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Safety evaluation of the food enzyme xylanase from *Bacillus pumilus* (strain BLXSC)

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Abstract

The food enzyme 1,4- β -D-xylan xylanohydrolase (EC 3.2.1.8) is produced with the non-genetically modified strain *Bacillus pumilus* (strain BLXSC) by Advanced Enzyme Technologies Ltd. The food enzyme is intended to be used in baking processes, grain treatment for the production of starch and gluten fractions, and distilled alcohol production. Since residual amounts of the food enzyme are removed by distillation and during grain treatment, dietary exposure was only calculated for baking processes. Based on the maximum recommended use levels for baking processes, and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.138 mg TOS/kg body weight (bw) per day. As the production strain of *B. pumilus* meets the requirements for a Qualified Presumption of Safety (QPS) approach, no toxicological data are required. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that under the intended conditions of use (other than distilled alcohol production), the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but is considered to be low. Based on the QPS status of the production strain and the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1332/2008¹ 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 1331/2008² 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 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 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 European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008¹ on food enzymes.

Three applications have been submitted by the company 'Advanced Enzyme Technologies Ltd.' and 'Novozymes A/S' of the food enzymes pectinase, polygalacturonase, pectinesterase, pectin lyase, and arabanase from *Aspergillus niger* (strain ANSC), xylanase from *Bacillus pumilus* (strain BLXSC) and asparaginase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-CK).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008², 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 (EC) requests the European Food Safety Authority (EFSA) to carry out safety assessments on the food enzymes pectinase, polygalacturonase, pectinesterase, pectin lyase,

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.

and arabanase from *Aspergillus niger* (strain ASNSC), xylanase from *Bacillus pumilus* (strain BLXSC) and asparaginase from a genetically modified strain of *Bacillus subtilis* (strain NZYM-CK) in accordance with Article 17.3 of Regulation (EC) No 1332/2008¹ 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 xylanase from a non-genetically modified microorganism *B. pumilus* (strain BLXSC).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme xylanase from non-genetically modified *B. pumilus* (strain BLXSC).

Additional information was requested from the applicant during the assessment process on 19 April 2018 and on 16 October 2018 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, 2009) and following the relevant existing guidance from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA CEF Panel, 2009) has been followed 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. Assessment⁴

IUBMB nomenclature: Endo-1,4- β -xylanase

Systematic name: 1,4- β -D-Xylan xylanohydrolase

Synonyms: endo-(1 \rightarrow 4)- β -xylan 4-xylanohydrolase; xylanase; β -1,4-xylanase; endo-1,4-xylanase; endo- β -1,4-xylanase; 1,4- β -xylan xylanohydrolase; β -xylanase; β -1,4-xylan xylanohydrolase

IUBMB No: EC 3.2.1.8

CAS No: 9025-57-4

EINECS No: 232-800-2

Xylanases catalyse the hydrolysis of 1,4- β -D-xylosidic endo-linkages in xylan (including arabinoxylan, which is xylan branched with arabinose), resulting in the generation of (1 \rightarrow 4)- β -D-xylan oligosaccharides of different lengths. It is intended to be used in baking processes, grain treatment for the production of starch and gluten fractions, and distilled alcohol production.

3.1. Source of the food enzyme⁵

The xylanase is produced with the non-genetically modified strain of *B. pumilus* designated BLXSC,⁶ which is deposited at [REDACTED]

with number [REDACTED]

The production strain was identified as *B. pumilus* by [REDACTED]

[REDACTED] analysis confirmed this identification.⁹

⁴ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 3.

⁵ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 18–22; Technical dossier/Additional data, 9 January 2019.

⁶ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 18–22; Technical dossier/Annex I1.

⁷ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 18; Technical dossier/Annex I1.

⁸ Technical dossier/Annex I1; Technical dossier/Additional data, 9 January 2019.

⁹ Technical dossier/Additional data, 9 January 2019.

The species *B. pumilus* is included in the 'Qualified Presumption of Safety' (QPS) list with the qualifications of absence of cytotoxic activity and antimicrobial resistance activity (EFSA, 2007; EFSA BIOHAZ Panel, 2017). The absence of cytotoxicity against Vero cells¹⁰ and of haemolytic activity on blood agar plates¹¹ was demonstrated for *B. pumilus* BLXSC. Antimicrobial resistance of *B. pumilus* BLXSC was tested [REDACTED] as recommended by EFSA FEEDAP Panel (2018).¹⁰ [REDACTED] of the strain BLXSC indicates that it does not contain any acquired antimicrobial gene.⁹

3.2. Production of the food enzyme¹²

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004¹³, with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP¹⁴), 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, 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 ultrafiltration steps in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity and analysis of the substances used to control the fermentation 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 xylanase is a single polypeptide chain of 228 amino acids.¹⁵ The molecular mass of the mature protein, based on the amino acid sequence, was calculated to be 24 kDa.¹⁶ The homogeneity of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. The gels presented for all batches are comparable and consistently showed one major protein band with an apparent molecular weight close to 24 kDa.¹⁷ No other side enzyme activities were reported by the applicant.¹⁸

The in-house determination of xylanase activity¹⁹ is based on hydrolysis of beechwood xylan to reducing carbohydrates, which reduce dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, producing a colour (reaction conditions: pH 6.0, temperature 50°C, incubation time 20 min). One bacterial xylanase unit (BXU) is defined as the amount of enzyme required that liberates 1 micromole of reducing sugars (measured as xylose equivalents) from beechwood xylan in 1 min under the standard assay conditions.

The food enzyme has a temperature optimum of around 55°C (pH 6.0) and a pH optimum of around 6.5 (T = 50°C).²⁰ Thermostability²¹ was tested after pre-incubation of the food enzyme for 2 h at different temperatures. Under the conditions (pH 6.0) of the applied temperature stability assay, the xylanase showed no residual activity above 70°C.

¹⁰ Technical dossier/Annex I2.

¹¹ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 37–38; Technical dossier/Annex I2.

¹² Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 23–26; Technical dossier/Annex G.

¹³ 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.

¹⁴ Technical dossier/Annex F.

¹⁵ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 5.

¹⁶ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 4.

¹⁷ Technical dossier/p. 4; Technical dossier/Annex B; Technical dossier/Additional data, 10 July 2018.

¹⁸ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 10.

¹⁹ Technical dossier/Annex A2 and C.

²⁰ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 11; Technical dossier/Annex C.

²¹ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 12.

3.3.2. Chemical parameters²²

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The average Total Organic Solids (TOS) of the three commercial batches was 87.45%. The three food enzyme batches presented in Table 1 are spray dried concentrates.

The average enzyme activity/mg TOS ratio of the three food enzyme batches for commercialisation is 51.18 BXU/mg TOS.

Table 1: Compositional data of the food enzyme preparation

Parameter	Unit	Batches ^(a)		
		1	2	3
Xylanase activity	BXU/g batch ^(b)	47,321	44,453	42,542
Protein	%	41.14	39.54	38.89
Ash	%	5.64	6.22	6.87
Water	%	5.79	6.34	6.79
Total Organic Solids (TOS) ^(c)	%	88.57	87.44	86.34
Xylanase activity/mg TOS	BXU/mg TOS	53.43	50.84	49.27

BXU: Bacterial Xylanase Unit; TOS: Total Organic Solids.

(a): Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 4, 6; Technical dossier/Annex A3; Annex D and Annex E.

(b): BXU/g: Bacterial Xylanase Unit/g (see Section 3.3.1).

(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches was below 0.5 mg/kg which complies with the specification for lead (≤ 5 mg/kg)²³ as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of heavy metals (arsenic, mercury, cadmium) were below the limits of detection (LODs) of the employed methodologies.²⁴

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.²⁵ No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).²⁶

The presence of mycotoxins (aflatoxin B1, B2, G1, G2, zearalenone, ochratoxin A, deoxynivalenol, T-2 toxin, HT-2 toxin, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine²⁷) was examined in three food enzyme batches and were below LOD of the applied analytical methods.²⁸

3.4. Toxicological data

No toxicological tests were provided by the applicant.

The Panel considers no toxicological studies other than assessment of allergenicity necessary. This is based on the QPS status of the production strain (see Section 3.1) and the absence of any hazards arising from the manufacturing of the food enzyme.

²² Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 4; Technical dossier/Annex A1, A2 and C.

²³ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 9; Technical dossier/Annex D; LOD/LOQ: Pb = 0.25 mg/kg.

²⁴ Technical dossier/Annex D; LOD/LOQ: As = 0.25 mg/kg; Cd = 0.25 mg/kg; Hg = 0.025 mg/kg.

²⁵ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 9; Technical dossier/Annex A1.

²⁶ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 9; Technical dossier/Annex J3.

²⁷ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 9; Technical dossier/Annex E.

²⁸ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 9; Technical dossier/Annex E: LOD for aflatoxin B1, B2, G1, G2, ochratoxin A < 1 µg/kg; LOD for zearalenone < 5 µg/kg; LOD for deoxynivalenol: < 25 µg/kg; LOD for T-2 toxin < 10 µg/kg, LOD for HT-2 toxin: < 50 µg/kg; LOD for ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine < 100 µg/kg.

3.4.1. Allergenicity²⁹

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 xylanase produced with *B. pumilus* (strain BLXSC) 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.³⁰

No information is available on oral sensitisation or elicitation reactions of this 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; Harris-Roberts et al., 2009; Martel et al., 2010; Budnik et al., 2017) and case reports (Tarvainen et al., 1991; Baur et al., 1998; Merget et al., 2001; Lipińska-Ojrzanowska et al., 2016). 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). Overall, allergic reactions upon oral ingestion of this xylanase, produced with the non-genetically modified *B. pumilus* strain BLXSC in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur is low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011³¹) are used as raw materials (██████████³²) in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

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 removed, as is the case for distilled alcohol production.

The Panel considered that, under the intended conditions of use (other than distilled alcohol production), 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

This food enzyme is intended to be used in three food manufacturing processes at the recommended use levels summarised in Table 2.

²⁹ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 39–40; Technical dossier/Additional data/10 July 2018.

³⁰ Technical dossier/Additional data/10 July 2018.

³¹ Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

³² Technical dossier/Appendix 2 of Annex G.

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

Food manufacturing process ^(b)	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	2.33–11.6 mg TOS/kg flour
Grain treatment for the production of starch and gluten fractions	Grain or flour	23.32–46.64 mg TOS/kg grain or flour
Distilled alcohol production	Milled grain	23.32–46.64 mg TOS/kg dry milled grain

TOS: Total Organic Solids.

(a): Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 29–33.

(b): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

In baking processes,³³ the xylanase is added during the preparation of the dough. It hydrolyses (arabino)xylans, which interact with gluten and bind water, thus reducing the dough viscosity and shortening the processing time. The decrease in viscosity facilitates the handling of the dough, results in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness).

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

In grain treatment for the production of starch and gluten fractions,³⁴ the xylanase is added during dough preparation. It is used to reduce technical difficulties (e.g. high viscosity), to give higher yield due to efficient hydrolysis of xylans, improving starch purity due to greater extraction yield of the high value fraction and efficient removal of fibres and proteins, resulting in more efficient processes and more consistent product quality.

Analytical data have been provided to investigate the transfer of food enzyme–TOS (several activities from different sources) into the starch and gluten fractions during grain treatment (Documentation provided to EFSA No. 5). The data provided includes a theoretical calculation of enzyme transfer based on measured amounts of intermediate and final fractions; and provision of measured enzymatic activities in the weighed intermediate and final products. Based on both data sets, a removal of > 99% of the amount of enzyme added to the raw material (e.g. grain, flour) during production of the final fractions (starch and gluten) can be estimated. The Panel considered the evidence as sufficient to conclude that TOS (including substances other than proteins) are efficiently removed by mechanical separation and repeated washing, resulting in negligible amount of residual TOS remaining in the starch and gluten fractions.

In distilled alcohol production, the xylanase food enzyme is applied during liquefaction and fermentation, and may also be added during slurry mixing and pre-saccharification. Endo-1,4- β -xylanase catalyses the hydrolysis of 1,4- β -D-xylosidic linkages in xylan. The reaction products are (1 \rightarrow 4)- β -D-xylan oligosaccharides of different lengths.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production (Documentation provided to EFSA No. 6). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed during grain treatment for the fractionation of starch and gluten and by distillation (see Section 3.5.1), dietary exposure to the food enzyme resulting from these two processes was not calculated.

For baking processes, 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 and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

³³ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 31.

³⁴ Technical dossier/Section 3: Subsection 3.2: Risk Assessment Data/p. 32.

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1/Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual 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 3 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 3: Summary of estimated dietary exposure to food enzyme-TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.002–0.032 (10)	0.024–0.069 (14)	0.028–0.067 (19)	0.015–0.043 (18)	0.011–0.027 (19)	0.011–0.024 (18)
Min–max 95th percentile (number of surveys)	0.013–0.138 (8)	0.061–0.118 (12)	0.055–0.126 (19)	0.034–0.087 (17)	0.025–0.052 (19)	0.023–0.041 (18)

TOS: Total Organic Solids.

Based on the maximum use levels recommended for baking processes and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme-total organic solids was estimated to be up to 0.138 mg TOS/kg bw per day.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

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

Sources of uncertainties	Direction of impact
Use of recipe fractions in disaggregation FoodEx categories	+/-
Use of technical factors in the exposure model	+/-
Exclusion of other processes from the exposure estimate:	
– distilled alcohol production	–
– grain treatment for the production of starch and gluten fractions	–

+: uncertainty with potential to cause overestimation of exposure; -: uncertainty with potential to cause underestimation of exposure.

FoodEx: a standardised food classification and description system; TOS: Total Organic Solids.

The conservative approach applied to the exposure estimate to food enzyme–TOS in baking processes, 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.

The exclusion of two food manufacturing processes (distilled alcohol production and grain treatment for the production of starch and gluten fractions – see Table 4) from the exposure assessment was based on > 99% of TOS removal during both processes and is not expected to have an impact on the overall estimate derived.

4. Conclusions

Based on the QPS status of the production strain and the data provided, the Panel concluded that the food enzyme xylanase produced with non-genetically modified strain *B. pumilus* (strain BLXSC) does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

- 1) Technical dossier 'Application for authorisation of xylanase from *Bacillus pumilus* (strain BLXSC) in accordance with Regulation (EC) No 1331/2008'. 21 October 2014. Submitted by Advanced Enzyme Technologies Ltd.
- 2) Summary report on technical data. Delivered by contractor Hylobates Consulting and BiCT, 29 January 2016.
- 3) Additional information. 10 July 2018. Submitted by Advanced Enzyme Technologies Ltd.
- 4) Additional information. 9 January 2019. Submitted by Advanced Enzyme Technologies Ltd.
- 5) Additional information on 'Grain processing/Fate of the food enzymes'. 26 April 2018 and 13 July 2018. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and Starch Europe. Unpublished document.
- 6) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry-over in glucose syrups'. 22 February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP). Unpublished document.

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Abbreviations

AMFEP Association of Manufacturers and Formulators of Enzyme Products



bw	body weight
BXU	Bacterial Xylanase Unit
CAS	Chemical Abstracts Service
CFU	colony forming units
█	█
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
FoodEx	a standardised food classification and description system
GM	genetically modified
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
█	█
QPS	Qualified Presumption of Safety
█	█
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization
█	█

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

Information provided in this appendix is shown in an excel file (downloadable <https://efsa.onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2019.5901/supinfo>).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

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