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SCIENTIFIC OPINION

EFSA Journal

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Safety evaluation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Aspergillus niger* (strain XEA)

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Abstract

The food enzyme is an endo-1,4-β-xylanase (EC 3.2.1.8) produced with a genetically modified strain of *Aspergillus niger* (strain XEA), by DSM Food Specialities B.V. The food enzyme is intended to be used in baking and brewing processes. Based on maximum use levels recommended for the food processes and individual consumption data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.310 mg TOS/kg body weight per day in European populations. Genotoxicity tests with the food enzyme did not indicate a genotoxic concern. A repeated dose 90-day oral toxicity study in rodents, carried out with this endo-1,4-β-xylanase, showed no concern with respect to systemic toxicity. The allergenicity was evaluated by searching for similarity of the amino acid sequence to those of known allergens; no match was found. The Panel considers that there are no indications for allergic sensitisation and elicitation reactions by dietary exposure to the food enzyme endo-1,4-β-xylanase. Based on the microbial source, the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, the dietary exposure assessment, the findings in the toxicological studies and the allergenicity assessment, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Keywords: food enzyme, endo-1, 4-β-xylanase, EC 3.2.1.8, *Aspergillus niger*, genetically modified microorganism

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Question number: EFSA-Q-2015-00045

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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 obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

- 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 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[1] on food enzymes.

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP), and by the companies "DSM Food Specialties B.V" and "Novozymes A/S" for the authorisation of the food enzymes Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

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[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

[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, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of the food enzyme endo-1,4-β-xylanase produced with a genetically modified *A. niger* (strain XEA).

1.3. Information on existing authorisation and evaluations

The applicant reports that the endo-1,4-β-xylanase activity from *A. niger* strain XEA has been evaluated and authorised as a feed additive in the EU, but not for food processing.

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 *A. niger* (strain XEA) deposited in the DSM internal culture collection under accession number DS 38163. The food enzyme is intended to be used in baking and brewing processes.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009) and following the relevant Guidances 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 by the CEF Panel for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with 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; β-D-xylanase; endo-1,4-β-D-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 food enzyme produced with a genetically modified strain of *A. niger* XEA is a single polypeptide of 408 amino acids including a signal peptide of 22 amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be about 42 kDa. The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis showed a prominent band at about 52 kDa and several bands of lower staining intensity.

Data on the chemical parameters of the food enzyme have been provided for three commercial food enzyme batches and one batch used for toxicological tests (Table 1).

The average total organic solids (TOS) of the three commercial food enzyme batches was 26.3% (w/w); the values ranged from 23.7% to 31.1%.

The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation was 72.5 xylanase activity Units/mg TOS (NXTU/mg TOS); the values ranged from 72.2 to 72.7 NTXU/mg TOS (Table 1).

Table 1: Compositional data of the food enzyme

Parameter	Units	Batches			
		1	2	3	4[(a)]
Xylanase activity	NXTU[(b)]/g	17,200	22,450	17,600	21,185
Protein	%	17.7	24.1	18.0	22.2
Ash	%	0.7	0.7	0.6	0.4
Water	%	75.6	68.2	75.2	70.7
Total organic solids (TOS)[(c)]	%	23.7	31.1	24.2	28.9
Xylanase activity /mg TOS	NXTU/mg TOS	72.6	72.2	72.7	73.3

(a): Batch used for the toxicological tests.
(b): NTXU: Xylanase Units (see Section 3.3).
(c): TOS calculated as 100% − % water − % ash

The food enzyme complies with the specification for lead (no more than 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 applicant provided data that demonstrate that the concentrations of mycotoxins (fumonisins, ochratoxin A) of the four food enzyme batches were below the limits of quantification (LOQ) of the applied analytical methods.[4]

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)/g.

The applicant has provided information on the identity of the antifoam agent used. 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 Panel considered the compositional data provided for the food enzyme as sufficient.

3.1.3. Properties of the food enzyme

Endo-1,4-β-xylanase catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylan (including arabinoxylan, i.e. xylan branched with arabinose) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different chain lengths. The endo-1,4-β-xylanase from *A. niger* strain XEA does not require cofactors.

The endo-1,4-β-xylanase activity is quantified based on the hydrolysis of *p*-nitrophenyl-β-D-xylopyranoside (pNP-X) to xylose and *p*-nitrophenol. After adjusting the pH with a sodium carbonate solution, the yellow colour resulting from *p*-nitrophenol is determined at 405 nm as a measure of the enzyme activity. One NTXU is defined as the amount of enzyme that liberates 0.06 μmol *p*-nitrophenol per minute under the conditions of the assay (pH 4.5, 37°C).

Endo-1,4-β-xylanase has been characterised regarding its activity depending on temperature and pH. The temperature profile has been measured from 20°C to 85°C. The xylanase shows a temperature optimum of 70–80°C (at pH 4.5). The pH profile has been measured from pH range of 3–8, with an optimum of 4.5 (at 37°C). The xylanase is inactivated when heated at 90°C for 15 min.

[4] LOQ: ochratoxin A: 0.1 μg/kg; fumonisins (B1, B2 and B3): 10 μg/kg each.

3.1.4. Information on the microbial source material

3.1.4.1. Information related to the genetically modified microorganism

According to the CEF Guidance, the certificate of deposition of the strain in a public validated culture collection should be provided. The applicant deposited the endo-1,4-β-xylanase production strain *A. niger* XEA only in the [redacted] under number [redacted]. The Panel noted that this would not allow a verification of the strain independently of the company.

The production strain XEA has been taxonomically identified [redacted]. Moreover, the taxonomic identification is supported by whole genome sequence (see Section 3.1.4.2).

3.1.4.2. Characteristics of the parental and recipient microorganism

[redacted]

3.1.4.3. Characteristics of the donor organisms

[redacted]

3.1.4.4. Description of the genetic modification process

[redacted]

[redacted]

3.1.4.5. Safety aspects of the genetic modification

[redacted]

3.1.5. Manufacturing process

The food enzyme is manufactured with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), according to the Food Hygiene Regulation (EC) No 852/2004[5], and in accordance with current Good Manufacturing Practice (GMP).

The food enzyme is produced by a pure culture in a contained, submerged, fed-batch fermentation system with conventional process controls in place. The identity and the purity of the culture are checked at each transfer step from frozen vials until the end of fermentation.

The downstream processing includes recovery, purification and concentration. The food enzyme produced is recovered from the fermentation broth after killing of mycelium using [redacted] and further biomass separation via filtration. Further purification and concentration involve a series of filtration steps including ultrafiltration, and final polish and germ filtration.

[redacted]

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

3.1.6. Safety for the environment

The production strain and its recombinant DNA were not detected in the final product. The Panel concluded that there is no safety concern for the environment.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in baking and brewing processes (Table 2).

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

Food manufacturing process	Raw material	Recommended use levels (mg TOS/kg RM)
Baking process	Flour	0.1–27.6 mg
Brewing process	Cereals	0.7–6.9 mg

TOS: total organic solids; RM: raw material.

[5] 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.

In baking processes, the xylanase 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 bind water, so contributing to the reduction of dough viscosity. The decrease in dough viscosity facilitates the handling of the dough, gives improved crumb structure and increases the volume.

In brewing processes, the food enzyme is added during the mashing step. The use of endo-1,4-β-xylanase results in the reduction of the viscosity of the process streams, which leads to an improvement of filterability and brewing yield, more flexibility in the choice of raw materials and better consistency in the quality of the product.

3.1.8. Reaction and fate in food

The enzyme endo-1-4-β-xylanase catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylan resulting in the production of (1→4)-β-D-arabinoxylan oligosaccharides of different lengths. Endo-1,4-β-xylanase is specific in its action, not known to catalyse other reactions than the endo-hydrolysis of xylans to shorter xylan chains, xylo-oligosaccharides and xylose. These reaction products are naturally present in xylan-containing foods. Owing to the substrate specificity of the xylanase, no unintended reaction products in foods are to be expected.

The data and information provided indicate that the endo-1,4-β-xylanase is inactivated during processing under the intended conditions of use.

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, 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,[6] 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[7]) 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 interpreted with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to possible subjects' 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 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.

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

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 3.1.7). 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 (recipe fraction of 0.7) and then multiplied by 27.6 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 1.93 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 separately for each individual in the database. Table 3 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 1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix C – Table 2.

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

	Estimated exposure (mg/kg body weight per day)					
Population group	**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)	0.019–0.084 (6)	0.072–0.174 (10)	0.076–0.165 (18)	0.045–0.110 (17)	0.037–0.075 (17)	0.034–0.059 (14)
Min–max of 95th percentiles (number of surveys)	0.112–0.237 (5)	0.162–0.294 (7)	0.142–0.310 (18)	0.081–0.218 (17)	0.074–0.153 (17)	0.064–0.104 (14)

TOS: total organic solids.

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 4.

Table 4: 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	+/–

Sources of uncertainties	Direction of impact
	Exposure to food enzyme–TOS
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories likely to contain the food enzyme	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solids.
+: uncertainty with potential to cause over-estimation 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 over-estimation of the exposure.

3.3. Toxicological data

The test item used for the toxicity studies is described in Table 1 (batch 4). This batch is a ultrafiltered concentrate, produced according to the procedure used for commercial production. Despite a lower ash content and a slightly higher specific activity per mg TOS, the Panel considers the batch 4 as representative for the commercial food enzyme.

3.3.1. Bacterial reverse mutation test

To investigate the potential of the food enzyme to induce gene mutations, a bacterial reverse mutation assay (Ames test) was performed according to the OECD Test Guideline 471 (OECD, 1997a), and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537) and *E. coli* WP2*uvrA* pKM 101, in the presence and absence of metabolic activation, applying the plate incorporation assay. The effect of xylanase activity on S9-mix was tested and it was observed that the test item did not inhibit the activity of S9-mix. Two independent experiments were carried out in triplicate using five concentrations of the food enzyme ranging from 50 to 5,000 μg dry matter/plate of the food enzyme (corresponding to 49–4,932 μg TOS/plate). Appropriate positive control chemicals and water as a negative control were used. All positive controls induced a significant increase of revertant colony numbers confirming the sensitivity of the tests and the efficacy of the metabolic activation; the negative controls were within the historical control ranges. No precipitation or significant cytotoxicity were observed in any strain at any dose level tested. Upon treatment with the food enzyme, there was no significant increase in the number of revertant colonies in any tester strain, both in the presence and absence of metabolic activation. 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.

3.3.2. *In vitro* mammalian chromosome aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP. Chinese hamster ovary cells (CHO) were treated with the food enzyme, purified water (negative control) or appropriate positive controls both in the absence and presence of metabolic activation. The effect of xylanase activity on S9-mix was tested and it was observed that the test item did not inhibit the activity of S9-mix. Based on the results obtained in a dose-range finding test, the cells were treated with 1,250, 2,500 and 5,000 μg dry matter/mL (corresponding to 1,233, 2,466 and 4,932 μg TOS/mL) applying a short-term treatment (3 + 17 h of recovery) in the presence and absence of S9-mix, and with 750, 3,000 and 5,000 μg dry matter/mL (corresponding to 740, 2,959 and 4,932 μg TOS/mL) applying a continuous treatment (20 + 0 h) in the absence of S9-mix. No precipitation or significant changes in pH were detected. Two hundred

metaphases were scored per experimental point. The positive controls induced statistically significant increases in chromosomal aberration frequency and the system was considered sensitive and valid. The negative controls were within the historical vehicle control ranges. Cytotoxicity, measured as mitotic inhibition, did not exceed 23% of concurrent negative control values at any concentration of the food enzyme. No statistically significant increase in the frequency of chromosomal aberrations was observed in the short term treated cultures compared to the negative controls both in the presence and absence of metabolic activation. After continuous treatment in the absence of S9-mix, a statistically significant increase in the frequency of aberrant cells was observed only at 5,000 μg dry matter/mL (0 vs 2.5% aberrant cells at 0 and 5,000 μg dry matter/mL, respectively). However, the increase was slightly above the historical negative control range (0–2) that was not considered robust because it was based only on six experiments. Therefore, the increase was not considered biological relevant and the Panel concluded that the food enzyme did not induce chromosomal aberrations under the experimental conditions employed for this study.

3.3.3. Repeated Dose 90-day Oral Toxicity Study in Rodents

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD test guideline 408 (OECD, 1998) and following GLP. Groups of ten male and ten female Wistar rats received daily via gavage for at least 90 days dose levels of 0 (double distilled water as vehicle), 400, 1,600 and 6,400 mg food enzyme/kg bw per day in a volume of 10 mL/kg bw per day, corresponding to 0, 116, 463 and 1,852 mg TOS/kg bw per day (referred to as control, low-, mid- and high dose groups).

No treatment-related deaths or effects on clinical signs, body weight and body weight gains, food consumption, ophthalmoscopic examinations, organ weights and organ weight ratios, and macroscopic or microscopic pathology were observed.

In the functional observation battery tests a lower grip strength value was observed in forelimbs of males in the low-dose group and a higher grip strength value in hindlimbs of the mid-dose group. In females a significantly higher grip strength value was observed in forelimbs of the mid- and high-dose groups and in hindlimbs of the high-dose group. All these changes were considered to be incidental findings since they lacked dose relationship. Significantly higher values of landing foot splay were observed in mid and high dose males and females and were also considered to be incidental, as there was no change in gait observed in these animals.

In haematology evaluation significant incidental increases were observed in mid-dose males for mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) (also in high dose males), platelets, prothrombin time, neutrophils and decreased values for lymphocytes. The higher levels of MCHC in mid- and high-dose males were considered incidental, as the corresponding changes were not observed in red blood cell counts and haemoglobin. In females, a significantly increased mean corpuscular volume (MCV) was seen in low- and mid-dose animals, a higher level of haematocrit in mid-dose females, and an increased neutrophil percentage with lower lymphocyte percentage in high-dose females. These changes were minor and were considered as incidental and attributed to normal biological variation.

In clinical chemistry evaluation some parameters were only affected in males. Minor increased sodium and chloride levels were observed in the high dose group which were considered to be attributed to normal biological variation. The dose-related increased creatinine levels at mid- and high-doses groups were considered as incidental, as there was no corresponding histopathological changes in the kidneys.

The Panel derived a no observed adverse effect level (NOAEL) based on the high-dose level of this repeated dose 90-day oral toxicity study of 1,852 mg TOS/kg bw per day.

A comparison of the NOAEL (1,852 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.019–0.174 mg/kg bw per day at the mean and from 0.064–0.310 mg TOS/ kg bw per day at the 95th percentile, resulted in margins of exposure (MOEs) above 5,974, indicating that there is no safety 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 potential allergenicity of the endo-1,4-β-xylanase produced with the genetically modified *A. niger* strain XEA was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and

microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.

Endo-1,4-β-xylanase from *A. niger* strain XEA is not listed as an allergen in the AllergenOnline[8] and the WHO/IUIS Allergen Nomenclature[9] database. No information is available on oral sensitisation and elicitation reactions of this endo-1,4-β-xylanase. Several cases of respiratory allergy following occupational inhalation of xylanase have been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma to an enzyme used in food 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). In addition, 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 on adverse reactions upon ingestion of endo-1,4-β-xylanase in individuals sensitised through the respiratory route has not been reported. Therefore, it can be concluded that an allergic reaction upon oral ingestion of endo-1,4-β-xylanase produced with the genetically modified *A. niger* strain XEA, in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such reaction to occur is considered to be low.

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. As no individuals were reported to be allergic to food enzymes, no conclusion can be drawn regarding the potential allergenicity of endo-1,4-β-xylanase from *A. niger*.

Taken together, the Panel considers that under the intended condition of use there are no indications for allergic sensitisation and elicitation reactions by dietary exposure to the food enzyme endo-1,4-β-xylanase produced with the genetically modified *A. niger* strain XEA.

4. Conclusions

Based on the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, the dietary exposure assessment, the findings in the toxicological studies and the allergenicity assessment, the Panel concludes that the food enzyme endo-1,4-β-xylanase from *Aspergillus niger* strain XEA does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Dossier "Application for authorisation of endo-1,4-ß-xylanase from a genetically modified strain of Aspergillus niger XEA". First submission data by January 2015. Submitted by DSM Food Specialties. Second submission data by September 2015.
2) Preparatory work reports on technical data, toxicological data and on the genetic modifications were delivered by FoBiG GmbH (Freiburg, Germany) on 22 August 2016 and by the Technical University of Denmark (Søborg, Denmark) on 1 March 2016, respectively.
3) Additional information received from DSM Food Specialities B.V. in January 2018.

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Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
bw	body weight
CAS	Chemical Abstracts Service
[redacted]	[redacted]
CFU	colony forming units
CHO	Chinese hamster ovary cells
EC	European Commission and Enzyme Commission
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
LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MOE	Margin of Exposure
NOAEL	no observed adverse effect level
NXTU	Xylanase Unit
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
pNP-X	*p*-nitrophenyl-β-D-xylopyranoside
QPS	Qualified Presumption of Safety
RM	raw material
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization
[redacted]	[redacted]

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	27.6
A.01.03	Grain milling products (unspecified)	1	1	27.6
A.01.03.001	Wheat milling products (unspecified)	1	1	27.6
A.01.03.001.001	Wheat flour, brown	1	1	27.6
A.01.03.001.002	Wheat flour, Durum	1	1	27.6
A.01.03.001.003	Wheat flour, white	1	1	27.6
A.01.03.001.004	Wheat flour, wholemeal	1	1	27.6
A.01.03.001.005	Graham flour	1	1	27.6
A.01.03.001.006	Wheat flour, gluten free	1	1	27.6
A.01.03.001.014	Wheat starch	1.2	1	27.6
A.01.03.002	Rye milling products (unspecified)	1	1	27.6
A.01.03.002.001	Rye flour, gluten free	1	1	27.6
A.01.03.002.002	Rye flour, light	1	1	27.6
A.01.03.002.003	Rye flour, medium	1	1	27.6
A.01.03.002.004	Rye flour, wholemeal	1	1	27.6
A.01.03.003	Buckwheat milling products (unspecified)	1	1	27.6
A.01.03.003.001	Buckwheat flour	1	1	27.6
A.01.03.004	Corn milling products (unspecified)	1	1	27.6
A.01.03.004.001	Corn flour	1	1	27.6
A.01.03.004.003	Corn starch	1.3	1	27.6
A.01.03.005	Oat milling products (unspecified)	1	1	27.6
A.01.03.005.002	Oat flour	1	1	27.6
A.01.03.005.004	Oat starch	1.2	1	27.6
A.01.03.006	Rice milling products (unspecified)	1	1	27.6
A.01.03.006.001	Rice flour	1	1	27.6
A.01.03.006.002	Rice flour white	1	1	27.6
A.01.03.006.003	Rice flour, instant	1	1	27.6
A.01.03.006.004	Rice starch	1.2	1	27.6
A.01.03.007	Spelt milling products	1	1	27.6
A.01.03.008	Other milling products (unspecified)	1	1	27.6
A.01.03.008.001	Amaranth flour	1	1	27.6
A.01.03.008.002	Barley flour	1	1	27.6
A.01.03.008.003	Chapatti flour	1	1	27.6
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	27.6
A.01.03.008.005	Millet flour	1	1	27.6
A.01.03.008.007	Sorghum flour	1	1	27.6
A.01.04	Bread and rolls (unspecified)	1	0.7	27.6
A.01.04.001	Wheat bread and rolls	1	0.7	27.6
A.01.04.002	Rye bread and rolls	1	0.7	27.6
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	27.6
A.01.04.004	Multigrain bread and rolls	1	0.7	27.6
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	27.6
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	27.6

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	27.6
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	27.6
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	27.6
A.01.04.005.005	Rusk, light	1	0.9	27.6
A.01.04.005.006	Rusk, wholemeal	1	0.9	27.6
A.01.04.005.007	Pita bread	1	0.7	27.6
A.01.04.005.008	Matzo	1	0.9	27.6
A.01.04.005.009	Tortilla	1	0.7	27.6
A.01.04.006	Other bread	1	0.7	27.6
A.01.04.007	Bread products	1	0.7	27.6
A.01.07	Fine bakery wares (unspecified)	1	0.5	27.6
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	27.6
A.01.07.001.001	Beignets	1	0.15	27.6
A.01.07.001.002	Buns	1	0.7	27.6
A.01.07.001.003	Cake from batter	1	0.25	27.6
A.01.07.001.004	Cheese cream cake	1	0.24	27.6
A.01.07.001.005	Cheese cream sponge cake	1	0.24	27.6
A.01.07.001.006	Chocolate cake	1	0.24	27.6
A.01.07.001.007	Chocolate cake with fruits	1	0.24	27.6
A.01.07.001.008	Cream cake	1	0.24	27.6
A.01.07.001.009	Cream cheese cake	1	0.24	27.6
A.01.07.001.010	Cream custard cake	1	0.24	27.6
A.01.07.001.011	Cream custard sponge cake	1	0.24	27.6
A.01.07.001.012	Croissant	1	0.5	27.6
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	27.6
A.01.07.001.014	Croissant, filled with cream	1	0.5	27.6
A.01.07.001.015	Croissant, filled with jam	1	0.5	27.6
A.01.07.001.016	Croquembouche	1	0.15	27.6
A.01.07.001.017	Doughnuts	1	0.24	27.6
A.01.07.001.018	Clair	1	0.15	27.6
A.01.07.001.019	Flan	1	0.5	27.6
A.01.07.001.020	Fruit cake	1	0.6	27.6
A.01.07.001.021	Fruit pie	1	0.15	27.6
A.01.07.001.022	Cheese pie	1	0.15	27.6
A.01.07.001.023	Fruit tart	1	0.15	27.6
A.01.07.001.024	Gingerbread	1	0.6	27.6
A.01.07.001.025	Gougere	1	0.15	27.6
A.01.07.001.026	Kringles	1	0.25	27.6
A.01.07.001.027	Nut cream cake	1	0.24	27.6
A.01.07.001.028	Pancakes	1	0.25	27.6
A.01.07.001.029	Profiterole	1	0.15	27.6
A.01.07.001.030	Pyramid cake	1	0.25	27.6
A.01.07.001.031	Rhubarb flan	1	0.15	27.6
A.01.07.001.032	Scone	1	0.5	27.6
A.01.07.001.033	Sponge dough	1	0.25	27.6
A.01.07.001.034	Sponge cake	1	0.25	27.6
A.01.07.001.035	Sponge cake roll	1	0.25	27.6
A.01.07.001.036	Muffins	1	0.25	27.6

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material[(a)]	Recipe fraction[(b)]	mg TOS/kg flour
A.01.07.001.037	Waffles	1	0.25	27.6
A.01.07.001.038	Apple strudel	1	0.15	27.6
A.01.07.001.039	Cream-cheese strudel	1	0.24	27.6
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	27.6
A.01.07.001.041	Croissant from puff pastry	1	0.6	27.6
A.01.07.001.042	Brioche	1	0.5	27.6
A.01.07.001.044	Lebkuchen	1	0.6	27.6
A.01.07.001.045	Dumpling	1	0.5	27.6
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	27.6
A.01.07.001.047	Marzipan pie	1	0.25	27.6
A.01.07.001.048	Baklava	1	0.15	27.6
A.01.07.002	Biscuits (cookies)	1	0.9	27.6
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	27.6
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	27.6
A.01.07.002.003	Biscuits, cream filling	1	0.81	27.6
A.01.07.002.004	Biscuits, fruit filling	1	0.81	27.6
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	27.6
A.01.07.002.006	Butter biscuits	1	0.81	27.6
A.01.07.002.007	Biscuit, iced	1	0.81	27.6
A.01.07.002.008	Speculaas	1	0.9	27.6
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	27.6
A.01.07.002.010	Biscuits, oat meal	1	0.9	27.6
A.01.07.002.011	Biscuits, spelt meal	1	0.9	27.6
A.01.07.002.012	Biscuits, salty	1	0.9	27.6
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	27.6
A.01.07.002.014	Sticks, salty	1	0.81	27.6
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	27.6
A.18.04.001	Find bakery products for diabetics	1	0.5	27.6
A.19.01.001	Sandwich and sandwich-like meal	1	0.32	27.6
A.19.01.002	Pizza and pizza-like pies	1	0.3	27.6
A14.01	Beer and beer-like beverage	1.37	0.19	6.9
A.14.01.001	Beer, strong	1.37	0.265	6.9
A.14.01.002	Beer, regular	1.37	0.19	6.9
A.14.01.003	Beer, light (reduced alcohol content)	1.37	0.135	6.9
A.14.01.004	Beer, alcohol-free	1.37	0.135	6.9
A.14.01.005	Beer-like beverages (malt drink)	1.37	0.19	6.9

TOS: total organic solids.
(a): Available at see http://wwwfaoorg/fileadmin/templates/ess/documents/methodology/tcfpdf
(b): Derived from publically available recipe information, and/or food label information (such as the Mintel's Global New Products Database http://wwwmintelcom/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.2018.5228/suppinfo).

The file contains two sheets, corresponding to two tables.

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

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