
Total organic solids (TOS)[(e)]	%	11.7	11.3	11.1	12.1	32.7	11.0
Activity/mg TOS	ALU/mg TOS	172	182	196	174	165	199

(a): Batch used for the first bacterial reverse mutation test, first chromosomal aberration test and first repeated dose 90-day oral rat study (Section 3.4.2.1).
(b): Batch used for the second reverse mutation test, second chromosomal aberration test, comet assay and the third repeated dose 90-day oral rat study (Section 3.4.2.3).
(c): Batch used for the second repeated dose 90-day oral rat study (Section 3.4.2.2).
(d): ALU: lactase units (see Section 3.3.1).
(e): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in one of the batches used for toxicological studies was below 0.05 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 detection (LoD) of the employed method.[21,22]

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).[23] No antimicrobial activity was detected in any of the tested batches.[24]

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 aflatoxin B1, B2, G1 and G2, ochratoxin A, sterigmatocystin, T2-toxin and zearalenone was examined in three food enzyme batches. All were below the limit of quantification (LoQ) of the applied analytical methods.[25,26] 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

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 was added to 40 mL of sterile water and five times 1 mL of this solution was plated on five non-selective agar plates and incubated at 30°C for 6 days with an intermediate check after 3 days. No colonies were produced. A positive control was included.[27]

3.4. Toxicological data

Toxicological tests included two bacterial gene mutation assays (Ames tests), two *in vitro* mammalian chromosomal aberration tests, a comet assay and three repeated dose 90-day oral toxicity studies in rats. Batches 4, 5 and 6 (Table 1) used in these studies are all considered suitable as test items.

[21] LoDs: Pb = 0.05 mg/kg; As = 0.1 mg/kg.
[22] Technical dossier/Additional information January 2021/Attachment C.
[23] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 36 and Annex IV.1.
[24] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 36 and Annex IV.1 and IV.2.
[25] LoQ: aflatoxins B1, B2, G1 and G2 = 0.5 μg/kg each; ochratoxin A = 0.5 μg/kg; zearalenone = 50 μg/kg; sterigmatocystin = 20 μg/kg; T2-toxin = 50 μg/kg and 100 μg/kg.
[26] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 38 and Annex IV.3 and I.3.
[27] Technical dossier/Additional information March 2021/Attachment A.

3.4.1. Genotoxicity

3.4.1.1. *In vitro* genotoxicity studies

3.4.1.1.1. Bacterial reverse mutation test (with batch 4)

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1997) with limitations and following Good Laboratory Practice (GLP).[28]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) were used in the presence or absence of metabolic activation (S9-mix) using the 'treat and wash' method. A preliminary toxicity test was carried out in triplicate with five concentrations of the food enzyme from 100 to 10,000 μg/plate (corresponding to 12.1, 39.9, 121, 399, 1,210 μg TOS/plate). No evidence of cytotoxicity was seen at any concentration.

A main experiment and a confirmatory study were carried out in triplicate at five concentrations of the food enzyme from 100 to 10,000 μg/plate (corresponding to 12.1, 39.9, 121, 399, 1,210 μg TOS/plate). No increase in revertant colony numbers was observed in any of the strains tested either in the presence or absence of S9-mix. The study was evaluated as negative, but of insufficient reliability due to the use of a limited battery of bacterial strains.

3.4.1.1.2. Bacterial reverse mutation test (using batch 5)

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

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation and 'treat and wash' method.

A preliminary study was carried out using the pre-incubation method with five concentrations of the food enzyme (3.5, 35, 350, 3,500 and 35,000 μg TOS/plate). An increase in revertant colony numbers was observed in *S*. Typhimurium TA100, TA1535, TA98 and TA1537 in the absence of S9-mix and in strain TA1535 also in the presence of S9-mix at the highest concentration tested (35,000 μg TOS/plate). At this concentration, an acceleration of the growth of background bacteria was observed in all strains in the absence and presence of S9-mix, considered to be caused by the presence of free amino acids in the food enzyme.

A dose-finding study was carried out applying the preincubation method in triplicate using six concentrations of the food enzyme (144, 432, 1,300, 3,890, 11,700 and 35,000 μg TOS/plate). An increase in revertant colony numbers two times or more than in the negative controls was observed in *S*. Typhimurium TA98 and TA1537 strains in the absence of S9-mix and in strains TA1535 and TA98 in the presence of S9-mix. An acceleration of the growth of background bacteria was observed at 11,700 and 35,000 μg TOS/plate in the absence and presence of S9-mix. A 'treat and wash' method was applied with *S*. Typhimurium TA98 and TA1537 in the absence of S9-mix and the strains TA1535 and TA98 in the presence of S9-mix at six concentrations of the food enzyme (144, 432, 1,300, 3,890, 11,700 and 35,000 μg TOS/plate). The number of revertants was less than two times that of the negative controls. The increases observed with the preincubation method were attributed to a histidine feeding effect considering that the test article contains a small amount of histidine.

The main study was performed in triplicate with the pre-incubation and the 'treat and wash' method. The preincubation method was applied using five concentrations of the food enzyme (2,190, 4,380, 8,750, 11,700 and 35,000 μg TOS/plate) in *S*. Typhimurium TA100, TA1535 and *E. coli* WP2uvrA in the absence of S9-mix and in *S*. Typhimurium TA100, TA1537 and *E. coli* WP2uvrA in the presence of S9-mix. An acceleration of the growth of background bacteria was observed at 11,700 and 35,000 μg TOS/plate in the absence and presence of S9-mix. The 'treat and wash' method was applied with *S*. Typhimurium TA98 and TA1537 strains treated at six concentrations of the food enzyme (1,090, 2,190, 4,380, 8,750, 17,500 and 35,000 μg TOS/plate) in the absence of S9-mix and with *S*. Typhimurium TA1535 and TA98 strains exposed to five concentrations of the food enzyme (2,190, 4,380, 8,750, 17,500 and 35,000 μg TOS/plate) in the presence of S9-mix. Growth inhibition of background bacteria was observed at 35,000 μg TOS/plate in the absence of S9-mix. No significant increase in revertant colony numbers above the control values was observed in any strain with or without S9-mix.

[28] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI. 6.
[29] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI. 5.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.1.1.3. In vitro *mammalian chromosomal aberration test (using batch 4)*

The *in vitro* mammalian chromosomal aberration test was carried out in cultured human peripheral lymphocytes according to recommendation of the United Kingdom Environmental Mutagen Society Sub-Committee on Guidelines for Mutagenicity Testing (Scott et al., 1990) and following GLP. Two separate experiments were carried out in duplicate.

Based on the results of a preliminary test, in the first experiment the cell cultures were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (151.3, 302.5 and 605 μg TOS/mL) for 20 h in the absence of metabolic activation (S9-mix) and for 3 h followed by 17 h recovery period in the presence of S9-mix. Cytotoxicity, measured as a reduction of the mitotic index to 59.5% and 57% of the control values, was observed at 5,000 μg/mL in the absence and presence of S9-mix, respectively. The frequency of structural chromosomal aberrations in treated cultures was comparable to the values detected in negative controls. In the second experiment, the cells were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 151.3, 302.5 and 605 μg TOS/mL) in the presence of S9-mix for 3 h followed by a 17-h recovery period and at 5,000 μg/mL (corresponding to 605 μg TOS/mL) for 3 h followed by a 41-h recovery period. In the absence of S9-mix, the cells were exposed continuously to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 151.3, 302.5 and 605 μg TOS/mL) for 20 h and at 5,000 μg/mL (corresponding to 605 μg TOS/mL) for 44 h. A statistically significant increase in the frequency of structural chromosomal aberrations was observed after short-term treatment at the concentration of 1,250 μg/mL (corresponding to 151.3 μg TOS/mL) with S9-mix. As this finding was limited to the lowest concentration and to one of the experiments, it was considered not to be biologically relevant.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study. However, the study had some limitations (e.g. short-term treatment experiments without S9-mix, data on historical controls were not provided and only 100 metaphases for each set of slides were scored for chromosomal aberrations).

3.4.1.1.4. In vitro *mammalian chromosomal aberration test (using batch 5)*

An *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 2014) and following GLP.[28] in Chinese hamster lung fibroblast cell lines (CHL/IU) with and without metabolic activation (S9-mix).

The cell growth inhibition test was performed at concentrations of 1,094, 2,188, 4,375, 8,750, 17,500 and 35,000 μg TOS/mL. No inhibition of cell growth by 50% (IC_{50}) was observed in a short-term treatment (6 h + 18 h recovery time) with or without S9-mix. The IC_{50} for a 24-h continuous treatment was calculated to be 27,333 μg TOS/mL. Based on these results, the cells were exposed to the food enzyme at 8,750, 17,500 and 35,000 μg TOS/mL in the short-term treatment with or without S9-mix, and at 4,375, 8,750, 17,500 and 35,000 μg TOS/mL in a 24-h continuous treatment in the absence of S9-mix.

After the short-term treatment, no inhibition of cell growth was observed and the frequencies of structural and numerical chromosomal aberrations were comparable to the values observed in negative controls and within the range of the historical control data. After 24-h continuous treatment, the relative cell growth rate at 17,500 μg TOS/mL was 48.6%. Cell cultures were scored for chromosomal aberrations at 4,375, 8,750 and 17,500 μg TOS/mL. A statistically significant, concentration-dependent increase in the structural chromosomal aberrations was recorded at concentrations of 8,750 and 17,500 μg TOS/mL (2.7% and 3.3% vs 0.3% in the control); however, these values were within the historical control range (0.0% to 4.7%). Therefore, a confirmatory study was conducted in which the cells were exposed to the concentrations of 2,500, 5,000, 8,750 and 17,500 μg TOS/mL for 24 h in the absence of S9-mix. A statistically significant increase in the structural chromosomal aberrations was observed at 17,500 μg TOS/mL (3% vs 0.3% in the control), however, within the range of historical control data. No evidence of an increase in polyploid cells was recorded.

The Panel concluded that the food enzyme did not induce chromosome aberrations in short-term treatment in the presence or absence of S9-mix. The results obtained in continuous treatment in the absence of S9-mix were considered equivocal.

3.4.1.2. *In vivo* genotoxicity studies

3.4.1.2.1. Alkaline Comet assay (using batch 5)

An *in vivo comet* assay with the food enzyme β-galactosidase from *A. oryzae* strain GL 470 was carried out with tissue from the glandular stomach and duodenum of rats to assess the DNA damage at the site of first contact. The study was conducted according to OECD Test Guideline 489 (OECD, 2016) and following GLP.[30]

In the main study, six male Sprague–Dawley (Crl:CD(SD)) rats in each group were dosed once daily by oral gavage with 1,750, 3,500 and 7,000 mg TOS/kg body weight (bw) per day for two consecutive days at 21-h intervals. The glandular stomach and duodenum were collected 3 h after the last treatment.

No mortality, changes in the body weight, body weight gain, treatment-related clinical signs or pathological–anatomical changes in the glandular stomach and duodenum were observed in any animal group. Five animals were processed for the comet analysis.

Measurements of tail intensity (% DNA in tail) were obtained from 150 cells/animal. No statistically significant increase in mean tail intensity values for animals treated with food enzyme were observed in the duodenum and glandular stomach of any treated group compared to the concurrent vehicle control group. Similarly, no differences in the frequencies of hedgehogs were observed.

The Panel concluded that the food enzyme did not induce DNA damage in the duodenum and glandular stomach of rats, administered via oral gavage.

3.4.1.3. Conclusions on genotoxicity studies

Two sets of studies carried out with different batches of food enzyme were evaluated for genotoxicity. The first set of studies, made with batch 4 (Table 1), consisting of an Ames test and an *in vitro* chromosomal aberration test, were of limited value due to limitations in the experimental protocols. However, these studies reported negative results for gene mutations in bacteria and for chromosomal aberrations in mammalian cells up to the concentrations inducing 50% cytotoxicity, as recommended by the OECD TG 473.

The second set of studies carried out with batch 5 (Table 1) including an Ames test, an *in vitro* chromosomal aberration test and an *in vivo* Comet assay perfectly complied with the requirements of the OECD guidelines. No increase of bacterial gene mutations was reported with the Ames test and equivocal results were obtained with structural chromosomal aberrations at the highest concentrations tested in the absence of S9-mix. As follow-up for the clastogenic effects observed *in vitro* an *in vivo* comet assay at the site of first contact (glandular stomach and duodenum) was carried out in rats at up to 7,000 mg TOS/kg and provided clear negative results.

On the basis of the results of the *in vitro* and *in vivo* studies, the Panel concludes that there is no concern for genotoxicity for the food enzyme tested.

The Panel noted the large difference in terms of cytotoxicity between the two batches evaluated. The range of concentrations, expressed as TOS/mL, tested in the *in vitro* chromosomal aberration studies were clearly different (from 151.3 to 605 μg TOS/mL for batch 4 and from 4,375 to 17,500 μg TOS/mL for batch 5). The concentration needed to obtain 50% cytotoxicity for 24 h continuous treatment with batch 4 (i.e. 605 μg TOS/mL) was about 29-fold lower than that for batch 5 (17,500 μg TOS/mL). However, a direct comparison could not be done because different cells were used in the two studies (e.g. peripheral human lymphocytes for batch 4 and Chinese hamster lung fibroblast for batch 5).

3.4.2. Subchronic toxicity

3.4.2.1. Repeated dose 90-day oral toxicity study in rodents (using batch 4, first study)

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1981) and following GLP.[31] Groups of 20 male and 20 female Sprague–Dawley rats received by gavage the food enzyme in doses corresponding to 97.7, 195.4 and 977 mg TOS/kg bw per day. Controls received the vehicle (distilled water).

No mortality was observed.

[30] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI. 7.
[31] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI.3.

The body weight was statistically significantly increased on days 10, 14, 17, 21, 24, 42 and 49 of administration in mid-dose females (+3% on average) and on days 7, 10, 17, 21, 38, 42, 59, 84 and 90 of administration in high-dose females (+3% on average). The Panel considered these changes as not toxicologically relevant as there were only recorded sporadically, they were only observed in one sex and the changes were small.

The feed consumption was statistically significantly increased on days 7–10 in mid- and high-dose females (+11% and +9%, respectively), decreased on days 59–63 in mid- and high-dose females (5% and 6%, respectively) and decreased on days 66–70 in the high-dose females (−3%). The Panel considered these changes as not toxicologically relevant as there were only recorded sporadically, they were only observed in one sex, the changes were small and there were no toxicologically relevant changes in the body weight.

The haematological investigation revealed a statistically significant decrease in absolute monocyte count (−15%) in high-dose females, a decrease in in eosinophils in low-dose males (−21%), a decrease in mean cell volume (MCV) in mid-dose males (−1%) and a decreased prothrombin time (PT) in high-dose males (−3%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship (except for PT and monocytes), the changes were small and there were no changes in other relevant parameters (i.e. for monocytes in the total white blood cell count; for MCV in the total red blood cell count and haematocrit; for PT in other blood coagulation parameters).

The clinical chemistry investigation revealed a statistically significant increase in alkaline phosphatase (ALP) (+16%) and urea levels (+10%), and a decrease in albumin/globulin (A/G) ratio (−6%) in high-dose males, an increase in gamma glutamyl transferase (GGT) levels in mid-dose males (+161%), and a decrease in potassium levels (K^+) in mid-dose females (−8%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship (GGT and K^+) and the change in ALP was small and not associated with changes in other hepatobiliary parameters (i.e. alanine aminotransferase, aspartate aminotransferase or bilirubin). The Panel considered that the changes in urea and A/G ratio could be related to histopathological changes in the kidney.

Statistically significant changes in organ weights included increases in absolute and relative weights of full caecum in low-dose males (+25% and +23%, respectively) and in high-dose males (+23% and +23%, respectively) and of mesenteric lymph nodes in low-dose males (+26% and +23%, respectively) and high-dose males (17% and +19%, respectively), decreases in relative weights of the liver (−5%) and testes (−4%) in the low-dose males, decreases in absolute weights of mesenteric lymph nodes in low- and mid-dose females (−16% and −15%, respectively), in relative weights of mesenteric lymph nodes in low-, mid- and high-dose females (−16%, −17% and −11%, respectively) and an increase in the absolute kidney weight in mid-dose females (+5%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (except for mesenteric lymph nodes), there was no dose–response relationship (all parameters), there was no consistency between the changes in males and females (mesenteric lymph nodes), the changes were small (liver, testes, kidneys) and there were no histopathological changes in the organs (all organs except the kidneys).

The microscopic examination revealed statistically significant differences in incidence of changes in lungs and kidneys in the treated groups compared to the control group.

In lungs, statistically significant increases were recorded in incidence of focal inflammatory cell infiltrates (13/20 vs 2/20 control) and of presence of pigment (10/20 vs 0/20 control) in low-dose males and of foci of foamy macrophages in mid-dose males (5/20 vs 0/20). The Panel considered these changes as not toxicologically relevant as there were only observed in one sex and there was no-dose response relationship. These changes could be related to unintentional deposition of small amounts of the test solution into airways during the gavage administration or regurgitation of the test solution.

Histological examination of kidneys of males revealed a statistically significant increase in the incidence of cell necrosis in the proximal tubules (0/20, 0/20, 5/20 vs 0/20 control) and of glomerular necrosis (0/20, 0/20, 8/20 vs 1/20 control) in the high-dose group and of tubular basophilia in all treated groups (11/20, 12/20 and 11/20 vs 3/20 control). The incidence of calcification in renal cortex was statistically significantly higher in mid-dose males (8/20 vs 2/20).

Histopathological changes in kidneys from the treated females were less severe than in males. Incidences of cell necrosis in the proximal tubules (0/20, 1/20, 1/20 vs 1/20) and of globular necrosis (0/20, 2/20, 4/20 vs 0/20) were not statistically significantly different from those in the control group.

Regarding tubular basophilia, a statistically significantly increased incidence was seen at the high dose (5/20, 4/20, 8/20 vs 2/20). Incidence of calcification of cortex was statistically significantly increased in the low-dose group (10/20, 8/20, 5/20 vs 3/20) and of papilla in the mid-dose group (3/20, 5/20, 1/20 vs 0/20), while no statistically significant difference was seen in the incidence of calcification in medulla in any treated female groups (11/20, 14/20 and 14/20 vs 8/20).

The Panel considered cell necrosis in proximal tubules in high-dose males as treatment-related based on statistically significantly increased incidence. The Panel noted, however, that spontaneous aetiology of cell necrosis in proximal tubules in the treated females could not be ruled out because this finding was recorded at the same single incidence in the control and mid- and high-dose females. Regarding glomerular necrosis, the Panel noted a single incidence in the control males. However, the Panel regarded glomerular necrosis in high-dose males as treatment-related considering a statistically significant increase in the incidence. The Panel also regarded glomerular necrosis in mid- and high-dose females as treatment-related because the increase in the incidence, although not statistically significant in mid- and high-dose females, appeared dose-related.

The Panel noted that tubular basophilia is a spontaneous change in rats seen more often in males than in females as a manifestation of spontaneous chronic progressive nephropathy (CPN). However, tubular basophilia can also be a manifestation of induced nephron injury in repeated dose toxicity studies (a low-grade toxicologic insult) or represent reparative processes when the cell is in recovery. The presence of tubular basophilia in some of control males and females and lack of a dose dependency in the increase of the incidence in the treated males could support a spontaneous aetiology of this finding as an early manifestation of CPN. However, a statistically significantly increased incidence in all treated male groups and in high-dose females signified a relation to the treatment with the test compound.

Finally, the Panel considered calcification in renal cortex, medulla and papilla as manifestation of nephrocalcinosis. Nephrocalcinosis is a common finding in rats. It is seen more often in females than in males. Calcification in cortex was observed not only in the treated groups of both sexes but also in some control males and females and in medulla in some control females. Despite the fact that the incidence of calcification was greater in the treated groups than in the controls, the lack of dose–response in the incidence of calcification of cortex in treated males and females, of medulla and papilla in treated females and lack of calcification of medulla and papilla in treated males indicated no relation to the treatment of this finding.

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

Overall, the Panel considered that no NOAEL could be identified in the study based on the histopathological changes in the kidneys.

3.4.2.2. Repeated dose 90-day oral toxicity study in rodents (using batch 6, second study)

In order to further investigate the kidney changes observed in the first study, a second repeated dose 90-day oral toxicity study in rats was conducted.

The lowest dose in the previous study (97.7 mg TOS/kg bw per day) was chosen as the highest dose level in this study.

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[32] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses corresponding to 50, 75 and 100 mg TOS/kg bw per day. Controls received the vehicle (distilled water for injection).

No mortality was observed.

The feed consumption was statistically significantly decreased on days 43–50 in low-dose females (−6%). The Panel considered the change as not toxicologically relevant as it was transient, it was only observed in one sex, there was no dose–response relationship and there -was no statistically significant change in the final feed consumption or in the body weight.

Haematological investigation revealed a statistically significant increase in activated partial thromboplastin time (APTT) (+13%) in high-dose males. The Panel considered the change as not toxicologically relevant as it was only observed in one sex and there were no changes in other coagulation parameters.

Clinical chemistry investigation revealed a statistically significant increase in sodium levels (+0.07%) and a decrease in albumin concentration (−6%) in mid-dose males and a decrease in blood

[32] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI.4.

urea nitrogen (BUN) levels in mid- and high-dose-females (−19%, −19%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (both parameters) and there were no changes in other relevant parameters (i.e. no changes in clinical pathology indices of liver and kidney function or in morphology of these organs).

The urinalysis revealed a statistically significant increased potassium level in mid-dose females (+1%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, there was no dose–response relationship and there were no changes in other relevant parameters (i.e. no histopathological changes in the kidney).

Statistically significant changes in organ weights included an increase in absolute kidney and seminal vesicle weights in mid-dose males (+4% and 7%, respectively), in absolute kidney (+10%) and pituitary (+14%) and in absolute and relative ovary weights (+22% and +19%, respectively) in low-dose females and an increase in relative liver weight in high-dose females (+6%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no consistency between males and females concerning a dose level at which the changes occurred (kidney), there was no dose–response relationship (all parameters except liver), the change was small (liver) and there were no histopathological correlations (all parameters).

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

The Panel identified a no observed adverse effect level (NOAEL) of 100 mg TOS/kg bw per day, the highest dose tested.

3.4.2.3. Repeated dose 90-day oral toxicity study in rodents (using batch 5)

As no adverse effects on kidneys were observed in the second study, a third repeated oral 90-day toxicity study was conducted in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[33] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses corresponding to 778, 2,333 and 7,000 mg TOS/kg bw per day. Controls received the vehicle (water). No mortality was observed.

In functional observations, a statistically significant decrease in hindlimb grip strength on day 89 in high-dose males (−26%) and an increase in forelimb grip strength in mid-dose females (+34%) were observed. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (both parameters) and there was no dose–response relationship (forelimb grip strength).

The feed consumption was statistically significantly decreased in week 12 in high-dose females (−12%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, it was transient and there were no statistically significant changes in the body weight or body weight gain.

The haematological investigation showed a statistically significant increase in partial thromboplastin time (APTT) (+13%) in high-dose females. The Panel considered the change as not toxicologically relevant as it was only observed in one sex, there were no changes in other coagulation parameters and the changes were within the historical control values.

Clinical chemistry investigation revealed statistically significant increases in concentrations of total cholesterol (+24%), BUN (+33%), α-2 globulin (+12%), β-globulin (+10%) and β-globulin ratio (+8%) in high-dose males, an increase in concentrations of chloride (+2%) and a decrease in concentration of calcium (−4%) in high-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), the changes were small (chloride, calcium) and there were no changes in other relevant parameters (i.e. for α-2 globulin, in absence of alteration of total protein concentration or any overt clinical pathology signs of inflammation; for BUN, in absence of any changes in creatinine concentration and of histopathological changes in kidneys).

The urinalysis revealed a statistically significant decrease in chloride (−38%) and sodium (−38%) concentrations in low-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters) and there was no dose–response relationship.

Statistically significant changes in organ weight included a statistically significant increase in the relative liver weight in high-dose males (+13%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and the change was small and there were no histopathological changes in the liver.

[33] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/Annex VI.8.

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

The Panel identified a no observed adverse effect level (NOAEL) of 7,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Conclusions on toxicity studies

The Panel noted that batch 4 caused 50% cytotoxicity at a 29-fold lower concentration in the first *in vitro* mammalian chromosomal aberration test compared to batch 5 in the second *in vitro* mammalian chromosomal aberration test. Similarly, batch 4 was associated with adverse effects on kidneys such as cell necrosis in the proximal tubules and glomerular necrosis in high-dose males (incidence of both changes was statistically significantly higher than in the control males), glomerular necrosis in mid- and high-dose females (incidence was not statistically significantly higher than in the control females) and tubular basophilia in treated males and females (incidence in low-, mid- and high-dose males and in high-dose females was statistically significantly higher than in the control group) at doses sevenfold smaller (for necrotic changes) and 70-fold smaller (for tubular basophilia) than the high dose of batch 5 (that did no cause adverse effects in the third repeated dose 90-day oral toxicity study).

The Panel considered that the apparent differences in cytotoxicity *in vitro* and the differences in toxicity *in vivo* between batches 4 and 5 cannot be explained with the available data on composition (Table 1). These data also cannot explain the occurrence of tubular basophilia at the low dose in the first repeated dose 90-day oral toxicity study with batch 4 and lack of histopathological changes in the kidneys at the same dose in the second repeated dose 90-day oral toxicity study with batch 6. Therefore, uncertainty associated with the toxicity manifested by batch 4 in the studies submitted by the applicant remains. Since the available data do not allow the identification of the origin of the cytotoxicity in *in vitro* studies and nephrotoxicity in *in vivo* studies with batch 4, and since the results with batch 6 indicated that the NOAEL for the enzyme–TOS could be substantially higher than the highest dose tested in the second repeated dose 90-day oral toxicity study, the Panel considered it appropriate to apply a NOAEL from the most recent 90-day oral toxicity study with batch 5 (for which the complete battery of *in vitro* studies and *in vivo* comet assay were also submitted) for the risk assessment of the food enzyme i.e. for the calculation of the margin of exposure (MOE).

3.4.4. 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 the *A. oryzae* strain GL 470 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.[34]

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).

Wheat bran, a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[35]) is used as a raw material. However, during the fermentation process, this product 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 potentially

[34] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/p. 66–69 and Annex VII.

[35] 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.

allergenic residues of this food employed as a protein source is not expected to be present. This was supported by the analysis of six batches of the enzyme by an enzyme-linked immunosorbent assay (ELISA), that indicated the absence of wheat proteins (at a detection limit of 1 mg/kg).[36]

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

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

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

Food manufacturing process(a)	Raw material (RM)	Recommended use level (mg TOS/kg RM)(b)
Lactose hydrolysis in milk processing	Milk	**13.1**
Production of fermented milk products	Milk	**13.1**
Whey processing	Liquid whey	**13.1**
Manufacture of enzyme-modified dairy ingredients	Cheese curd or slurry	**13.1**
Manufacture of galacto-oligosaccharides	Lactose	**670**

TOS: total organic solids.
(a): The name has been harmonised 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): Additional data January 2021.

Schematic diagrams were provided to show the point of addition of the food enzyme during the various food manufacturing processes. Milk or whey can be treated with this food enzyme for the hydrolyses of lactose to release glucose and galactose. The treatment makes milk more suitable for lactose-intolerant individuals and sweeter. Treating milk before coagulation can be used to produced lactose-reduced cheese. Treating the curd or cheese slurry would result in enzyme-modified cheese. Adding β-galactosidase together with microbial cultures during fermentation can be used to produce lactose-reduced yoghurt. Treatment of the cheese whey or whey permeate would result in lactose-reduced and sweeter whey syrups. No separation step is applied to remove the food enzyme–TOS from the treated milk, fermented milk products, enzyme modified cheese or whey syrup.[37]

When the food enzyme is added to whey permeate or to lactose, due to the high concentration of lactose, the β-galactosidase will react with the lactose to produce GOS by transglycosylation. Ion exchange chromatography applied after the enzymatic treatment is expected to remove the enzyme TOS in the GOS products.[38] However, the applicant did not provide analytical data to establish the extent of removal. Consequently, the dietary exposure to the food enzyme TOS via this food manufacturing process cannot be waived (EFSA CEP Panel, 2021a).

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

[36] Technical dossier/spontaneous data submission March 20/CD-rom 2/technical dossier/pp. 68–69.
[37] Additional data January 2021/Attachment E1, E2, E3 and E4.
[38] Additional data January 2021/Attachment E5 & Additional data March 2021.

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 1.388 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)	0.190–0.566 (11)	0.063–0.639 (15)	0.104–0.422 (19)	0.022–0.157 (21)	0.020–0.070 (22)	0.010–0.064 (22)
Min–max 95th percentile (number of surveys)	0.636–1.388 (9)	0.572–1.192 (13)	0.230–0.698 (19)	0.071–0.328 (20)	0.063–0.195 (22)	0.064–0.150 (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 a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (7,000 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.010–0.639 mg TOS/kg bw per day at the mean and from 0.063–1.388 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure of at least 5,043.

4. Conclusions

Based on the data provided and considering the most recent complete toxicological data set and the derived margin of exposure, the Panel concluded that the food enzyme β-galactosidase from *A. oryzae* strain GL 470 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Application for the Authorisation of Lactase (β-galactosidase) from *Aspergillus oryzae* Strain GL 470 as a Food Enzyme in the European Union. February 2020. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. January 2021. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. March 2021. Submitted by Shin Nihon Chemical Co., Ltd.

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Abbreviations

A/G	albumin/globulin
ALP	alkaline phosphatase
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
CPN	chronic progressive nephropathy
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
LoQ	limit of quantification
MCV	mean cell volume
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PT	prothrombin time
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
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.2022.7572#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).