SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 September 2018

doi: 10.2903/j.efsa.2018.5447

Safety evaluation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Bacillus subtilis* (strain LMG S-24584)

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Abstract

The food enzyme endo-1,4-β-xylanase (EC 3.2.1.8) is produced with the genetically modified *Bacillus subtilis* strain LMG S-24584 by Puratos N. V. The genetic modifications do not give rise to safety concerns. The Panel noted that, although the production strain was not detected in the food enzyme, recombinant DNA was present in all batches of the food enzyme tested. The food enzyme is intended to be used in baking processes. Based on the maximum use levels recommended for the baking processes and individual consumption data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.017 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. A comparison of the no observed adverse effect level of 37 mg TOS/kg bw per day from this study with the dietary exposure results in a sufficiently high margin of exposure. The amino acid sequence of the food enzyme did not match those of known allergens. The Panel considered that, under the intended condition 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 occurring is considered to be 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, xylanase, endo-1, 4-β-xylanase, EC 3.2.1.8, 4-β-D-xylan xylanohydrolase, *Bacillus subtilis*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2015-00065

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Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once decision on confidentiality will be received from the European Commission.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mortensen A, Riviere G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Glandorf B, Penninks A, Želježić D, Andryszkiewicz M, Gomes A, Kovalkovičová N, Liu Y, Engel KH and Chesson A, 2018. Scientific Opinion on the safety evaluation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Bacillus subtilis* (strain LMG S-24584). EFSA Journal 2018;16(10):5447, 16 pp. https://doi.org/10.2903/j.efsa.2018.5447

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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.

Five applications have been introduced by the companies "Novozymes A/S", "Puratos NV sa", "Neova Technologies Inc." and "Amano Enzyme Inc." for the authorisation of the food enzymes Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CE), Xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzymes Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *Bacillus licheniformis* (strain

[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, p. 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, p. 1–6.

NZYM-CE), Xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT) in accordance with the 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 request to carry out the safety assessment of food enzyme xylanase from *B. subtilis* strain LMG S-24584 submitted by Puratos N. V.

1.3. Information on existing authorisations and evaluations

The applicant reports that the French food authorities have evaluated and authorised the use of the food enzyme from a genetically modified *B. subtilis* strain [redacted] (with a xylanase from [redacted]) for a number of food manufacturing processes.[3]

2. Data and methodologies

2.1. Data

The applicant submitted a dossier supporting the application for authorisation of the food enzyme endo-1,4-β-xylanase produced with a genetically modified microorganism (GMM) *B. subtilis* (strain LMG S-24584). The food enzyme is intended to be used in baking processes.

2.2. Methodologies

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

The current guidance on the submission of a dossier for safety evaluation of a food enzyme (EFSA, 2009) has been followed for the evaluation of this application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Endo-1,4-β-xylanase
Systematic name:	4-β-D-Xylan xylanohydrolase
Synonyms: Xylanase:	endo-1,4-D-β-xylanase
IUBMB No:	EC 3.2.1.8
CAS No:	9025-57-4
EINECS No:	232-800-2.

Endo-1,4-β-xylanase catalyses the hydrolysis of 1,4-glycosidic linkages in xylans (including arabinoxylans in which the xylan chain is substituted with arabinose residues) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths. It is intended to be used in baking.

3.1. Source of the food enzyme

The endo-1,4-β-xylanase is produced with a genetically modified bacterium *B. subtilis*. The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *B. subtilis* LMG S-24584 [redacted] is deposited in the Belgian Coordinated Collections of Microorganisms with accession number LMG S-24584.[4]

[3] Technical Dossier: Annex 17.
[4] Additional information April 2018: Annex 4.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *B. subtilis* [redacted]

The recipient strain *B. subtilis* [redacted]

The recipient strain [redacted], as well as the production strain *B. subtilis* LMG S-24584, were confirmed as *B. subtilis* through [redacted]

3.1.2. Characteristics of the introduced sequences

The sequence encoding the [redacted]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to express endo-1,4-β-xylanase from [redacted]

The production strain *B. subtilis* LMG S-24584 was developed from the recipient strain [redacted]

3.1.4. Safety aspects of the genetic modification

The production strain *B. subtilis* LMG S-24584 differs from the recipient strain [redacted] only in its capacity to produce the endo-1,4-β-xylanase from [redacted]. The presence and the location of the enzyme encoding gene were confirmed by Southern analysis. The phenotypic stability of the *B. subtilis* LMG S-24584 strain was confirmed by its capacity to produce a constant level of the enzyme endo-1,4-β-xylanase measured in relation to the TOS in three independent batches of the food enzyme and its genetic stability was demonstrated by Southern analysis with DNA isolated from three independent cultures.

The absence of the AMR genes used during the genetic modification was confirmed by Southern analysis. Results showed that no AMR genes are present in the production strain *B. subtilis* LMG S-24584.

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

[5] Additional information April 2018: Annex 5.
[6] Technical dossier: Annex 26 and List of missing data.
[7] Technical Dossier: Annex 28.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation[9] and in the subsequent downstream processing of the food enzyme.

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 endo-1,4-β-xylanase produced from the genetically modified *B. subtilis* strain LMG S-24584 is a [redacted] The molecular mass of the mature protein, derived from the amino acid sequence (without the signal peptide), was calculated to be [redacted]. The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. The apparent molecular mass based on this technique is about [redacted] which is consistent with the calculated value. The gels presented showed one main protein band and some minor protein bands of lower and higher molecular mass.[11] The three batches for commercialisation were tested for protease and α-amylase activities and no relevant activities were detected.[12] No other enzymatic side activities were reported.

The in-house determination of xylanase activity is based on hydrolysis of the substrate beechwood xylan to reducing carbohydrates (reaction conditions: pH 6.5, temperature 25°C, reaction time 15 min). The xylanase activity is quantified relative to an enzyme standard and expressed in Xylanase Units/mL (GDXU/mL). One GDXU is defined as the amount of enzyme that produces 1 μmol of product (measured in xylose equivalents) per minute and per mL in the incubation mixture under the assay conditions (pH 6.5, temperature 25°C, reaction time 15 min).[13]

The food enzyme has been characterised with regard to its temperature and pH profiles. The xylanase has a temperature optimum around 30°C (pH 6.5) and a pH optimum around pH 6.5 (temperatures ranging from 25°C to 50°C). Thermostability was tested at different temperatures and pH 6.5. The xylanase activity decreased rapidly above 50°C, showing no residual activity after 10 min incubation at 60°C.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for five food enzyme batches, three batches to be used for commercialisation and two batches used for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 3.58% (range 3.16–3.79%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 104 and was used for subsequent calculations.

[8] 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, p. 3–21.
[9] Technical dossier p. 37–38.
[redacted]
[11] Technical Dossier: Annexes 5a and 5b.
[12] Technical dossier: Annex 6.
[13] Technical dossier: Annex 03.
[14] Technical dossier: p. 27–28 and additional information April 2018.

Table 1: Compositional data of the food enzyme.

Parameter	Unit	Batch				
		1	2	3	4[(a),(f)]	5[(b),(g)]
Endo-1,4-β-xylanase activity	GDXU/mL batch[(c)]	4,284	4,004	2,946	289	1,999
Protein	%	1.87	2.13	1.65	NA[(d)]	1.17
Ash	%	4.99	4.69	4.86	NA[(d)]	1.27
Water	%	91.22	91.53	91.97	NA[(d)]	96.26
Total organic solids (TOS)[(e)]	%	3.79	3.78	3.17	2.49	2.47
Activity/mg TOS	GDXU/mg TOS	113	106	93	12	81

(a): Batch used for the genotoxicity studies.
(b): Batch used for the repeated dose 90-day oral toxicity study.
(c): GDXU: Xylanase Units (see Section 3.1.3).
(d): NA: not analysed.
(e): TOS calculated as 100% - % water - % ash.
(f): Technical dossier: Annex 36 and List of missing data.
(g): Technical dossier: additional information April 2018, annexes 1.3.A, 1.3.B, 1.3.C.

3.3.3. Purity

The lead content in the three commercial batches and in the batches used for toxicological studies was below 0.1 mg/kg which complies with the specification for lead (< 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[15]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 CFU (Colony Forming Unit) per gram.[16] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[17]

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 in three independent batches analysed in triplicate. [redacted]

Recombinant DNA was detected in three enzyme batches, tested in triplicate, by polymerase chain reaction (PCR) [redacted]

Recombinant DNA was present in all batches of the food enzyme tested.

3.4. Toxicological data

The food enzyme batch 4 used for the genotoxicity studies and batch 5 used for the repeated dose 90-day oral toxicity study have a lower activity/mg TOS than the three batches for commercialisation (Table 1). This indicates a lower purity than the commercial batches and, thus, batches 4 and 5 were considered suitable for the toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test[19]

A bacterial reverse mutation assay (Ames test) was made according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP). Five strains of *Salmonella* Typhimurium (TA 1535, TA 1537, TA 98, TA 100 and TA 102) were used in the presence or absence of metabolic activation, applying the direct plate incorporation method. Two separate experiments were carried out

[15] CoA in Annex 4a and in additional information April 2018: annex 1.3.B.
[16] Technical dossier: annex 4a.
[17] Annex 4b and in additional information April 2018: annex 1.3.C.
[redacted]
[19] Technical dossier: Annex 19.

using five different concentrations of the food enzyme (500, 1,500, 5,000, 15,000 and 50,000 μg food enzyme/mL, corresponding to 0, 12, 37, 125, 374 and 1,245 μg TOS/mL), and appropriate positive and negative controls. No cytotoxic effects were observed at any tested food enzyme concentration. Upon treatment with the food enzyme, there was no increase in revertant colony numbers above the control values in any of the strains, with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test[20]

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP. The food enzyme was tested for its ability to induce chromosomal aberrations in human peripheral blood lymphocytes with and without metabolic activation (S9 mix) at concentrations up to 5,000 μg food enzyme/mL (corresponding to 124.5 μg TOS/mL final culture concentration). In the short-treatment (4 + 20 h), two experiments were performed: the first experiment, in the presence and absence of the S9 mix, and the second experiment in the presence of the S9 mix. In both experiments, the concentrations scored for the chromosome aberration test were 1,250, 2,500 and 5,000 μg food enzyme/mL, corresponding to 31.1, 62.3 and 124.5 μg TOS/mL In the long-treatment (24 + 0 h in absence of the S9 mix concentrations), the concentrations scored for chromosome aberration were 2,500, 3,750 and 5,000 μg food enzyme/mL corresponding to 62.3, 93.4 and 124.5 μg TOS/mL. Cytotoxic effects were observed at higher concentrations (18% mitotic inhibition at 5,000 μg/mL in the presence of S9 in the in the short treatment; up to 73% mitotic inhibition at 5,000 μg/mL and 43% mitotic inhibition at 3,750 μg/mL in the continuous treatment experiment without metabolic activation). 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 endo-1,4-β-xylanase did not induce chromosome aberrations in cultured human blood lymphocytes, under the test conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents[21]

The repeated dose 90-day oral toxicity study in rodents was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Sprague–Dawley rats received by gavage the food enzyme corresponding to 11, 18.5 or 37 mg TOS/kg bw per day. Controls received the vehicle (water).

No mortality was observed.

Statistically significant differences in body weight or body weight gain as compared to controls included a lower body weight on day 8, a lower body weight gain in days 1–8 and a higher body weight gain in days 8–15 for all treated female groups and a higher body weight gain in all treaded male groups in several intervals (i.e. days 29–36; days 36–43; days 71–78 and days 78–85). As the body weights of treated male and female groups were not statistically significantly different from controls at the end of the clinical phase, the differences were recorded sporadically and with no apparent dose relationship, the Panel considered these body weight and/or body weight gain changes to reflect normal biological variation. The latter was further supported by the fact that the body weights of the control and treated animals were within the historical control data.

In ophthalmological examination, unilateral chorioretinopathy was observed in one high-dose male. This finding was not considered to be treatment-related as it was observed in only one animal and is a common finding in rats of this strain and age.

Haematological investigation revealed a statistically significantly lower mean cell haemoglobin concentration (MCHC) in mid-dose males.

Clinical chemistry investigation revealed that the potassium concentration was statistically significantly decreased in low-dose males and increased in mid-dose females. In addition, decreased calcium levels and increased creatinine concentrations were observed in mid- and high-dose males, respectively.

All the changes in haematology and blood chemistry parameters were considered by the Panel as not treatment-related because the differences were small and without an apparent dose dependency.

[20] Technical dossier: Annex 20.
[21] Technical dossier: Additional information April 2018: annex 6.

There was a small but statistically significant increase in spleen weight in high-dose males and decreased relative brain weights in the low- and high-dose males. Adrenal weight was slightly but statistically significantly decreased in low-dose females. The Panel considered the changes in organ weights as incidental because these changes were small and the values were within the normal variability of relevant historical control ranges in the laboratory, there was no apparent dose–response relationship and the changes were not accompanied by histopathological findings.

No other statistically significant differences to controls were observed. Based on the above, the Panel identified a no observed adverse effect level (NOAEL) at the highest dose tested of 37 mg TOS/kg bw per day.

3.4.3. Allergenicity[22]

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 endo-1,4-β-xylanase produced with the genetically modified *B. subtilis* strain LMG S-24584 was assessed by comparison of its amino acid sequence with those of known allergens according to the report of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology (FAO/WHO, 2001). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

No information is available on oral sensitisation or elicitation reactions of this endo-1,4-β-xylanase. However, respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al.,1998; Merget et al., 2001). However, several studies have shown that adults with occupational asthma to an enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Such information is not reported for xylanase. Overall, the likelihood of an allergic reaction upon oral ingestion of this endo-1,4-β-xylanase, produced with the genetically modified *B. subtilis* strain LMG S-24584 in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed.

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 occurring is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at an intended use level of up to 150 UI/kg flour, corresponding to 1.44 mg TOS/kg flour.

In baking processes, the food enzyme is added to the raw materials during the preparation of the dough. It is used to hydrolyse (arabino)xylans, which interact with gluten and water, thus contributing to reduce the viscosity of the dough. The decrease in dough viscosity facilitates the handling of the dough, resulting in more uniform products with slightly increased volume and an improved crumb structure.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the endo-1,4-β-xylanase is inactivated during baking processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[23] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

[22] Technical dossier: Annex 23.
[23] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed average 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg/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)	0.000–0.004 (10)	0.003–0.009 (14)	0.003–0.008 (19)	0.002–0.005 (18)	0.001–0.003 (19)	0.001–0.003 (18)
Min–max 95th percentile (number of surveys)	0.002–0.017 (8)	0.008–0.015 (12)	0.007–0.016 (19)	0.004–0.011 (17)	0.003–0.006 (19)	0.003–0.005 (18)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), 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 survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
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 based on the description of the food process provided by the applicant	+
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 regarding 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 (37 mg TOS/kg bw per day) from the 90-day study with the exposure estimates of 0–0.009 mg TOS/kg bw per day at the mean and 0.002–0.017 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) above 2176.

4. Conclusions

Based on the data provided the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *B. subtilis* strain LMG S-24584 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. The Panel noted that recombinant DNA was present in all batches of the food enzyme tested.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of endoβ(1-4)xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584 in accordance with Regulation (EC) No 1331/2008', January 2015. Submitted by Puratos N. V.
2) Additional information was received from Puratos in April 2018.

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Abbreviations

AMR	antimicrobial resistance
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
GDXU	Xylanase Units
GLP	Good Laboratory Practice
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LMG	Laboratory of Microbiology, university of Gent
MCHC	mean cell haemoglobin concentration
MIC	minimum inhibitory concentration

MOE	Margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid
SDS–PAGE	sodium dodecyl sulfate–poly acrylamide 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 here).

The file contains two sheets, corresponding to two tables.

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

Table 2: The contribution of FoodEx categories to the food enzyme-TOS dietary exposure

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(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).