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Safety evaluation of the food enzyme β-galactosidase from the genetically modified *Aspergillus niger* strain TOL

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Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the genetically modified *Aspergillus niger* strain TOL by DSM Food Specialties B.V. The genetic modifications did not give rise to safety concerns. The food enzyme was considered free from viable cells of the production organism and recombinant DNA. The food enzyme is intended to be used in whey processing. Dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 0.197 mg TOS/kg body weight (bw) per day in European populations. The toxicity studies were carried out with an asparaginase from *A. niger* strain ASP. The Panel considered this food enzyme as a suitable substitute for the β-galactosidase to be used in the toxicological studies, because the genetic differences between the production strains are not expected to result in a different toxigenic potential and the raw materials and manufacturing processes of both food enzymes are comparable. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,038 mg TOS/kg bw per day, the highest dose tested. This results in a margin of exposure of at least 5,269. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, β-Galactosidase, β-D-galactoside galatohydrolase, EC 3.2.1.23, lactase, *Aspergillus niger*, genetically modified microorganism

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

Three applications have been introduced by the companies 'Takabio' and 'DSM Food Specialties B.V.' for the authorisation of the food enzymes Microbial Rennet from *Rhizomucor miehei*, Acid prolyl endopeptidase from a genetically modified strain *Aspergillus niger* (strain GEP) and Beta-galactosidase from a genetically modified strain *Aspergillus niger* (strain TOL).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three applications fall within the scope of the food enzyme 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 a safety assessments of the food enzymes Microbial Rennet from *Rhizomucor miehei*, Acid prolyl endopeptidase from a genetically modified strain *Aspergillus niger* (strain GEP) and Beta-galactosidase from a

[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.

genetically modified strain *Aspergillus niger* (strain TOL) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the terms of reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme β-galactosidase from a genetically modified *Aspergillus niger* strain TOL.

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 genetically modified *Aspergillus niger* (strain TOL).

Additional information was requested from the applicant during the assessment process on 8 October 2020 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 guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	β-galactosidase
Systematic name	β-D-galactoside galactohydrolase
Synonyms	Lactase; β-D-lactosidase
IUBMB No	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-galactose and D-glucose. The substrates include ß-galactosides including lactose and various glycoproteins. It is intended to be used in whey processing.

3.1. Source of the food enzyme

The β-galactosidase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain TOL, which is deposited in the Westerdijk Fungal Biodiversity Institute culture collection (CBS, The Netherlands) with deposition certificate [redacted].[4]

The production strain was taxonomically identified as *A. niger* by [redacted].[5]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted][6]

[4] Technical dosier/Additional information June 2021/Annex II-1.
[5] Technical dossier/Annex II-2.
[6] Technical dossier/Annex II-3.

[redacted]

3.1.1.1. Characteristics of introduced sequences

[redacted][7][8]

3.1.1.2. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise β-galactosidase [redacted][9]

3.1.2. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

[redacted][10][11]

No issues of concern arising from the genetic modifications were identified by the panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[12], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.

[7] Technical dossier/Annex II-6 and Annex II-8.
[8] Technical dossier/Annex II-7 and Annex II-9.
[9] Technical dossier/Annex II-10.
[10] Technical dossier/Annex II-12.
[11] Technical dossier/Additional information June 2021/Annex II-2.
[12] 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.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, cells are killed and the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is 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. 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.[13]

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 987 amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 108 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 single major protein band corresponding to an apparent molecular mass of about 116 kDa, consistent with the expected mass of the enzyme.[13] No other enzymatic activities were reported.

The in-house determination of β-galactosidase activity is based on the hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (reaction conditions: pH 4.5, 37°C, 15 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenol spectrophotometrically at 420 nm. The enzyme activity is expressed in acid lactase units (ALU)/g. One ALU is defined as the amount of enzyme required to release one μmole of *o*-nitrophenol from *o*-nitrophenyl-β-D-galactopyranoside in 1 min under the conditions of the assay.

The food enzyme has a temperature optimum around 55°C (pH 4.5) and a pH optimum around pH 4.0 and 4.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 4.5). β-Galactosidase activity decreased above 55°C, showing no residual activity above 75°C.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[14] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 20.6% and the mean enzyme activity/TOS ratio is 182 ALU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches		
		1	2	3
β-galactosidase activity	ALU/g batch[(a)]	44,800	34,500	33,300
Protein	%	18.2	13.9	14.1
Ash	%	0.2	0.1	0.2
Water	%	76.0	81.1	80.7
Total organic solids (TOS)[(b)]	%	23.8	18.8	19.1
Activity/mg TOS	ALU/mg TOS	188.2	183.5	174.3

(a): ALU: Acid lactase unit (see Section 3.3.1).
(b): TOS calculated as 100%–% water–% ash.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies (see Section 3.4.1) was below 5 mg/kg[15] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

[13] Technical dossier/Additional information June 2021.
[14] Technical dossier/Additional information June 2021/Annex I-3.
[15] Technical dossier/Annex I-3.

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[15]

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 mycotoxins (zearalenone, fumonisins, trichothecenes, aflatoxins, ochratoxin A) was examined in the three food enzyme preparation batches and were below the limits of detection (LoD) of the applied analytical methods.[16] Adverse effects caused by the potential presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated [redacted].[17]

The absence of recombinant DNA in the food enzyme concentrate before formulation was demonstrated [redacted].[18]

3.4. Toxicological data

3.4.1. Choice of test item

No toxicological studies were provided for the β-galactosidase food enzyme produced with the *A. niger* strain TOL. Instead, the applicant argued that the assessment of this β-galactosidase produced by *A. niger* strain TOL could be based on toxicological data from another food enzyme – an asparaginase produced with the *A. niger* strain ASP, previously submitted to EFSA (Question No EFSA-Q-2013-00895) following the EFSA guidance (EFSA, 2009a).

The production strain of the asparaginase was developed from the same recipient strain [redacted] as that for the β-galactosidase under assessment (*A. niger* TOL) using the same genetic modification system [redacted], with a specific gene of interest in each case. The genetic modification in *A. niger* ASP only differs from that of *A. niger* TOL in the gene of interest [redacted] and in the location of the expression cassettes [redacted]. No rounds of mutagenesis have been applied in the development of the production strains from the recipient and all the genetic modifications have been described throughout. No partial inserts of the TOL expression cassette were introduced into the genome which was confirmed by WGS analysis.[11] Therefore, the genetic differences between *A. niger* TOL and *A. niger* ASP are not expected to result in a different toxigenic potential.

The batch of asparaginase from *A. niger* ASP, used for toxicological studies, was produced according to a standard procedure similar to the one described in Section 3.2. According to the applicant, the raw materials used and the steps involved in the manufacturing of the asparaginase and the β-galactosidase food enzymes are comparable in both processes, and the temperature and pH conditions used during fermentation are similar. Small differences in raw materials were noted, including some salts and excipients. The Panel concluded that none of these differences raised concern.[19] The batch used for toxicological studies had a TOS content of 89.7%.

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats was provided, all made with the substitute food enzyme.

[16] LoD: Zearalenone: 3 μg/kg, Fumonisins 10 μg/kg each toxin, Trichothecenes: 10 μg/kg (each toxin), Aflatoxins 0.1 μg/kg each toxin, Ochratoxin A 0.1 μg/kg.
[17] Technical dossier/Additional information June 2021/Annex II-4.
[18] Technical dosier/Additional information June 2021/Annex II-3.
[19] Technical dossier/Additional information June 2021.

3.4.2. Genotoxicity

3.4.2.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was made according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[20] Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537, TA98) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the 'plate incorporation assay'. One experiment in triplicate was performed using five different concentrations of the food enzyme (62, 185, 556, 1,667 and 5,000 μg/plate, corresponding to ca. 56, 166, 499, 1,495 and 4,484 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. A slightly denser background lawn was observed at all strains at the highest concentrations tested.

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

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

3.4.2.2. *In vitro* mammalian chromosomal aberration test

An *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in human peripheral blood lymphocytes with and without metabolic activation (S9-mix).[21]

Two separate chromosomal aberration tests were conducted in duplicate cultures.

In a first experiment, the cultures were exposed at concentrations of 2,000, 3,000 and 5,000 μg of food enzyme/ml (corresponding to ca. 1,794, 2,690 and 4,484 μg TOS/ml) applying a 4-h treatment followed by 20-h recovery period in the presence and absence of S9-mix. In a second experiment, 3,000, 4,000 and 5,000 μg of food enzyme/ml (corresponding to ca. 2,690, 3,587 and 4,484 μg TOS/ml) were tested in a short-term treatment 4 h + 20 h with the S9-mix and in a continuous 24 h treatment in the absence of S9-mix. A slight cytotoxicity was observed after the short-term treatment with and without metabolic activation. The test substance was clearly cytotoxic at the highest concentration analysed after a continuous treatment (mitotic index was reduced to 46% of that of the concurrent controls). The enzyme preparation did not induce a significant increase in structural or numerical chromosome aberrations in cultured human blood lymphocytes, in either of the two independently repeated experiments.

The Panel concluded that the food enzyme did not induce chromosome aberrations in cultured human blood lymphocytes, under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[22] Groups of 20 male and 20 female Wistar WU rats (Crl:WI(Wu)) received in feed 0.2%, 0.6% or 1.8% of the food enzyme. These dietary concentrations corresponded to 117, 351 or 1,038 mg TOS/kg bw per day for males and to 135, 405 or 1,194 mg TOS/kg bw per day for females based on the registered feed intake. Controls received the feed with no food enzyme added.

No mortality was observed.

The haematological examination revealed a statistically significant increase in the absolute differential count (+46%) and in the relative differential count (+38%) of monocytes (MONO) in the high-dose males in week 2, a decrease in the absolute differential count of basophils (BASO) in the low-, mid- and high-dose males (−38%, −31%, −46%) and in the relative differential count of BASO in low-dose and high-dose males (−33% and −33%, respectively) at the end of the treatment. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (MONO, BASO), they were only recorded sporadically (MONO), there was no dose–response relationship (BASO), the changes were small (MONO, BASO) and there were no changes in other relevant parameters (i.e. in a total white blood cell count).

[20] Technical dossier/Annex I-8.
[21] Technical dossier/Annex I-9.
[22] Technical dossier/2subm09042015_valid/1subm09122014/Confidential/Annexes Part I/Annex I-11 90d study test.

The clinical chemistry investigation revealed a statistically significant decrease in activity of sorbitol dehydrogenase (SDH) in high-dose males (−22%) and in mid- and high-dose females (−19% and −22%, respectively) in week 2, a decrease in activity of alanine aminotransferase (ALT) in low-dose males (−13%) in week 2 and an increase in urea concentration in low- and mid-dose females in week 7 (+11% and +29%, respectively). The Panel considered the changes as not toxicologically relevant as they were only recorded sporadically (all parameters), they were only observed in one sex (ALT, urea), there was no dose–response relationship (ALT, urea) and the change was small (ALT).

The urinalysis revealed a statistically significant increase in triple phosphate crystals in urinary sediment from high-dose males in week 13. The Panel considered the change as not toxicologically relevant as it was only observed in one sex, and there were no changes in other relevant parameters (e.g. in weight and morphology of the kidneys).

Statistically significant changes in organ weight included a decrease in relative weighs of testes (−9%) and epididymides (−7%) in low-dose males. The Panel considered the changes as not toxicologically relevant as there was no dose–response relationship (both parameters) and the changes were small (both parameters).

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

No adverse effects were observed at the highest dietary concentrations tested, equal to 1,038 mg TOS/kg bw per day for males and to 1,194 mg TOS/kg bw per day for females. The Panel identified a no observed adverse effect level (NOAEL) of 1,038 mg TOS/kg bw per day.

3.5. 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 enzyme ß-galactosidase produced with the genetically modified strain of *Aspergillus niger* TOL 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.

No information on oral and respiratory sensitisation or elicitation reactions of the ß-galactosidase under evaluation have been reported.

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

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

3.6. Dietary exposure

3.6.1. Intended use of the food enzyme

The food enzyme is intended to be used in whey processing at a recommended use level of up to 0.22 mg TOS/g lactose present in whey or processed whey.[23] Considering that the lactose content in cheese whey and processed whey (whey permeate) is 4.5% (w/w) and 4.7% (w/w),[24] respectively, the recommended use level is converted to 9.9 mg TOS/kg cheese whey and 10.3 mg TOS/kg whey permeate. The use level 9.9 mg TOS/kg whey is used for calculation.

In whey processing, the β-galactosidase can be added to the whey directly, the whey permeate or the concentrated whey permeate.[25] The effect of the enzymatic conversion with the help of ß-galactosidase is the hydrolysis of lactose in whey and whey-derived products to reduce the lactose

[23] Technical dossier/p.68.
[24] Additional data June 2021.
[25] Technical dossier/pp. 65–67.

levels and liberate glucose and galactose. Reduction of lactose in whey and whey-derived ingredients make it more suitable for lactose-intolerant individuals, and due to the enhanced sweetness, the amount of sugar added to the final foods can be reduced. It also decreases the sandiness in products like ice cream.

The food enzyme remains in the whey and whey-derived ingredients. Based on data provided on thermostability (see Section 3.3.1), it is expected that the ß-galactosidase is inactivated during the pasteurisation step.

3.6.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 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). The highest dietary exposure to the food enzyme–TOS was estimated to be about 0.197 mg TOS/kg bw per day in infants at the 95th percentile.

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
Min–max mean (number of surveys)	0.002–0.051 (11)	0.007–0.043 (15)	0.008–0.019 (19)	0.002–0.009 (21)	0.002–0.007 (22)	0.002–0.006 (22)
Min–max 95th (number of surveys)	0.011–0.197 (9)	0.016–0.170 (13)	0.016–0.043 (19)	0.006–0.025 (20)	0.006–0.021 (22)	0.004–0.014 (21)

3.6.3. Uncertainty analysis

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

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–

Sources of uncertainties	Direction of impact
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
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	+/–

+: 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.

3.7. Margin of exposure

A comparison of the NOAEL (1,038 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.002–0.051 mg TOS/kg bw per day at the mean and from 0.004–0.197 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 5,269.

4. Conclusions

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

The CEP Panel considers the food enzyme free from viable cells of the production organism and recombinant DNA.

5. Documentation as provided to EFSA

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Additional information. June 2021. Submitted by DSM Food Specialties B.V.

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Abbreviations and acronyms

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 Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
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
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
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.2022.7570#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).