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Safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Bacillus subtilis* strain XAN

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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 genetically modified microorganism *Bacillus subtilis* strain XAN by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The production strain of the food enzyme contains antimicrobial resistance genes. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk. The food enzyme 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 0.02 mg TOS/kg body weight (bw) per day in European populations. As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considered that toxicological tests are not needed for the assessment of this food enzyme. A search for 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, *Bacillus subtilis*, genetically modified microorganism

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Table of contents

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list (when established) 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.

On 10 November 2021, a new application has been introduced by the applicant "DSM Food Specialties B.V." for the authorization of the food enzyme endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* (strain XAN).

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: Endo-1,4-β-xylanase from a genetically modified strain of *Bacillus subtilis* (strain XAN), in accordance with Regulation (EC) No 1331/2008[2] establishing a common authorization procedure for food additives, food enzymes and food flavourings.

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 endo-1,4-β-xylanase from the genetically modified *B. subtilis* strain XAN.

[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.

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 genetically modified *B. subtilis* strain XAN.

Additional information was requested from the applicant during the assessment process on 22 September 2022 and received on 27 September 2022 (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the relevant guidance documents of the EFSA Scientific Committee.

The current '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).

2.3. Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[3] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 2 March to 23 March 2023. No comments were received.

3. Assessment[4]

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-β-D-xylosidic linkages in xylans (including arabinoxylans) resulting in the generation of (1- > 4)-β-D-xylan oligosaccharides.

The food enzyme under assessment is intended to be used in baking processes and cereal-based processes.

3.1. Source of the food enzyme[5]

The enzyme is produced with the genetically modified bacterium *B. subtilis* strain XAN, which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands) with the deposit number CBS 147476.[6] It was identified as *B. subtilis* by whole genome sequencing (WGS) analysis, which showed an average nucleotide identity (ANI) of 99.98% with several *B. subtilis* strains, including the type strain *B. subtilis* 168 from which it derives.[7]

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[4] Technical dossier/Risk assessment/Section 05. Identify of the food enzyme and specifications; Section 12. Intended use in food; Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 3.

[5] Technical dossier/Risk assessment/Section 06. Source of the food enzyme; Technical dossier/Risk assessment/Annex 3; Annex 4; Annex 5; Annex 6; Annex 7; Annex 8; Annex 20.

[6] Technical dossier/Risk assessment/Annex 8.

[7] Technical Dossier/Risk assessment/Annex 3.

The species *B. subtilis* is included in the list of organisms for which the Qualified Presumption of Safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance genes and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020). A cytotoxicity test made with supernatants indicated that the production strain *B. subtilis* XAN did not induce cell damage to CHO-K1 (Chinese Hamster Ovary epithelial cell line) cells using the lactate dehydrogenase (LDH) assay.[8] WGS analysis of the production strain showed the presence of the *aadK* gene, which encodes aminoglycoside 6-adenyltransferase, a streptomycin-modifying enzyme. This gene is also present in the genome of the parental strain *B. subtilis* 168. The production strain also carries the genes showing homology to *tet(L)* and *rphB*, which confer resistance to tetracycline and rifampicin, and are not found in the WGS of the parental strain.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is *B. subtilis* strain 168 (DS 20887). The recipient strain DS [redacted] was developed from the parental strain by classical mutagenesis and selection for non-sporulation and for the absence of α-amylase production [redacted] and [redacted] respectively). This was followed by genetic modification steps for the disruption of [redacted] and [redacted] and insertion of [redacted].[9] Genetic modifications were confirmed by WGS.[7]

During the development of the recipient strain, plasmids were used containing genes conferring resistance to bleomycin, kanamycin and neomycin. The absence of backbone sequences from these vectors was confirmed by comparing WGS analysis.[7] [redacted] remained in the genome of the production strain.

3.1.2. Characteristics of introduced sequences

The plasmid vectors [redacted] and [redacted] are derived from [redacted] (from [redacted] and [redacted] from [redacted]. [redacted] and [redacted]) designed from [redacted] under the control of [redacted] promoter and [redacted] terminator from the [redacted] gene of [redacted], were inserted, respectively, in the plasmid vectors [redacted] and [redacted], resulting in plasmids [redacted] and [redacted] used for transformation. Plasmids [redacted] and [redacted] contain genes conferring resistance to [redacted] from [redacted] from [redacted] and to [redacted] from [redacted]. The latter gene was [redacted]. The helper plasmid [redacted] contains the [redacted] gene, [redacted] and the marker genes [redacted] and [redacted] (from [redacted], conferring [redacted] and [redacted] resistance, respectively.[10]

3.1.3. Description of the genetic modification process[11]

The purpose of genetic modification was to enable the production strain to overproduce endo-1,4-β-xylanase. For this purpose, two insertion cassettes were constructed, both consisting of the [redacted] and the [redacted] expression cassettes described above. One of the cassettes was flanked by the 3′ and 5′ flanking regions of the [redacted] gene ([redacted], while the other was flanked by the 3′ and 5′ flanking regions of the [redacted] gene ([redacted]).

The production strain XAN was obtained from the recipient DS [redacted] by the following genetic modifications:

- Transformation of the recipient strain with plasmid [redacted] and integration of the insertion cassette in the [redacted] by double homologous recombination. Transformants were selected for their resistance to [redacted] and sensitivity to [redacted].
- Transformation of the resulting strain with plasmid [redacted] and integration of the insertion cassette in the [redacted] by double homologous recombination. Transformants were selected for their resistance to [redacted] and sensitivity to [redacted]. One transformant was selected as the production strain *B. subtilis* XAN.

[8] Technical Dossier/Risk assessment/Annex 4.
[9] Technical Dossier/Risk assessment/Annex 5.
[10] Technical Dossier/Risk assessment/Annex 6; Annex 7.
[11] Technical dossier/Risk assessment/Annex 5.

After each step of genetic modification, plasmid [redacted] was transiently introduced to remove the [redacted] gene present in the insertion cassettes by the [redacted]. After each use, [redacted] was [redacted].[12]

The modifications were confirmed by comparing the WGS of the production strain XAN with the parental strain genome and the vector sequences.[7]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *B. subtilis* XAN differs from the recipient strain DS [redacted] in its capacity to overproduce the endo-1,4-β-xylanase. The absence of the antibiotic resistance genes used during the genetic modifications was confirmed by analysing the WGS of the production strain.[7]

The introduced genetic modifications did not raise safety concerns.

3.2. Production of the food enzyme[13]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[14], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[15]

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 or centrifugation. The filtrate/supernatant containing the enzyme is stabilised and 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.[16] 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.[17]

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.[18] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 22 kDa.[19] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 22 kDa, consistent with the expected mass of the enzyme. The protein profile also included bands of lesser staining intensity.[20] No other enzymatic activities were reported.[21]

The in-house determination of enzyme activity[22] is based on hydrolysis of wheat arabinoxylan (reaction conditions: pH 6, 30°C, 30 min). The enzymatic activity is determined by measuring the release of reducing groups, with a hexacyanoferrate reagent (Hoffman reagent) which is measured spectrophotometrically at 420 nm. The enzyme activity is expressed in New Bakery Xylanase Units

[12] Technical Dossier/Risk assessment/Annex 7.
[13] Technical dossier/Risk assessment/Annex 12; Annex 13; Annex 14; Technical dossier/Risk assessment/Section 07. Manufacturing process.
[14] 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.
[15] Technical dossier/Risk assessment/Annex 12; Technical dossier/Risk assessment/Section 07. Manufacturing process.
[16] Technical dossier/Risk assessment/Annex 13.
[17] Technical dossier/Risk assessment/Annex 14; Technical dossier/Additional data, 27 September 2022.
[18] Technical dossier/Risk assessment/Annex 3/Appendix 3.
[19] Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 3.
[20] Technical dossier/Risk assessment/Annex 17; Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 3.
[21] Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 4.
[22] Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 4; Technical dossier/Risk assessment/Annex 16.

(NBXU/g). One NBXU is defined as the amount of enzyme that produces 0.5 mg of xylose equivalents under the assay conditions.[23]

The food enzyme has a temperature optimum between 40 and 50°C (pH 6) and a pH optimum between pH 5.5 and 6.5 (30°C).[24] Thermostability was tested at different temperatures (51–75°C; pH 6.0) and for different time periods (2–60 min).[24] The enzyme is completely inactivated after 2 min at 70°C or after 60 min at 55°C.[25]

3.3.2. Chemical parameters[26]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The mean total organic solids (TOS) of the three food enzyme batches was 7.4% and the mean enzyme activity/TOS ratio was 486.7 NBXU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Endo-1,4-β-xylanase activity	NBXU/g[(a)]	50,350	25,900	33,750
Protein	%	5.8	3.3	4.2
Ash	%	0.8	0.9	1.0
Water	%	90.8	92.7	91.6
Total organic solids (TOS)[(b)]	%	8.4	6.4	7.4
Endo-1,4-β-xylanase activity/TOS	NBXU/mg TOS	599.4	404.7	456.1

(a): NBXU: New Bakery Xylanase Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity[27]

The lead content in the three commercial batches was below 5 mg/kg[28,29] 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 criteria for total coliforms, *Escherichia coli* and *Salmonella* 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.[29]

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[30]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. One gram of product was plated in non-selective agar and incubated at 45°C for 3 days. No colonies of the production strain were detected. A positive control was included.[31]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify a 497-bp fragment specific for the junction at the insertion site in the [redacted], with a limit of detection of 1 ng spiked DNA/mL food enzyme.[32]

[23] Technical dossier/Risk assessment/Annex 16.
[24] Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity/p. 5; Technical dossier/Risk assessment/Annex 18; Technical dossier/Additional data, 27 September 2022.
[25] Technical dossier/Risk assessment/Annex 18.
[26] Technical dossier/Risk assessment/Annex 1; Annex 15; Technical dossier/Risk assessment/Section 08. Chemical composition properties and purity.
[27] Technical dossier/Risk assessment/Annex 1; Annex 2; Technical dossier/Risk assessment/Section 05. Identity of the food enzyme and specifications.
[28] Technical dossier/Risk assessment/Annex 1; Limit of detection (LoD): Pb = 0.01 mg/kg.
[29] Technical dossier/Risk assessment/Annex 1.
[30] Technical dossier/Risk assessment/Annex 9; Annex 10.
[31] Technical Dossier/Risk assessment/Annex 9.
[32] Technical Dossier/Risk assessment/Annex 10.

3.4. Toxicological data

Although all other requirements for the QPS have been met, the production strain carries acquired antimicrobial resistance genes and therefore cannot be considered as suitable for the QPS approach. However, no risk is expected from the presence of these antimicrobial resistance genes in the production strain, as the enzyme has been shown not to contain viable cells and DNA (Section 3.3.4). As no other concerns arising from the microbial source and its subsequent genetic modification or from the manufacturing process have been identified, the Panel considers that no toxicological studies other than assessment of allergenicity are needed for the assessment of this food enzyme.

3.4.1. Allergenicity[33]

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 enzyme endo-1,4-β-xylanase produced with the genetically modified *Bacillus subtilis* strain XAN 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.

No information is available on oral and respiratory 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 (Tarvainen et al., 1991; Baur et al., 1988; 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).

Yeast extract, 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 microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues 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 processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[(b)]

Food manufacturing process	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(a)]
Baking processes	Flour	0.10–**1.54**
Cereal-based processes	Flour	0.21–**1.03**

TOS: total organic solids.
(a): The numbers in bold were used for calculation.
(b): Technical dossier/Risk assessment/Section 12. Intended use in food; Section 13. Dietary exposure assessment; Section 17. Use levels.

In baking and cereal-based processes, the food enzyme is added to flour during the preparation of the dough.[34] The endo-1,4-β-xylanase hydrolyses (arabino)xylans, which interact with gluten and bind

[33] Technical dossier/Risk assessment/Annex 10; Annex 19.
[34] Technical dossier/Risk assessment/Section 12. Intended use in food.

water, thus, reducing the dough viscosity and shortening the processing time. The decrease in viscosity facilitates the handling of the dough, which results in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness). The food enzyme–TOS remains in the final foods.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes,[35] it is expected that the enzyme is inactivated by heat during baking processes 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 body weight (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 one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed 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 about 0.018 mg TOS/kg bw per day in infants 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)	0.001–0.005 (11)	0.004–0.010 (15)	0.005–0.009 (19)	0.003–0.006 (21)	0.002–0.004 (22)	0.002–0.004 (22)
Min–max 95th percentile (number of surveys)	0.003–0.018 (9)	0.010–0.016 (13)	0.009–0.017 (19)	0.005–0.012 (20)	0.004–0.008 (22)	0.003–0.006 (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.

[35] Technical dossier/Risk assessment/Section 11. Reaction and fate in food.

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

TOSL: 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

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *Bacillus subtilis* strain XAN does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

5. Documentation as provided to EFSA

Technical dossier Application for authorization of food enzyme endo-1,4-β-xylanase from the genetically modified *Bacillus subtilis* (strain XAN) in accordance with Regulation (EC) No 1331/2008. 10 November 2021. Submitted by DSM Food Specialties B.V.

Additional information. 27 September 2022. Submitted by DSM Food Specialties B.V.

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Abbreviations

ANI	average nucleotide identity
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CHO-K1 cells	Chinese Hamster Ovary, epithelial cell line
EFSA BIOHAZ Panel	EFSA Panel on Biological Hazards
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	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
GM	genetically modified
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDH	lactate dehydrogenase
LoD	limit of detection
NBXU	New Bakery Xylanase Units
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
RM	raw material
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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

Information provided in this Appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.8017#support-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 one 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).