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Safety evaluation of the food enzyme endo-1,4-β-xylanase from *Bacillus subtilis* (strain XAS)

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Abstract

The food enzyme is an endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) produced with the genetically modified *Bacillus subtilis* strain XAS. Antibiotic resistance genes are present in the production organism on a self-replicative vector. The endo-1,4-β-xylanase is intended to be used in baking processes. Based on the maximum use levels, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.014 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 rats. The Panel identified a no-observed-adverse-effect level (NOAEL) of 55 mg TOS/kg bw per day that, compared with the estimated dietary exposure, results in a sufficiently high margin of exposure (MOE) (of at least 3,600). 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, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood to occur is considered to be low. Since the absence of viable cells in the food enzyme has not been adequately demonstrated, the Panel cannot conclude on the risks associated with the possible spread of a genetically modified bacterial strain carrying antimicrobial resistance determinants.

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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Amendment: An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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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:

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

Two applications have been introduced by the companies Novozymes A/S and DSM Food Specialties B.V. for the authorisation of the food enzymes serine protease (chymotrypsine) from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-RH) and endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* (strain XAS).

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 two applications fall within the scope of the food enzyme Regulation and contains 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 (EFSA) to perform the safety assessments on the food enzymes serine protease (chymotrypsine) from a genetically modified

[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.

strain of *Bacillus licheniformis* (strain NZYM-RH) and endo-1,4-β-xylanase obtained with from a genetically modified strain of *Bacillus subtilis* (strain XAS) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.3. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of food enzyme endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* (strain XAS).

1.2. Information on existing authorisation and evaluations

The applicant reports that the French food authorities have evaluated and authorised the use of xylanase produced by genetically modified *B. subtilis* strain XAS in a number of food- and beverage-manufacturing processes.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase produced with a genetically modified *B. subtilis* (strain XAS).

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 10 March 2015 and 4 October 2016 and was consequently provided (see 'Documentation provided to EFSA'). However, some of the data requested in October 2016 were not provided. Consequently, the Panel concluded this assessment on the basis of the available data.

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) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following the relevant existing guidance's of the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (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. 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.

The food enzyme catalyses the hydrolysis of endo-1,4-β-D-xylose 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. It is intended to be used in baking processes.

3.1. Source of the food enzyme

The endo-1,4-β-xylanase is produced with the genetically modified bacterium *B. subtilis* strain XAS, which is only deposited in the [redacted] The Panel noted that this would not allow a verification of the strain independently of the company.

[redacted][4]

[4] Technical Dossier: Annex II-2 and II-4.

[redacted][5]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted][6]

3.1.2. Characteristics of the introduced sequences

[redacted][7]

3.1.3. Description of the genetic modification process

[redacted]

3.1.4. Safety aspects of the genetic modification

The production strain *B. subtilis* XAS differs from the recipient strain *B. subtilis* [redacted] by its enhanced expression of endo-1,4-β-xylanase [redacted]

The genetic stability of the production strain *B. subtilis* XAS was demonstrated [redacted][8] The consistency of enzyme activity observed in three batches intended for commercialisation (Table 1) indicates that the production strain is phenotypically stable.

[redacted]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP)[10] and in accordance with current Good Manufacturing Practice (GMP).

The applicant originally proposed two alternative methods for elimination of the viable cells from the food enzyme, but then informed that only the one assessed in this opinion is used. The production strain is grown as a pure culture using a typical industrial medium in a contained, submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the 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

[5] Technical Dossier/Annex II-5.
[6] Technical Dossier/Additional information November 2015/Annex 3.
[7] Technical Dossier: Annex II-7 and II-8.
[8] Technical Dossier/p 94.
[9] 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.
[10] Technical dossier: Annex I-5.

concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded as well as final germ filtration. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[11]

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 is a single polypeptide chain of 213 amino acids including a signal peptide of 28 amino acids, which is cleaved off during secretion of the enzyme protein. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 22 kDa. The homogeneity of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. Gels presented for three food enzyme batches used for commercialisation and the batch used for toxicological testing (Table 1) are comparable, showing one main protein band of the expected size, accompanied by several minor bands.[12] No other enzymatic side activities were reported.

The in-house determination of enzyme activity is based on hydrolysis of the substrate wheat arabinoxylan (reaction conditions: pH 6.0, 30°C, 30 min). The enzyme activity is determined by measuring the release of reducing carbohydrates, after subsequent addition of a hexacyanoferrate reagent (Hoffman reagent) and heating to 100°C to develop a colour, which is measured spectrophotometrically at 420 nm. The enzyme activity is expressed in New Bakery Xylanase Units/g (NBXU/g). One NBXU is defined as the amount of enzyme that produces 0.5 mg of xylose equivalents in the incubation mixture under the assay conditions (pH 6.0, 30°C, incubation time 30 min).[13]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 45°C (pH 6) and a pH optimum around pH 5–7 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 2, 5, 15, 30 and 60 min at different temperatures. Under the conditions (pH 6) of the applied temperature stability assay, endo-1,4-β-xylanase activity decreased above 52°C showing no residual activity above 65°C when pre-incubated for 2 min.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological tests (Table 1). The average total organic solids (TOS) content of the three food enzyme batches for commercialisation was 7.1% (range 6.0–8.9%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 396.

Table 1: Compositional data of the food enzyme

Parameter	Units	Batches			
		1	2	3	4[(a)]
Endo-1,4-β-xylanase activity	NBXU/g batch[(b)]	33,500	22,600	27,500	17,400
Protein	%	4.4	2.9	3.2	3.3
Ash	%	1.3	1.0	0.8	0.9
Water	%	89.8	92.6	93.2	93.6
Total organic solids (TOS)[(c)]	%	8.9	6.4	6.0	5.5
Activity/mg TOS	NBXU/mg TOS	376.4	353.1	458.3	314.6

(a): Batch used for the toxicological studies.
(b): NBXU: New Bakery Xylanase Units (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

[11] Technical dossier: Section 3.2.1.2.5.
[12] Technical Dossier/Additional information November 2015.
[13] Technical dossier/Annex I-2.
[14] Technical Dossier/pp. 45–46 and Additional information November 2015.

3.3.3. Purity

The lead content in the three commercial batches was below 2 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 *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[16]

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 tested [redacted] Although requested, insufficient information was provided on the methodology applied, so the Panel is not able to conclude on the absence of viable cells in the product. The Panel considers this as a critical issue, because the production strain carries a multicopy plasmid with antimicrobial resistance genes.

The absence of recombinant DNA [redacted] was demonstrated [redacted][17]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1) used in these studies has similar protein pattern and similar chemical purity, and thus is considered suitable for testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test[18]

The Ames test was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP)[18] in four strains of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98) and *Escherichia coli* WP2uvrA in the presence or absence of metabolic activation (S9-mix). The direct plate incorporation method was applied. A single experiment in triplicate was carried out using five different concentrations of the food enzyme (62, 185, 556, 1,667 and 5,000 μg dry matter/plate, corresponding to 54, 159, 478, 1,433 and 4,297 μg TOS/plate). Upon treatment with the food enzyme there was no toxicity and no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test[19]

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP[19] in cultured human peripheral blood lymphocytes. Two experiments were performed, in concentrations of 1,250, 2,500 and 5,000 μg dry matter/mL (corresponding to 1,075, 2,150 and 4,300 μg TOS/mL) and 3,000, 4,000 and 5,000 μg dry matter/mL (corresponding to 2,580, 3,440 and 4,300 μg TOS/mL), respectively. In the first experiment in the presence and in the absence of S-9 mix, the treatment/harvest times were 4/24 h (pulse treatment)

[15] Technical dossier/Annex I-3. LOD = 0.006 mg/L sample solution.
[16] Technical dossier/Annex I-3.
[17] Technical dossier: Annex II-11.
[18] Technical dossier: Annex I-16
[19] Technical dossier: Annex I-17.

and in the absence of S-9 24/24 h (continuous treatment). In the second test, in the presence of S-9 mix, the treatment/harvest times were 4/24 h and 4/48 h (pulse treatment) and, in the absence of S-9 mix, 24/24 h and 48/48 h (continuous treatment).

For all food enzyme concentrations used, the frequency of cells with chromosomal aberrations was similar to that of negative controls. A decrease in the mitotic index was observed in both experiments after exposure to food enzyme, but it did not exceed 61% of negative control. The Panel concluded that the food enzyme xylanase did not induce chromosomal aberration in cultured human peripheral blood lymphocytes when tested up to 5,000 μg food enzyme dry matter/ml (corresponding to ca. 4,300 μg TOS/mL), under the experimental conditions employed for this study.

Therefore, the Panel concluded that on the basis of the *in vitro* studies there is no concern for genotoxicity for the xylanase tested.

3.4.2. Repeated dose 90-day oral toxicity study in rodents[20]

A repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[20] Four groups of 10 male and 10 female Wistar (WU) rats received by gavage the food enzyme in doses corresponding to 2.8, 11 and 55 mg TOS/kg body weight (bw) per day. Controls received the vehicle (double distilled water).

All animals survived until the scheduled termination.

Hair thinning with regrowth, randomly distributed among the groups was observed. This is a common finding in laboratory rat and as such of not toxicological relevance.

Body weight and body weight gain of the test male groups were similar to those of the control group. In the test female groups, statistically significantly increased body weight at the low dose in weeks 8, 9 and 12, and body weight gain in weeks 3–9 and week 12 at the low dose, in week 8 at the mid dose and in week 3 at the high dose were recorded. These effects were not dose-related and/or transitory and therefore of no toxicological concern.

Feed intake was statistically significantly higher in low-dose males in weeks 6 and 10, and in low-dose females in weeks 3 and 4, and in week 9 in the high-dose females. As these were isolated findings without dose–response relationship they were considered incidental.

Among functional observation parameters the only statistically significant differences to controls were higher hind limb foot splay values in mid-dose males, and lower hind limb foot splay values in all test female groups. In view of lack of dose–response relationship, of different direction of change between males and females, and that the values were within the range of historical control data for the laboratory, these findings were considered not of toxicological significance.

Haematological examination revealed a statistically significantly increased relative neutrophil count and a statistically significantly decreased absolute and relative lymphocyte count in high-dose males. Both changes appeared to be dose related but this effect was not observed in females. Red blood cell count and haematocrit values were significantly decreased in high-dose females. These changes were recorded in one sex only, and were considered to represent normal biological variation.

Among clinical chemistry parameters a statistically significantly increased albumin level in mid-dose males and a statistically significantly increased sodium concentration in high-dose females were observed. As none of these effects were dose-related and in one sex only, they were considered not to be of toxicological significance.

A significant decrease of the relative adrenal weight was observed in the mid-dose females, which is considered incidental as no corresponding gross and histopathological changes were noted.

No other significant effects were reported. The Panel identified a no-observed-adverse-effect level (NOAEL) of 55 mg TOS/kg bw per day, the highest dose tested.

3.5. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of endo-1,4-β-xylanase produced with the genetically modified *B. subtilis* (strain XAS) has been assessed by comparing its amino acid sequence with those of known allergens according to the Guidance on allergenicity assessment of genetically modified plants (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

[20] Technical dossier: Annex I-18.

No information is available on oral sensitisation or elicitation reactions of this endo-1,4-β-xylanase. 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 XAS in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

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.

3.6. Dietary exposure

3.6.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at a recommended use level of up to 1.28 mg TOS/kg flour.

In baking processes, the food enzyme is added to flour during the preparation of dough. It hydrolyses (arabino)xylans, which interact with gluten and bind water, thus contributing to the reduction of dough viscosity. The decrease in viscosity facilitates the handling of the dough, gives improved crumb structure and increases the volume.

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.

3.6.2. Dietary exposure estimation

For the 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[21] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant 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).

[21] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Table 2: Summary of estimated dietary exposure to food enzyme-TOS in six age classes

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 of means (number of surveys)	0.000–0.004 (10)	0.003–0.008 (14)	0.003–0.007 (19)	0.002–0.005 (18)	0.001–0.003 (19)	0.001–0.003 (18)
Min–max of 95th percentiles (number of surveys)	0.001–0.015 (8)	0.007–0.013 (12)	0.006–0.014 (19)	0.004–0.010 (17)	0.003–0.006 (19)	0.002–0.005 (18)

TOS: total organic solid.

3.6.3. Uncertainty analysis

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

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

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

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.

3.7. Margin of exposure

A comparison of the NOAEL (55 mg TOS/kg bw per day) from repeated dose 90-day oral toxicity study in rats with the derived exposure estimated of 0.000–0.008 mg TOS/kg bw per day at the mean and from 0.001 to 0.015 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) of at least 3,667.

4. Conclusions

Based on the data provided, the intended food production process and the derived margin of exposure, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *B. subtilis* strain XAS does not give rise to toxicological safety concerns under the intended conditions of use.

Antibiotic resistance genes are present on a multicopy plasmid in the production organism. Since the absence of viable cells in the food enzyme has not been demonstrated, the Panel cannot conclude on the risks associated with the possible spread of a genetically modified bacterial strain carrying antimicrobial resistance determinants.

Documentation provided to EFSA

1) Dossier "Application for authorisation of endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* XAS". December 2013. Submitted by DSM Food Specialties.
2) Application for authorisation of endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* XAS. Additional information September 2014. Submitted by DSM Food Specialties.
3) Application for authorisation of endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* XAS. Additional information November 2015. Submitted by DSM Food Specialties.
4) Application for authorisation of endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* XAS. Additional information November 2016. Submitted by DSM Food Specialties.
5) Summary report on genotoxicity, subchronic toxicity study and allergenicity related to endo-1,4-β-xylanase produced with a strain of *Bacillus subtilis* (strain XAS) by DSM Food Specialties. December 2014. Delivered by FoBiG GmbH (Freiburg, Germany).

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CHO	Chinese hamster ovary
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
MOE	margin of exposure
MTT	3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide
NBXU	New Bakery Xylanase Units
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, 2011).

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	mg TOS/kg flour
A.01	Grains and grain-based products (unspecified)	0.8	1	1.28
A.01.03	Grain milling products (unspecified)	1	1	1.28
A.01.03.001	Wheat milling products (unspecified)	1	1	1.28
A.01.03.001.001	Wheat flour, brown	1	1	1.28
A.01.03.001.002	Wheat flour, Durum	1	1	1.28
A.01.03.001.003	Wheat flour, white	1	1	1.28
A.01.03.001.004	Wheat flour, wholemeal	1	1	1.28
A.01.03.001.005	Graham flour	1	1	1.28
A.01.03.001.006	Wheat flour, gluten free	1	1	1.28
A.01.03.001.014	Wheat starch	1.2	1	1.28
A.01.03.002	Rye milling products (unspecified)	1	1	1.28
A.01.03.002.001	Rye flour, gluten free	1	1	1.28
A.01.03.002.002	Rye flour, light	1	1	1.28
A.01.03.002.003	Rye flour, medium	1	1	1.28
A.01.03.002.004	Rye flour, wholemeal	1	1	1.28
A.01.03.003	Buckwheat milling products (unspecified)	1	1	1.28
A.01.03.003.001	Buckwheat flour	1	1	1.28
A.01.03.004	Corn milling products (unspecified)	1	1	1.28
A.01.03.004.001	Corn flour	1	1	1.28
A.01.03.004.003	Corn starch	1.3	1	1.28
A.01.03.005	Oat milling products (unspecified)	1	1	1.28
A.01.03.005.002	Oat flour	1	1	1.28
A.01.03.005.004	Oat starch	1.2	1	1.28
A.01.03.006	Rice milling products (unspecified)	1	1	1.28
A.01.03.006.001	Rice flour	1	1	1.28
A.01.03.006.002	Rice flour white	1	1	1.28
A.01.03.006.003	Rice flour, instant	1	1	1.28
A.01.03.006.004	Rice starch	1.2	1	1.28
A.01.03.007	Spelt milling products	1	1	1.28
A.01.03.008	Other milling products (unspecified)	1	1	1.28
A.01.03.008.001	Amaranth flour	1	1	1.28
A.01.03.008.002	Barley flour	1	1	1.28
A.01.03.008.003	Chapatti flour	1	1	1.28
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	1.28
A.01.03.008.005	Millet flour	1	1	1.28
A.01.03.008.007	Sorghum flour	1	1	1.28
A.01.04	Bread and rolls (unspecified)	1	0.7	1.28
A.01.04.001	Wheat bread and rolls	1	0.7	1.28
A.01.04.002	Rye bread and rolls	1	0.7	1.28
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	1.28
A.01.04.004	Multigrain bread and rolls	1	0.7	1.28
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	1.28
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	1.28
A.01.04.005.002	Crisp bread, rye, light	1	0.9	1.28

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[a]	Recipe fraction	mg TOS/kg flour
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	1.28
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	1.28
A.01.04.005.005	Rusk, light	1	0.9	1.28
A.01.04.005.006	Rusk, wholemeal	1	0.9	1.28
A.01.04.005.007	Pita bread	1	0.7	1.28
A.01.04.005.008	Matzo	1	0.9	1.28
A.01.04.005.009	Tortilla	1	0.7	1.28
A.01.04.006	Other bread	1	0.7	1.28
A.01.04.007	Bread products	1	0.7	1.28
A.01.07	Fine bakery wares (unspecified)	1	0.5	1.28
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	1.28
A.01.07.001.001	Beignets	1	0.15	1.28
A.01.07.001.002	Buns	1	0.7	1.28
A.01.07.001.003	Cake from batter	1	0.25	1.28
A.01.07.001.004	Cheese cream cake	1	0.24	1.28
A.01.07.001.005	Cheese cream sponge cake	1	0.24	1.28
A.01.07.001.006	Chocolate cake	1	0.24	1.28
A.01.07.001.007	Chocolate cake with fruits	1	0.24	1.28
A.01.07.001.008	Cream cake	1	0.24	1.28
A.01.07.001.009	Cream cheese cake	1	0.24	1.28
A.01.07.001.010	Cream custard cake	1	0.24	1.28
A.01.07.001.011	Cream custard sponge cake	1	0.24	1.28
A.01.07.001.012	Croissant	1	0.5	1.28
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	1.28
A.01.07.001.014	Croissant, filled with cream	1	0.5	1.28
A.01.07.001.015	Croissant, filled with jam	1	0.5	1.28
A.01.07.001.016	Croquembouche	1	0.15	1.28
A.01.07.001.017	Doughnuts	1	0.24	1.28
A.01.07.001.018	Clair	1	0.15	1.28
A.01.07.001.019	Flan	1	0.5	1.28
A.01.07.001.020	Fruit cake	1	0.6	1.28
A.01.07.001.021	Fruit pie	1	0.15	1.28
A.01.07.001.022	Cheese pie	1	0.15	1.28
A.01.07.001.023	Fruit tart	1	0.15	1.28
A.01.07.001.024	Gingerbread	1	0.6	1.28
A.01.07.001.025	Gougere	1	0.15	1.28
A.01.07.001.026	Kringles	1	0.25	1.28
A.01.07.001.027	Nut cream cake	1	0.24	1.28
A.01.07.001.028	Pancakes	1	0.25	1.28
A.01.07.001.029	Profiterole	1	0.15	1.28
A.01.07.001.030	Pyramid cake	1	0.25	1.28
A.01.07.001.031	Rhubarb flan	1	0.15	1.28
A.01.07.001.032	Scone	1	0.5	1.28
A.01.07.001.033	Sponge dough	1	0.25	1.28
A.01.07.001.034	Sponge cake	1	0.25	1.28
A.01.07.001.035	Sponge cake roll	1	0.25	1.28
A.01.07.001.036	Muffins	1	0.25	1.28

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[(a)]	Recipe fraction	mg TOS/kg flour
A.01.07.001.037	Waffles	1	0.25	1.28
A.01.07.001.038	Apple strudel	1	0.15	1.28
A.01.07.001.039	Cream-cheese strudel	1	0.24	1.28
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	1.28
A.01.07.001.041	Croissant from puff pastry	1	0.6	1.28
A.01.07.001.042	Brioche	1	0.5	1.28
A.01.07.001.044	Lebkuchen	1	0.6	1.28
A.01.07.001.045	Dumpling	1	0.5	1.28
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	1.28
A.01.07.001.047	Marzipan pie	1	0.25	1.28
A.01.07.001.048	Baklava	1	0.15	1.28
A.01.07.002	Biscuits (cookies)	1	0.9	1.28
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	1.28
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	1.28
A.01.07.002.003	Biscuits, cream filling	1	0.81	1.28
A.01.07.002.004	Biscuits, fruit filling	1	0.81	1.28
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	1.28
A.01.07.002.006	Butter biscuits	1	0.81	1.28
A.01.07.002.007	Biscuit, iced	1	0.81	1.28
A.01.07.002.008	Speculaas	1	0.9	1.28
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	1.28
A.01.07.002.010	Biscuits, oat meal	1	0.9	1.28
A.01.07.002.011	Biscuits, spelt meal	1	0.9	1.28
A.01.07.002.012	Biscuits, salty	1	0.9	1.28
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	1.28
A.01.07.002.014	Sticks, salty	1	0.81	1.28
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	1.28
A.18.04.001	Find bakery products for diabetics	1	0.5	1.28
A.19.01.002	Pizza and pizza-like pies	1	0.3	1.28

TOS: total organic solid.

(a): Food and Agriculture Organization of the United Nations. Technical Conversion Factors for Agricultural Commodities. Available online: http://www.fao.org/economic/the-statistics-division-ess/methodology/methodology-systems/technical-conversion-factors-for-agricultural-commodities/en/

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

Information provided in this appendix is shown in a supplementary excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5550)

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

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