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Safety evaluation of the food enzyme endo-1,4-β-xylanase from the non-genetically modified *Aspergillus tubingensis* strain LYX

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Abstract

The food enzyme endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase, EC 3.2.1.8) is produced with the non-genetically modified microorganism *Aspergillus tubingensis* strain LYX by DSM Food Specialties B.V. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in baking processes and cereal-based processes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.106 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate 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 of 227 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 2,142. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

Keywords: food enzyme, endo-1,4-β-xylanase, 4-β-D-xylan xylanohydrolase, EC 3.2.1.8, *Aspergillus tubingensis*, non-genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[1] 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;
- its use does not mislead the consumer.

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

The 'Guidance on submission of a dossier on 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 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.

Four applications have been introduced by the companies 'Puratos NV sa.', 'Novozymes A/S.', 'Meito Sangyo Co., Ltd' and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzymes Inulinase from a genetically modified strain of *Aspergillus oryzae* (strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger* 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 four applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Inulinase from a genetically modified strain of *Aspergillus oryzae*

[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.

(strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger* in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] 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 the food enzyme cellulase, glucanase and hemicellulase covering xylanase and mannanase from *Aspergillus niger*, submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00340/ 2018-01034/2018-01035. During the risk assessment phase, it was found that the technical dossier is too generic to be evaluated. A solution was found on 16 March 2020 via an ad hoc meeting between EFSA, the European Commission and representatives from the AMFEP. It was agreed that joint dossier will be split into 14 individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00340/2018-01034/2018-01035. This data package, identified as EFSA-Q-2022-00776, concerns the food enzyme endo-1,4-β-xylanase produced with a strain of *A. niger* (LYX) and submitted by DSM Food Specialties B.V.

Recent data identified the production microorganism as *Aspergillus tubingensis* (Section 3.1). Therefore, this name will be used in this opinion instead of *A. niger*.

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 from a non-genetically modified *A. tubingensis* strain LYX. The data package was submitted on 4 November 2022.

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 guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment[5]

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

Endo-1,4-β-xylanases catalyse the random hydrolysis of 1,4-ß-D-xylosidic linkages in xylans (including arabinoxylans) resulting in the generation of (1–4)-ß-D-xylan oligosaccharides of different lengths. The enzyme under assessment is intended to be used in baking processes and cereal-based processes.

[4] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/ 2011 with regard to specific data required for risk assessment of food enzymes. Text with EEA relevance. OJ L 168, 28.6.2012, pp. 21–23.

[5] Technical dossier/p. 4–5, 7, 15–16, 31, 34, 79.

3.1. Source of the food enzyme[6]

The endo-1,4-β-xylanase is produced with the non-genetically modified filamentous fungus *A. tubingensis* strain LYX, which is deposited at the [redacted] with the deposit number [redacted][7]

The production strain was identified as *A. tubingensis* by [redacted] showing [redacted][8]

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, and in accordance with current Good Manufacturing Practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[11] 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.[12]

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 three identified endo-1,4-β-xylanases of *A. tubingensis* strain LYX consist of [redacted] and [redacted] amino acids.[13] The molecular masses of the mature enzymes, calculated from the amino acid sequences, are [redacted] and [redacted] kDa, respectively.[13] The food enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.[14] A consistent protein pattern was observed showing multiple bands in all three food enzyme batches. The observed complexity of the protein profiles reflects the fact that the food enzyme is derived from a wild-type fungal strain without protein fractionation. No other enzymatic activities were reported.[15]

The in-house determination of enzyme activity is based on the hydrolysis of arabinoxylan (reaction conditions: pH 2.75, 47°C, 15 min), which reduces the viscosity of the reaction mixture. The xylanase activity is expressed in LYX/g. One LYX is defined as the amount of enzyme that causes a decrease in specific viscosity for a standardised arabinoxylan solution at a rate of 8.08 min^{-1} under the conditions of the assay.[16]

The food enzyme has a temperature optimum between 50 and 60°C (pH 3.5) and a pH optimum around pH 4.5 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 10, 30 and 40 min at different temperatures (pH 5.2). Enzyme activity decreased above 45°C showing no residual activity at 64°C, after 30 min incubation.[17]

[6] Technical dossier/p. 4, 8, 38; Technical dossier/Annex 16; Annex 17.
[7] Technical dossier/Annex 17.
[8] Technical dossier/Annex 16.
[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/p. 9, 42; Technical dossier/Annex 5.
[11] Technical dossier/p. 9, 15, 42–48; Technical dossier/Annex 6.
[12] Technical dossier/Annex 7.
[13] Technical dossier/p. 34; Technical dossier/Annex 15.
[14] Technical dossier/p. 32–33.
[15] Technical dossier/p. 35.
[16] Technical dossier/p. 35; Technical dossier/Annex 2.
[17] Technical dossier/p. 8, 35–37; Technical dossier/Annex 2.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation including one (batch 2) that was used in the genotoxicity studies, and for an additional batch (batch 4) produced for the repeated dose 90-day oral toxicity study (Table 1).[18] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 15.8% and the mean enzyme activity/TOS ratio was 71.8 LYX units/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2[(c)]	3	4[(d)]
Endo-1,4-β-xylanase activity	LYX/g[(a)]	10,150	13,150	10,800	23,000
Protein	%	9.7	12.6	10.7	19.3
Ash	%	0.7	0.6	0.7	44.6[(e)]
Water	%	85.2	81.7	83.7	10.0
Total organic solids (TOS)[(b)]	%	14.1	17.7	15.6	45.4
Activity/TOS	LYX/mg TOS	72.0	74.3	69.2	50.7

(a): LYX: Endo-1,4-β-xylanase Units (see Section 3.3.1).
(b): TOS calculated as 100% - % water - % ash.
(c): Batch used for the genotoxicity studies.
(d): Batch used for the repeated dose 90-day oral toxicity study in rats.
(e): Added [redacted]

3.3.3. Purity

The lead content in the three commercial batches and in the batch 4 used for the 90-day oral toxicity study was below 5 mg/kg[19, 20] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*,[19] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[19]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of fumonisins (B1, B2 and B3) and ochratoxin A was examined in all three commercial food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[21,22] Adverse effects caused by the possible presence of other secondary metabolites was addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme was sufficient.

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches of enzyme concentrate analysed in triplicate. Three × 1 mL of sample were incubated in 100 mL of non-selective medium at 30°C for 6 days for resuscitation. From these, 10 μL were inoculated on agar plates and incubated at 30°C for 6 days. No colonies were produced. A positive control was included.[23]

3.4. Toxicological data[24]

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian cell micronucleus test and a repeated dose 90-day oral toxicity study in rats, has been provided.

[18] Technical dossier/p. 32, 60; Technical dossier/Annex 1; Annex 2; Annex 3; Annex 11; Annex 12; Annex 14.
[19] Technical dossier/p. 34, 60; Technical dossier/Annex 3; Annex 4.
[20] Technical dossier/Annex 3: LoDs for lead = 0.003 mg/kg and 0.007 mg/kg.
[21] Technical dossier/ p. 34, 60; Technical dossier/Annex 3; Annex 4.
[22] Technical dossier/Annex 4: LoDs: fumonisins (B1, B2 and B3) = 10 μg/kg each; ochratoxin A = 1 μg/kg.
[23] Technical dossier/Annex 18.
[24] Technical dossier/p. 11, 15, 56–61.

The batch 2 (Table 1) used in genotoxicity studies was one of the examples of batches used for commercialisation. Batch 4 used in the repeated dose 90-day oral toxicity study, had a lower activity/TOS value than the batches used for commercialisation. Both were considered suitable as test items.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[25]

Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used with or without metabolic activation (S9-mix), applying the standard plate incorporation method (the first experiment) and pre-incubation method (the second experiment).

The first experiment was carried out in triplicate, using eight different concentrations of the food enzyme ranging from 3 to 5,000 μg TOS/plate. The second experiment was carried out in triplicate, using six different concentrations of the food enzyme ranging from 33 to 5,000 μg TOS/plate.

Toxic effects, evident as a reduction in the number of revertant colonies, occurred in *Escherichia coli* WP2uvrA at 2,500 and 5,000 μg TOS/plate in the second experiment without S9-mix. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.[26]

Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix). In the first experiment, cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 305, 533 and 933 μg TOS/mL in a short-term treatment (4 h exposure and 16 h recovery period) either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme and scored for MNBN at concentrations of 533, 933 and 1,633 μg TOS/mL in a long-term treatment (20 h exposure without recovery period) without S9-mix.

At the end of treatment, precipitation in the culture medium was observed at the highest concentrations of 933 and 1,633 μg TOS/mL in experiments I and II, respectively.

Cytotoxicity of 11%, evaluated as a decrease of the proliferation index, was observed at the highest concentration tested in the long-term treatment. The frequency of MNBN was not statistically significantly different to the negative controls at any concentrations tested.

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce an increase in the frequency of MNBN under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1981) and following GLP.[27] Groups of 10 male and 10 female Wistar rats SPF received by gavage the food enzyme in doses of 20, 100 or 500 mg/kg body weight (bw) per day, corresponding to 9, 45 or 227 mg TOS/kg bw per day. Controls received the vehicle (distilled water).

No mortality was observed.

The water consumption was statistically significantly increased on weeks 1 to 6 of administration in low-dose females (+16%, +13%, +11%, +14%, +19% and +16%, respectively) and decreased on weeks 6 to 13 of administration in mid-dose females (−12%, −13%, −16%, −18%, −12%, −14%, −13% and −11%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex and there was no dose–response relationship.

[25] Technical dossier/Annex 11.
[26] Technical dossier/Annex 12.
[27] Technical dossier/Annex 13, Annex 14.

Clinical chemistry investigations revealed a statistically significant increase in chloride level in high-dose males (+2%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex, the change was small and there were no histopathological changes in kidneys.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 227 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considered only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the food enzyme endo-1,4-β-xylanase produced with the non-genetically modified *A. tubingensis* strain LYX 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, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[28]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-1,4-β-xylanase.

Cases of occupational allergy following exposure by inhalation of xylanase have been reported in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) as well as in case reports (Baur et al., 1998; Merget et al., 2001). Several studies have shown that adults with occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). No food allergic reactions to xylanase have been reported in the literature.

[redacted] a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from this source are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two food manufacturing processes at the recommended use levels summarised in Table 2.[29]

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	flour	0.70–**8.36**
Cereal-based processes	flour	1.39–**11.14**

TOS: total organic solids.

(a): The name has been harmonised 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.

(b): The numbers in bold were used for calculation.

In baking and cereal-based processes, the food enzyme is added to flour during the preparation of the dough.[30] The endo-1,4-β-xylanase hydrolyses (arabino)xylans, which interact with gluten and bind water, thus, reducing the dough viscosity and shortening the processing time. The decrease in viscosity

[28] Technical dossier/p. 11, 61–78; Technical dossier/Annex 15, Annex 16, Annex 19.
[29] Technical dossier/p. 10, 50, 52, 85–86.
[30] Technical dossier/p. 50–51.

facilitates the handling of the dough, which results in more uniform products. The food enzyme–TOS remains in the final processed foods.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes,[31] it is expected that the enzyme is inactivated during baking and cereal-based processes.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for bw. 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 1 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 mean 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.106 mg TOS/kg bw per day in children at the 95th percentile.

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)	0.005–0.031 (11)	0.029–0.072 (15)	0.032–0.061 (19)	0.016–0.037 (21)	0.010–0.025 (22)	0.009–0.023 (22)
Min–max 95th (number of surveys)	0.027–0.099 (9)	0.061–0.099 (13)	0.054–0.106 (19)	0.030–0.070 (20)	0.022–0.044 (22)	0.018–0.039 (21)

TOS: total organic solids.

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	+/–

[31] Technical dossier/p. 50, 82–84.

Sources of uncertainties	Direction of impact
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 to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solids.
+: 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 overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (227 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.005–0.072 mg TOS/kg bw per day at the mean and from 0.018–0.106 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 2,142.

4. Conclusions

Based on the data provided, and the derived margin of exposure for the food manufacturing processes, the Panel concluded that the food enzyme endo-1,4-β-xylanase from the non-genetically modified *A. tubingensis* strain LYX does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Technical dossier "Application for authorization of food enzyme endo-1,4-β-xylanase from *Aspergillus tubingensis* in accordance with Regulation (EC) No 1331/2008". The data package was submitted by DSM Food Specialties B.V. on 31 October 2022.

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[redacted]

Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
[redacted]	[redacted]
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	standardised food classification and description system
GLP	good laboratory practice

GM	genetically modified
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
LYX	endo-1,4-β-xylanase unit
MNBN	bi-nucleated cells with micronuclei
MoE	margin of exposure
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OECD	Organisation for Economic Cooperation and Development
RM	raw material
SPF	specific-pathogen-free
TOS	total organic solids
[redacted]	[redacted]
WHO	World Health Organization

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

Appendix A can be found in the online version of this output (in the 'Supporting information' section). 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 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(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).