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Safety evaluation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Bacillus licheniformis* (strain NZYM-CE)

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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 a genetically modified *Bacillus licheniformis* (strain NZYM-CE) by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. This xylanase is intended to be used in baking and cereal-based processes. Based on the maximum use levels recommended for the respective food processes and individual 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 0.012 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a genotoxic concern. The systemic toxicity was assessed by a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level (NOAEL) of at least 1,020 mg TOS/kg bw per day, the highest dose tested. When the NOAEL value is compared to the estimated dietary exposure, this results in a margin of exposure (MoE) of at least 85,000. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is considered to be low. Overall, the Panel concluded that based on the data provided and the derived MoE, this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, xylanase, endo-1,4-β-xylanase, 4-β-D-xylan xylanohydrolase, EC 3.2.1.8, *Bacillus licheniformis*, 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 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 European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) its use does not mislead the consumer.

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

Five applications have been introduced by the companies 'Novozymes A/S', 'Puratos NV sa', 'Neova Technologies Inc'. and 'Amano Enzyme Inc'. for the authorisation of the food enzyme Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-CE), Xylanase from a genetically modified strain of *Bacillus subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] mplementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

[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, pp. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments of the food enzyme Asparaginase from a genetically modified strain of *A. oryzae* (strain NZYM-OA), Xylanase from a genetically modified strain of *B. licheniformis* (strain NZYM-CE), Xylanase from a genetically modified strain of *B. subtilis* LMG S-24584, Protease complex consisting of trypsin, chymotrypsin, elastase and carboxypeptidase from pig pancreas, and Cellulase from *Trichoderma viride* (strain AE-CT) 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's request to carry out the safety assessment of food enzyme endo-1,4-β-xylanase from a genetically modified *B. licheniformis* (strain NZYM-CE).

2. Data and methodologies

2.1. Data

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

Additional information was requested from the applicant during the assessment phase on 6 March 2017 and 24 September 2018 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following the relevant existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Endo-1,4-β-xylanase
Systematic name:	4-beta-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-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 lengths. It is intended to be used in baking and cereal-based processes.

3.1. Source of the food enzyme

The endo-1,4-β-xylanase is produced with the genetically modified bacteria *B. licheniformis* (strain NZYM-CE), which is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany) with the deposit number [redacted].[4]

3.1.1. Characteristics of the parental and recipient microorganism

The parental strain [redacted] was identified as *B. licheniformis* by [redacted]. It is deposited in the DSMZ with the deposition number [redacted].[5] The strain shows no cytotoxic activity

[4] Technical dossier/Annex 4/Annex A3.
[5] Technical dossier/Annex 4/Annex A1 and A2.

in Chinese hamster ovary cells (Pedersen et al., 2002). The absence of cytotoxicity has also been shown by the same methodology in strain [redacted], an intermediate strain [redacted] in the development of the recipient strain from [redacted] (Pedersen et al., 2002).

The recipient strain [redacted]

Additionally, [redacted]

For the development of the recipient strain [redacted]

3.1.2. Characterisation of the introduced sequences

The xylanase-coding gene, [redacted]

3.1.3. Description of the genetic modification process

The production strain *B. licheniformis* NZYM-CE was developed from the recipient strain [redacted] The resulting strain was called NZYM-CE.

[redacted]

As a consequence of the genetic modification, *B. licheniformis* NZYM-CE [redacted]

3.1.4. Safety aspects of the genetic modification

The production strain *B. licheniformis* NZYM-CE differs from the parental strain [redacted] by its capability to produce the xylanase [redacted]

The genetic stability of the production strain was demonstrated by Southern analysis with DNA isolated from the production strain culture and three commercial batches (Table 1).[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.

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 leaving a supernatant containing the food enzyme. 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 weight material passes the filtration membrane and is discarded. 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.[10]

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 produced with the genetically modified *B. licheniformis* (strain NZYM-CE) is a single polypeptide of 407 amino acids. The 45.4 kDa molecular mass of the protein was calculated based on the amino acid sequence.[11] The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis consistently showed one major protein band in all batches, migrating at approximately at 45 kDa.[12] The food enzyme (batches 1, 2 and 3 in Table 1) was tested for α-amylase, glucoamylase, lipase and protease activities and no activities were detected.[13,14] No other enzymatic side activities were reported.

The in-house determination of the endo-1,4-β-xylanase activity is based on the hydrolysis of wheat arabinoxylan to reducing carbohydrates (reaction conditions: pH 6.0, 50°C, 400 s). The reaction is stopped by adding an alkaline reagent to form a complex with the reducing sugar. The absorbance is

[7] Technical dossier/Annex 4.
[8] Technical dossier/Annex 4/Annex D3.
[9] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[10] Technical dossier/Annex 6.
[11] Technical dossier/Annex 1.
[12] Technical dossier/Figure 1.
[13] LoD = 0.3 FAU(F)/g for α-amylase, LoD = 0.825 AGU/g for glucoamylase, LoD = 0.02 KLU/g for lipase and LoD = 0.0056 AU (N)/g for protease.
[14] Technical dossier/Table 5.

then measured at 405 nm. The endo-1,4-β-xylanase activity is measured relative to an internal enzyme standard and expressed in New Xylanase Units/g (NXU/g).[15,16]

This food enzyme was characterised regarding its activity dependence on pH and temperature. The endo-1,4-β-xylanase has a temperature optimum range between 45 and 60°C (at pH 6) and a pH optimum between 6 and 8 (at 37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 minutes at different temperatures. Under the conditions (pH 6.0) of the applied temperature stability assay, the endo-1,4-β-xylanase activity decreased rapidly above 50°C showing no residual activity above 70°C.[17]

3.3.2. Chemical parameters

Data on chemical parameters were provided for four food enzyme batches, of which three (batches 1, 2 and 3) were used for commercialisation and one (batch 4) was for the toxicological testing (Table 1). The average total organic solids (TOS) content of the three commercial batches was 7.3% (w/w); the values ranged from 5.9% to 8.1%. The enzyme activity/TOS ratio ranged from 56 to 66 NXU/mg TOS. Considering the low variability of the enzyme activity/TOS ratio in these three commercial enzyme batches, the average enzyme activity/TOS ratio of 60 NXU/TOS was used for subsequent calculations.

Table 1: Compositional data provided for the food enzyme after concentration

Parameter	Unit	Batches			
		1[(c)]	2[(c),(d)]	3[(c),(d)]	4[(e)]
Xylanase activity	NXU[(b)]/g	4,550	4,700	3,880	3,670
Protein	%	3.9	3.4	2.5	Not provided
Ash	%	0.9	1.9	1.5	2.0
Water	%	91.0	90.2	92.6	88.3
TOS[(a)]	%	8.1	7.9	5.9	9.7
Xylanase activity/mg TOS	NXU/mg TOS	56	59	66	38

(a): TOS calculated as 100% – % water – % ash.
(b): NXU: new xylanase units.
(c): Batches used to characterise the chemical and microbiological parameters.
(d): Batches used to characterise the genetic stability of production strain.
(e): Batch used in toxicological studies.

3.3.3. Purity

The lead content was below the level of detection in any of the four batches, which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium and mercury were below their respective limits of detection (LoD) of the employed methodologies.[18,19] Trace amounts of arsenic were detected in two batches (0.14 and 0.15 mg/kg, respectively), but were found to be below the specification levels set for food additives (1–3 mg/kg arsenic).[20]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. In addition, the total viable cell count was 100–200 CFU/g in three other commercial food enzyme batches and in the batch for toxicological tests.[21]

The Panel considered the compositional data provided for the food enzyme as sufficient.

[15] LoD = 22.8 NXU/g for xylanase.
[16] Technical dossier/Annex 3.01.
[17] Technical dossier/Annex 9.
[18] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[19] Technical dossier/Table 2 and 6.
[20] Commission Regulation (EU) No 232/2012 of 16 March 2012 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the conditions of use and the use levels for Quinoline Yellow (E 104), Sunset Yellow FCF/Orange Yellow S (E 110) and Ponceau 4R, Cochineal Red A (E 124). OJ L 354, 31.12.2008, p. 16.
[21] Technical dossier/Table 4 and 6.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent production batches analysed in triplicate. One gram of product was incubated [redacted] No growth of the *B. licheniformis* production strain was detected.[22]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction analysis of three batches in triplicate. No DNA was detected [redacted], with a limit of detection of 1 ng spiked DNA/g food enzyme.[23]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian micronucleus assay, and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 4 (see Table 1) was used for toxicological testing. It has a lower specific activity compared to the batches used for commercialisation, and thus is considered cruder and suitable for toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

To investigate the potential of the endo-1,4-β-xylanase to induce gene mutations, a bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA1535, TA 1537, TA98 and TA100) and *E. coli* WP2*uvrA* (pKM101), in the presence and absence of metabolic activation, applying the 'treat and plate' assay. Two experiments were carried out in triplicate plating using six concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/mL). The highest tested concentration of 5,000 μg dry matter/mL corresponds to 4,145 μg TOS/mL. No significant cytotoxicity or increase in the mean number of revertant colonies were observed at any tested concentration in any test strain with or without S9-mix.[24]

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce gene mutations under the conditions employed.

3.4.1.2. *In vitro* mammalian micronucleus assay

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Whole blood cell cultures were exposed to three concentrations of the food enzyme (3,000, 4,000 and 5,000 μg food enzyme/mL, corresponding to 291, 388 and 485 μg TOS/mL) following a short treatment in the presence and absence of S9-mix (3 + 21 h of recovery) and a continuous treatment without S9-mix (24 +24 h recovery). No cytotoxicity was observed at any concentration tested and experimental condition. Frequencies of micronuclei were comparable to the negative controls at any concentration tested.[25]

The Panel concluded that the food enzyme endo-1,4-β-xylanase did not induce micronucleus under the test conditions employed for this study.

3.4.2. 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.[26] Groups of 10 male and 10 female Sprague–Dawley rats (of the Ntac:SD strain, 5 weeks old) received by gavage the food enzyme at doses corresponding to 102, 337 and 1,020 mg TOS/kg bw per day. A control group received ion-exchanged water which served as a vehicle.

[22] Technical dossier/Annex 4/Annex E1.
[23] Technical dossier/Additional information February 2019.
[24] Technical dossier/Annex 7.01.
[25] Technical dossier/Annex 7.02.
[26] Technical dossier/Annex 7.03.

One female of the low-dose group was sacrificed on day 45 due to the formation of a tumour larger than 2 cm at the chest region (an adenocarcinoma arising in the mammary gland). This was considered being an incidental finding with no relation to treatment.

Hair loss was observed in 11 animals during the study. As hair loss is often seen in rats and it was observed in both control and treated animals, it was considered not related to the treatment.

Statistically significant differences from controls were observed in water intake and some urine analysis parameters. In high-dose males, water intake during the study on eight occasions and the overall for the treatment period was higher. Furthermore, a larger volume of urine was collected from these animals, and the urine had a lighter colour and a higher gravity. In the mid- and high-dose females, an overall lower water intake (not statistically significantly different from the control group) was seen, together with a tendency towards a higher gravity urine. Since the overall water intake was affected differently for the two sexes, and no other observations in the study indicated changes in renal function, these findings were considered not to be of toxicological importance.

No other statistically significant effects were observed.

The Panel identified a no observed adverse effect level (NOAEL) of 1,020 mg TOS/kg bw per day, the highest dose tested.

3.4.3. 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 by the genetically modified strain *B. licheniformis* (strain NZYM-CE) was assessed by comparing its amino acid sequence with those of known allergens according to the Guidance on allergenicity assessment of genetically modified plants (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

No information is available on oral sensitisation or elicitation reactions of this endo-1,4-β-xylanase. Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al., 1998; Merget et al., 2001). However, several studies have shown that adults with occupational asthma to an enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Such information is not reported for xylanase. Overall, the likelihood of an allergic reaction upon oral ingestion of this endo-1,4-β-xylanase, produced with the genetically modified *B. licheniformis* strain NZYM-CE in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction is considered to be low.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. However, under the intended conditions of use of this food enzyme (see Section 3.5.1), the food enzyme–TOS remains in final foods.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions is considered to be 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 maximum recommended use levels of the food enzyme as provided by the applicant[27]

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	1 mg TOS/kg flour (up to 60 NXU/kg flour)
Cereal-based processes	Flour	0.5 mg TOS/kg flour (up to 30 NXU/kg flour)

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

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

The food enzyme remains in the final foods. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the endo-1,4-β-xylanase is inactivated during baking and cereal-based processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[28] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 3: Summary of estimated dietary exposure to the 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 mean (number of surveys)	0.001–0.003 (10)	0.003–0.006 (14)	0.003–0.006 (19)	0.002–0.004 (18)	0.001–0.002 (19)	0.001–0.002 (18)
Min–max 95th percentile (number of surveys)	0.002–0.012 (8)	0.006–0.010 (12)	0.005–0.011 (19)	0.003–0.008 (17)	0.002–0.005 (19)	0.002–0.004 (18)

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, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

[27] Technical dossier/Additional information May 2017.
[28] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

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 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	+/–
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	+/–
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 a considerable overestimation of the exposure.

3.6. Margin of exposure

The so derived NOAEL of 1,020 mg TOS/kg bw per day was compared to the exposure estimates of 0.001–0.006 mg TOS/kg bw per day at the mean and from 0.002 to 0.012 mg TOS/kg bw per day at 95th percentile, resulting in a Margin of Exposures of at least 85,000.

4. Conclusions

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

The CEP Panel considers the food enzyme endo-1,4-β-xylanase free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of endo-1,4-β-xylanase from a genetically modified strain of *B. licheniformis* (strain NZYM-CE)', March 2015, submitted by Novozymes A/S.
2) Additional information. May 2017, submitted by Novozymes A/S.
3) Additional information. February 2019, submitted Novozymes A/S.
4) Summary report on the genetically modified microorganism part. Delivered by Technical University of Denmark (Copenhagen, Denmark).
5) Summary report on genotoxicity and subchronic toxicity studies related to endo-1,4-β-xylanase produced with a strain of *B. licheniformis* (strain NZYM-CE). Delivered by FoBiG GmbH (Freiburg, Germany).

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming unit
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice

GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
rRNA	ribosomal ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

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

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5685).

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 FoodEx categories to the dietary exposure of the food enzyme–TOS

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, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).