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Safety evaluation of food enzyme xylanase from a genetically modified *Bacillus subtilis* (strain LMG S-27588)

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Abstract

The food enzyme considered in this opinion is an endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) produced from the genetically modified *Bacillus subtilis* strain LMG S-27588 by the company Puratos N. V. The production strain was not detected in the food enzyme. The endo-1,4-β-xylanase is intended to be used in baking processes. Based on the maximum use levels recommended 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 0.325 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests with the food enzyme indicated no genotoxic concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. A no observed adverse effect level (NOAEL) was derived (443 mg TOS/kg bw per day), which, compared with the dietary exposure, results in a sufficiently high margin of exposure. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens; no match was found. The Panel considered that there are no indications for food allergic reactions to this endo-1,4-β-xylanase by dietary exposure. Based on the genetic modifications, the manufacturing process, the compositional and biochemical data, the dietary exposure assessment, the findings in the toxicological studies and allergenicity assessment, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use. The Panel noted that recombinant DNA was present in all batches of the food enzyme tested.

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Keywords: food enzyme, xylanase, endo-1,4-β-xylanase, EC 3.2.1.8, 4-β-D-xylan xylanohydrolase, *Bacillus subtilis*, 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 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, and
- its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on a food enzyme for 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[1] on food enzymes.

Five applications have been introduced by the companies "Danisco US Inc." for the authorisation of the food enzyme Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21); "Novozymes A/S." for the authorisation of the food enzyme Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN); "Puratos NV" for the authorisation of the food enzyme Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG S-27588); the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Beta-galactosidase from *Kluyveromyces lactis* and "AB Enzymes GmbH" for the authorisation of the food enzyme Lysophospholipase from a genetically modified strain of Trichoderma reesei (strain RF7206).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[2] implementing Regulation (EC) No 1331/2008[3], the Commission has verified that the five applications

[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] 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, p. 15–24.

[3] 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.

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 Hexose oxidase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jza21), Pectin lyase from a genetically modified strain of *Aspergillus niger* (strain NZYM-PN), Xylanase from a genetically modified strain of *Bacillus subtilis* (strain LMG S-27588), Beta-galactosidase from *Kluyveromyces lactis* and Lysophospholipase from a genetically modified strain of Trichoderma reesei (strain RF7206), 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 *Bacillus subtilis* strain LMG S-27588 submitted by Puratos N. V.

1.3. Information on existing authorisations and evaluations

The applicant only reports either authorised food enzymes other than xylanase produced by the same production organism, *B. subtilis*, or authorised xylanase from production organisms other than *B. subtilis*.

2. Data and methodologies

2.1. Data

The applicant submitted a dossier in support of the application for authorisation of the food enzyme xylanase produced from genetically modified *B. subtilis* (strain LMG S-27588). 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, 2009b) and following the relevant Guidances from the EFSA Scientific Committee.

The current guidance on the submission of a dossier for safety evaluation of a food enzyme (EFSA, 2009a) has been followed by the CEF Panel for the evaluation of the 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

3.1. Technical data

3.1.1. Identity of the food enzyme

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.

3.1.2. Chemical parameters

The endo-1,4-β-xylanase produced from the genetically modified *B. subtilis* strain LMG S-27588 consists of a [redacted], including a signal peptide of [redacted], which is cleaved off during secretion of the enzyme protein. The molecular mass of the mature protein of [redacted] was calculated based on the amino acid sequence. The protein homogeneity status of the

food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The apparent molecular mass based on the SDS-PAGE pattern is about [redacted]. The gels presented showed one main protein band and some minor protein bands of higher molecular mass. The food enzyme did not show protease activity and very low alpha-amylase activity. No other enzymatic activities have been reported by the applicant.

Data on the chemical parameters of the food enzyme were provided for six food enzyme batches, three batches to be used for commercialisation and three batches used for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 4.03%; the values ranged from 2.66% to 5.13%.

The enzyme activity/mg TOS ratios of the three food enzyme batches for commercialisation ranged from 13.6 to 40.9 Xylanase Units (ADXU)/mg TOS (Table 1). The average value of 27.6 ADXU/mg TOS was used for subsequent calculations.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batch					
		1	2	3	4[(a)]	5[(b)]	6[(c)]
Endo-1,4-β-xylanase activity	ADXU/mL batch[(d)]	1,211	1,089	696	224	3,264	1,216
Protein	%	1.4	0.9	1.5	NA	15.2	NA
Ash	%	0.6	0.5	0.6	0.5	5.4	1.8
Water	%	95.1	96.8	94.3	97.0	57.0	93.4
Total organic solids (TOS)[(e)]	%	4.3	2.7	5.1	2.4	37.6	4.8
Activity/mg TOS	ADXU/mg TOS	28.2	40.9	13.6	9.2	8.7	25.3

NA: not analysed.
(a): Batch used for the *in vitro* micronucleus test.
(b): Batch used for the repeated dose 90-day oral toxicity study.
(c): Batch used for the bacterial reverse mutation test.
(d): ADXU: Xylanase Units (see Section 3.1.3).
(e): TOS calculated as 100% – % water – % ash.

The lead content on the three commercial batches and the batches used for toxicological studies was below 0.5 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). No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).

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 *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming units (CFU) per gram.

The applicant has provided information on the identity of the antifoam agent used and the method for analysis. Taking into account the nature and properties of the antifoam agent, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers its use as of no safety concern.

The compositional data provided for the food enzyme batches are considered sufficient.

3.1.3. Properties of the food enzyme

The food enzyme catalyses the hydrolysis of endo-1,4-β-D-xylosidic linkages in xylan (including arabinoxylan, which is xylan branched with arabinose) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths (1,4-β-xylan; 1,4-β-arabinoxylan). This xylanase does not require co-factors.

The enzymatic activity is determined based on the hydrolysis of beechwood xylan and is expressed in Xylanase Units/mL (ADXU/mL). The analytical principle is based on hydrolysis of the xylan to reducing carbohydrates (reaction conditions: pH = 6, T = 70°C, incubation time = 15 min). One ADXU is defined as the amount of enzyme that produces 1 μm of product (measured in xylose equivalents) per minute and per ml from xylan under the assay conditions.

The food enzyme has been characterised regarding its temperature and pH profiles. The xylanase showed maximum activity in the assay at 100°C, the highest temperature tested, and was active within a pH range of 5–9 (with an optimum of pH 6–7). The enzyme activity decreases gradually upon

treatments at temperatures above 90°C. Approximately 30% enzyme activity remains after 4 h of thermal treatment at 100°C. This indicates the food enzyme is thermostable.

3.1.4. Information on the microbial source material

3.1.4.1. Information relating to the genetically modified microorganism

The endo-1,4-β-xylanase production strain *B. subtilis* LMG S-27588 [redacted] is deposited in the LMG – Culture Collection, a node of the Belgian Co-ordinated Collection of Microorganisms located at the University of Gent, under deposit code LMG S-27588.

3.1.4.2. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *B. subtilis* [redacted]

3.1.4.3. Characteristics of the donor microorganisms

[redacted]

3.1.4.4. Description of the genetic modification process

The production strain *B. subtilis* LMG S-27588 was developed from the recipient strain [redacted]

3.1.4.5. Safety aspects of the genetic modification

[redacted]

[redacted]

3.1.5. Manufacturing process

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[4] and in accordance with current Good Manufacturing Practice (GMP). A dataset related to the manufacturing process including a list of raw materials used and a production flow process from fermentation and downstream processes was provided.

The food enzyme is produced by a pure culture in a contained, submerged, fermentation system with conventional process controls in place.

After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated by ultrafiltration. The production strain could not be detected in three different batches of the food enzyme. From each batch, before formulation, a [redacted]

Recombinant DNA was detected in three enzyme batches, tested in triplicate, by polymerase chain reaction (PCR) amplifying a fragment of about [redacted] of the xylanase coding sequence.

The Panel considered the information provided on the manufacturing process as sufficient.

3.1.6. Safety for the environment

The production strain could not be detected in the food enzyme (see Section 3.1.5). However, recombinant DNA was demonstrated to be present in all batches tested. The applicant provided laboratory transformation tests with samples from production batches which were negative and argued that presence of recombinant DNA was therefore not a concern. However, the CEF Panel did not consider these laboratory-based data as relevant for the risk assessment.

On the other hand, no sequences that cause safety concern (such as AMR marker genes or genes encoding known toxins) have been introduced in the production strain, therefore the Panel is of the opinion that the recombinant DNA present in the food enzyme does not pose a risk to the environment.

3.1.7. Case of need and intended conditions of use

As proposed by the applicant, the food enzyme is intended for use in baking processes at an intended use level of up to 800 ADXU/kg flour, corresponding to 29 mg TOS/kg flour.

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.

3.1.8. Reaction and fate in food

The xylanase catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylan resulting in the production of (1→4)-β-D-xylan and (1→4)-β-D-arabinoxylan oligosaccharides of different lengths. Xylanase is specific in its action, not known to catalyse other reactions than this endo-hydrolysis of xylans to shorter xylans chains, xylooligosaccharides and xylose. These reaction products are naturally

[4] 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.

present in xylan-containing foods. Based on the substrate specificity of the xylanase, no unintended reaction products are expected in foods.

The data and information provided indicate that the xylanase is largely inactivated during baking processes under the intended use conditions.

3.2. Dietary exposure

Exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel et al., 2016). The assessment of the food processes covered in this opinion involved selection of relevant food groups and application of process and technical conversion factors (Appendix B). These input data were subject to a stakeholder consultation through open calls,[5] and adjusted in accordance with feedback received.

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (hereafter the EFSA Comprehensive Database[6]) has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011a).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be made with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to subjects possibly underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database is the best available source of food consumption data across Europe.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Appendix A).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

3.2.2. Exposure assessment methodology

Chronic exposure was calculated based on individual consumption, averaged over the total survey period, excluding surveys with only one day per subject. 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, 2011a).

The exposure per FoodEx category was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of survey days and normalised for individual body weight (bw), resulting in an individual average exposure/day per kg bw for the survey period. This was done for all individuals in the survey and per age class, resulting in distributions of individual average exposure per survey and age class. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class.

3.2.3. Exposure to food enzyme–TOS according to the intended use proposed by the applicant

Exposure to the food enzyme–TOS was based on intended use and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Section 3.1.8). Food enzyme–TOS exposure was calculated from foods produced involving a baking process.

Relevant food groups and/or individual foods were selected from the Comprehensive Database and were assumed to always contain the food enzyme–TOS at the maximum recommended use level. This will result in an overestimation of exposure to food enzyme–TOS.

To facilitate matching of the reported use levels for baking processes with foods identified in the Comprehensive Database, the selected foods were disaggregated to ingredient level as appropriate, and converted into the corresponding raw material, i.e. flour, via the application of conversion factors (Appendix B). For example, consumption of 100 g of bread was converted into an intake of 70 g flour

[5] http://www.efsa.europa.eu/en/data/call/161110
[6] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

(recipe fraction of 0.7) and then multiplied by 29 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 2.03 mg TOS/100 g bread.

Exposure to the food enzyme–TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight (kg bw) separately for each individual in the database. Table 2 provides an overview of the derived exposure estimates. The average and 95th percentile exposure to the food enzyme-TOS per age class, country and survey are reported in Appendix C – Table C.1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix C – Table C.2.

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

Population group	Estimated exposure (mg/kg bw 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)	0.020–0.088 (6)	0.076–0.183 (10)	0.080–0.173 (18)	0.047–0.115 (17)	0.034–0.070 (17)	0.033–0.061 (14)
Min–max 95th percentile (number of surveys)	0.118–0.249 (5)	0.172–0.309 (7)	0.149–0.325 (18)	0.085–0.228 (17)	0.067–0.138 (17)	0.062–0.110 (14)

bw: body weight.

3.2.4. 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
	Exposure to food enzyme–TOS
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
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 (based on examples given by applicant)	+
Use of recipe fractions in disaggregation FoodEx categories likely to contain the food enzyme	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solid.
+: 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.

3.3. Toxicological data

Batches 4–6 (Table 1) were used for the toxicological assays (batch 4 used for the *in vitro* micronucleus test; batch 5 for the repeated dose 90-day oral toxicity study; batch 6 for the bacterial reverse mutation test). They were obtained by the same steps (microfiltration, ultrafiltration, sterile

filtration) as applied to the commercial batches, except for an additional concentration step via freeze-drying for batch 5. Batches 4 and 5 have the lowest specific activity (enzyme activity/mg TOS), which indicates that they are cruder than the three batches for commercialisation and their use for toxicological testing is considered suitable.

3.3.1. Genotoxicity

3.3.1.1. Bacterial Reverse Mutation Test

In order to investigate the potential of the food enzyme endo-1,4-β-xylanase to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in five strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537 and TA102), in the presence or absence of metabolic activation. Two experiments were carried out applying the plate incorporation assay (first experiment) and the pre-incubation method (second experiment) using five different concentrations: 312.5, 625, 1,250, 2,500 and 5,000 μg food enzyme/plate, corresponding to 15, 30, 60, 120 and 240 μg TOS/plate. Appropriate positive control chemicals and water for injection (as vehicle control) were evaluated concurrently. All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while negative controls were within the historical control ranges. No precipitate was observed at any tested concentration in any tester strain with or without S9-mix. No significant toxicity, as evident by the absence or reduction in the mean number of revertant colonies and absence or reduction in the background bacterial lawn, was observed in any strain or test condition used.

No significant increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix. Exceptions were strains TA1537 and TA98 in the absence of metabolic activation, where increases in the number of revertants were observed at the highest doses. However, these effects were not reproducible and were not considered to be biologically relevant. Therefore, the Panel concluded that the food enzyme did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.3.1.2. *In vitro* micronucleus test

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Duplicate cultures of whole blood were treated with culture medium (vehicle control), the food enzyme or appropriate positive controls. Two experiments were performed applying a short treatment (4 + 20 hours recovery) in the presence and absence of S9-mix, and a continuous treatment (20 + 28 hours recovery) without S9-mix. Three concentrations of the food enzyme per experimental condition were selected for the analysis of micronuclei (MN) (i) 1,250, 2,500 and 5,000 μg food enzyme/mL, corresponding to 50.38, 100.75 and 201.5 μg TOS/mL (short treatment); (ii) 3,000, 4,000 and 5,000 μg food enzyme/mL corresponding to ca. 71, 234 and 355 μg TOS/mL (continuous treatment). Two thousand cells were scored per concentration. The positive controls induced statistically significant increases in the frequency of micronucleated binucleated cells (MNBN), demonstrating the sensitivity of the test system and the efficacy of the S9-mix. No significant cytotoxicity was observed after treatments both in the presence and absence of S9-mix. No statistically significant increase in the frequency of MNBN was observed at any concentration and treatment condition. The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce an increase in the frequency of cells containing micronuclei in cultured human lymphocytes under the test conditions employed for this study.

3.3.2. Repeated dose 90-day oral toxicity study in rodents

A 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 the food enzyme (batch 5) at the dose levels of 1,920, 2,880 and 3,840 ADXU/kg bw per day, corresponding to 221, 332 and 443 mg TOS/kg bw per day (referred as low, mid and high dose, respectively) by oral gavage during a 13 week period. The control group received the same amount of water (1.17 mL/kg bw per day) by gavage as given to the highest dose group with food enzyme.

There were no premature deaths or unscheduled sacrifices during the study, apart from a single male given low dose, sacrificed prematurely in week 13 after blood sampling. During the 13-week treatment period, hypersalivation was observed in some test item-treated groups in both sexes. These observations

were considered as test item treatment related but were considered as non-adverse. No treatment-related changes in the Functional Observation Battery tests, mean body weight, food intake, ophthalmology, organ weight, macroscopic and microscopic pathology were observed. In haematology decreases in mean cell haemoglobin and mean cell haemoglobin concentration were observed in high dose females. Blood biochemistry investigations showed a statistically significant increase in alkaline phosphatase activity in high–dose males and females. There was a statistically significant increase of cholesterol in mid and high–dose females. These changes were attributed to the test item treatment but were considered of limited toxicological significance as they were without any microscopic correlates.

Overall, the Panel derived a NOAEL at the high dose level of 443 mg TOS/kg bw per day for both males and females.

A comparison of the NOAEL (443 mg TOS/kg bw per day) from the 90-day study with the highest estimated dietary exposure, calculated to be up to 0.325 mg TOS/kg bw per day, resulted in a margin of exposure (MOE) of 1,363, indicating that there is no concern.

3.4. 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 allergenicity of endo-1,4-β-xylanase produced from the genetically modified *B. subtilis* strain LMG S-27588 has been assessed by comparison of its amino acid sequence with those of known allergens, according to the EFSA 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 criterion, no match was found.

Several cases of respiratory allergy following occupational inhalation of aerosols containing xylanase (Elms et al., 2003) or other enzymes have been reported (Martel et al., 2010). However, several studies have shown that adults with occupational asthma to an enzyme (such as α-amylase) can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). However, only incidental cases have been described where ingestion of α-amylase led to adverse reaction in patients sensitised through the respiratory route (Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information is not reported for xylanase. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of xylanase produced from the genetically modified *B. subtilis* strain LMG S-27588, in individuals respiratory sensitised to xylanase cannot be excluded, but is considered to be low. In addition, no information is available on oral sensitisation or elicitation reactions of this xylanase. The potential cross reactivity of food enzymes was studied by Bindslev-Jensen et al. (2006) There were no indications of cross reactivity between 19 different commercial food enzymes and the main allergens represented by 400 patients (allergic to inhalation allergens, food allergens, allergens of bee or wasp or drugs) included in this study.

Taken together, the Panel considers that there are no indications for allergic reactions by dietary exposure to the food enzyme endo-1,4-β-xylanase produced from the genetically modified *B. subtilis* strain LMG S-27588.

Conclusions

Based on the genetic modifications, the manufacturing process, the compositional and biochemical data, the dietary exposure, the findings in the toxicological studies and allergenicity assessment, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced from *B. subtilis* strain LMG S-27588 by the company Puratos N. V. does not give rise to safety concerns under the intended conditions of use.

The Panel noted that recombinant DNA was demonstrated to be present in all batches of the food enzyme tested.

Remark

The carrier of the commercial enzyme preparation is food grade [redacted]. As [redacted] contains substances and products causing allergies (respiratory and food allergies) and intolerances (gluten intolerance) (Regulation (EU) No 1169/2011)[8], the food enzyme preparation might contain [redacted] allergens and gluten, which may give rise to safety concerns in [redacted]-allergic and gluten-intolerant consumers.

Documentation provided to EFSA

1) Dossier "Application for authorisation of endo-1.4-β-xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-27588 in accordance with Regulation (EC) No 1331/2008", June 2015. Submitted by Puratos N. V.
2) Additional information was received from Puratos in August 2017.

References

Armentia A, Díaz-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernández S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et immunopathologia, 37, 203–204. https://doi.org/10.1016/j.aller.2009.05.001

[redacted]

Baur X and Czuppon AB, 1995. Allergic reaction after eating alpha-amylase (Asp o 2)-containing bred. A case report. Allergy, 50, 85–87.

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P and Brinch DS, 2006. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. Food and Chemical Toxicology, 44, 1909–1915. https://doi.org/10.1016/j.fct.2006.06.012

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502; quiz 502, 426.

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal a-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? Allergy, 52, 346–349.

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;1305, 1–26. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009; 7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Karenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares Pocas MF, Tlustos C, Wolfle D, Zorn H, Zugravu C-A, Arcella D, Liu Y and Engel K-H, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016; 14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740. Available online: www.efsa.europa.eu/efsajournal

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

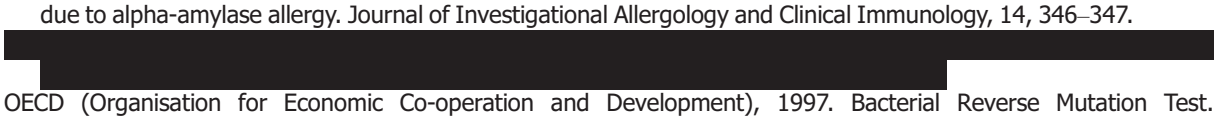
Elms J, Fishwick D, Walker J, Rawbone R and Jeffrey P, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmental Medicine, 60, 802–804. https://doi.org/10.1136/oem.60.10.802

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf

Kanny G and Moneret-Vautrin DA, 1995. Alpha-Amylase contained in bread can induce food allergy. Journal of Allergy and Clinical Immunology, 95(1 Pt 1), 132–133.

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN–75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.EN–75

Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to alpha-amylase allergy. Journal of Investigational Allergology and Clinical Immunology, 14, 346–347.



OECD (Organisation for Economic Co-operation and Development), 1997. Bacterial Reverse Mutation Test. Guideline 471, adopted 21.07.1997. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-operation and Development), 1998. Repeated Dose 90-day Oral Toxicity Study in Rodents. Guideline 408, adopted 21.09.1998. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

OECD (Organisation for Economic Co-operation and Development), 2010. Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test. OECD Publishing, Paris. Available online: https://doi.org/10.1787/9789264091016-en

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition and Food Research, 48, 413–423. https://doi.org/10.1002/mnfr.200400029

Abbreviations

ADXU	Xylanase Units
AMFEP	Association of Manufacturers and Formulators of Enzyme Product
AMR	Antimicrobial resistance
Bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
FAO	Food and Agricultural Organisation
GLP	Good Laboratory Practice
GM	Genetically Modified
GMO	Genetically Modified Organisms
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
LMG	Laboratory of Microbiology, university of Gent
MOE	Margin of exposure
MN	micronuclei
MNBN	micronucleated binucleated (cells)
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 – 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, Finland, Germany, Italy, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, 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, 2011a).

Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01	Grains and grain-based products (unspecified)	0.8	1	29
A.01.03	Grain milling products (unspecified)	1	1	29
A.01.03.001	Wheat milling products (unspecified)	1	1	29
A.01.03.001.001	Wheat flour, brown	1	1	29
A.01.03.001.002	Wheat flour, Durum	1	1	29
A.01.03.001.003	Wheat flour, white	1	1	29
A.01.03.001.004	Wheat flour, wholemeal	1	1	29
A.01.03.001.005	Graham flour	1	1	29
A.01.03.001.006	Wheat flour, gluten free	1	1	29
A.01.03.001.014	Wheat starch	1.2	1	29
A.01.03.002	Rye milling products (unspecified)	1	1	29
A.01.03.002.001	Rye flour, gluten free	1	1	29
A.01.03.002.002	Rye flour, light	1	1	29
A.01.03.002.003	Rye flour, medium	1	1	29
A.01.03.002.004	Rye flour, wholemeal	1	1	29
A.01.03.003	Buckwheat milling products (unspecified)	1	1	29
A.01.03.003.001	Buckwheat flour	1	1	29
A.01.03.004	Corn milling products (unspecified)	1	1	29
A.01.03.004.001	Corn flour	1	1	29
A.01.03.004.003	Corn starch	1.3	1	29
A.01.03.005	Oat milling products (unspecified)	1	1	29
A.01.03.005.002	Oat flour	1	1	29
A.01.03.005.004	Oat starch	1.2	1	29
A.01.03.006	Rice milling products (unspecified)	1	1	29
A.01.03.006.001	Rice flour	1	1	29
A.01.03.006.002	Rice flour white	1	1	29
A.01.03.006.003	Rice flour, instant	1	1	29
A.01.03.006.004	Rice starch	1.2	1	29
A.01.03.007	Spelt milling products	1	1	29
A.01.03.008	Other milling products (unspecified)	1	1	29
A.01.03.008.001	Amaranth flour	1	1	29
A.01.03.008.002	Barley flour	1	1	29
A.01.03.008.003	Chapatti flour	1	1	29
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	29
A.01.03.008.005	Millet flour	1	1	29
A.01.03.008.007	Sorghum flour	1	1	29
A.01.04	Bread and rolls (unspecified)	1	0.7	29
A.01.04.001	Wheat bread and rolls	1	0.7	29
A.01.04.002	Rye bread and rolls	1	0.7	29
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	29
A.01.04.004	Multigrain bread and rolls	1	0.7	29
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	29
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	29

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction[b]	mg TOS/kg flour
A.01.04.005.002	Crisp bread, rye, light	1	0.9	29
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	29
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	29
A.01.04.005.005	Rusk, light	1	0.9	29
A.01.04.005.006	Rusk, wholemeal	1	0.9	29
A.01.04.005.007	Pita bread	1	0.7	29
A.01.04.005.008	Matzo	1	0.9	29
A.01.04.005.009	Tortilla	1	0.7	29
A.01.04.006	Other bread	1	0.7	29
A.01.04.007	Bread products	1	0.7	29
A.01.07	Fine bakery wares (unspecified)	1	0.5	29
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	29
A.01.07.001.001	Beignets	1	0.15	29
A.01.07.001.002	Buns	1	0.7	29
A.01.07.001.003	Cake from batter	1	0.25	29
A.01.07.001.004	Cheese cream cake	1	0.24	29
A.01.07.001.005	Cheese cream sponge cake	1	0.24	29
A.01.07.001.006	Chocolate cake	1	0.24	29
A.01.07.001.007	Chocolate cake with fruits	1	0.24	29
A.01.07.001.008	Cream cake	1	0.24	29
A.01.07.001.009	Cream cheese cake	1	0.24	29
A.01.07.001.010	Cream custard cake	1	0.24	29
A.01.07.001.011	Cream custard sponge cake	1	0.24	29
A.01.07.001.012	Croissant	1	0.5	29
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	29
A.01.07.001.014	Croissant, filled with cream	1	0.5	29
A.01.07.001.015	Croissant, filled with jam	1	0.5	29
A.01.07.001.016	Croquembouche	1	0.15	29
A.01.07.001.017	Doughnuts	1	0.24	29
A.01.07.001.018	Clair	1	0.15	29
A.01.07.001.019	Flan	1	0.5	29
A.01.07.001.020	Fruit cake	1	0.6	29
A.01.07.001.021	Fruit pie	1	0.15	29
A.01.07.001.022	Cheese pie	1	0.15	29
A.01.07.001.023	Fruit tart	1	0.15	29
A.01.07.001.024	Gingerbread	1	0.6	29
A.01.07.001.025	Gougere	1	0.15	29
A.01.07.001.026	Kringles	1	0.25	29
A.01.07.001.027	Nut cream cake	1	0.24	29
A.01.07.001.028	Pancakes	1	0.25	29
A.01.07.001.029	Profiterole	1	0.15	29
A.01.07.001.030	Pyramid cake	1	0.25	29
A.01.07.001.031	Rhubarb flan	1	0.15	29
A.01.07.001.032	Scone	1	0.5	29
A.01.07.001.033	Sponge dough	1	0.25	29
A.01.07.001.034	Sponge cake	1	0.25	29

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[(a)]	Recipe fraction[(b)]	mg TOS/kg flour
A.01.07.001.035	Sponge cake roll	1	0.25	29
A.01.07.001.036	Muffins	1	0.25	29
A.01.07.001.037	Waffles	1	0.25	29
A.01.07.001.038	Apple strudel	1	0.15	29
A.01.07.001.039	Cream-cheese strudel	1	0.24	29
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	29
A.01.07.001.041	Croissant from puff pastry	1	0.6	29
A.01.07.001.042	Brioche	1	0.5	29
A.01.07.001.044	Lebkuchen	1	0.6	29
A.01.07.001.045	Dumpling	1	0.5	29
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	29
A.01.07.001.047	Marzipan pie	1	0.25	29
A.01.07.001.048	Baklava	1	0.15	29
A.01.07.002	Biscuits (cookies)	1	0.9	29
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	29
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	29
A.01.07.002.003	Biscuits, cream filling	1	0.81	29
A.01.07.002.004	Biscuits, fruit filling	1	0.81	29
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	29
A.01.07.002.006	Butter biscuits	1	0.81	29
A.01.07.002.007	Biscuit, iced	1	0.81	29
A.01.07.002.008	Speculaas	1	0.9	29
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	29
A.01.07.002.010	Biscuits, oat meal	1	0.9	29
A.01.07.002.011	Biscuits, spelt meal	1	0.9	29
A.01.07.002.012	Biscuits, salty	1	0.9	29
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	29
A.01.07.002.014	Sticks, salty	1	0.81	29
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	29
A.18.04.001	Find bakery products for diabetics	1	0.5	29
A.19.01.001	Sandwich and sandwich-like meal	1	0.32	29
A.19.01.002	Pizza and pizza-like pies	1	0.3	29

TOS: total organic solids.
(a): Available at see http://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf
(b): Derived from publically available recipe information, and/or food label information (such as Mintel's Global New Products Database http://www.mintel.com/global-new-products-database).

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

Information provided in this appendix is shown in an excel file (downloadable http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2018.5169/suppinfo).

The file contains two sheets, corresponding to two tables.

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

Table C.2: The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure.