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Safety evaluation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Trichoderma reesei* (strain DP-Nzd22)

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Abstract

The food enzyme endo-1,4-β-xylanase (EC 3.2.1.8) is produced with a genetically modified *Trichoderma reesei* (strain DP-Nzd22) by DuPont. 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. The endo-1,4-β-xylanase is intended to be used in distilled alcohol production, bakery and brewery. Residual amounts of total organic solids (TOS) are removed during the production of distilled alcohol, consequently dietary exposure was not calculated. For baking and brewing processes, based on the proposed maximum use levels, dietary exposure to the food enzyme–TOS was estimated to be up to 0.416 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. The Panel identified a No Observed Adverse Effect Level of 1,000 mg TOS/kg bw per day. A comparison of the no observed adverse effect level with the dietary exposure results in a sufficiently high margin of exposure (at least 2,400). Similarity of the amino acid sequence to those of known allergens was searched and no matches were 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 of such reactions to occur is considered to be low. Based on the data provided, the removal of TOS during the production of distilled alcohol and the derived margin of exposure for baking and brewing processes, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, glucoamylase, endo-1,4-β-xylanase, EC 3.2.1.3, *Trichoderma reesei*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

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

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 an approval via an EU Community list.

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Five applications have been introduced by the companies "Genencor international B.V.", "Amano Enzyme Inc" and "DSM Food Specialties B.V." for the authorisation of the food enzymes Endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* (DP-Nzd22), Acylglycerol Lipase from *Penicillium camemberti* (strain AE-LG), Beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), Beta-galactosidase from *Bacillus circulans* (strain AE-LT), and Arabinofuranosidase from *Aspergillus niger* (strain ARF).

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

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 Endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a

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

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

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

genetically modified strain of *Trichoderma reesei* (DP-Nzd22), Acylglycerol Lipase from *Penicillium camemberti* (strain AE-LG), Beta-galactosidase from *Kluyveromyces lactis* (strain AE-KL), Beta-galactosidase from *Bacillus circulans* (strain AE-LT), and arabinofurariosidase from *Aspergillus niger* (strain ARF) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of food enzyme endo-1,4-β-xylanase from a genetically modified strain of *T. reesei* (DP-Nzd22).

1.3. Information on existing authorisation and evaluations

The applicant reports that the Australia/New Zealand, Canada and France authorities have evaluated and authorised the use of endo-1,4-β-xylanase from genetically modified *T. reesei* in a number of food- and beverage-manufacturing processes. The food enzyme object of the present dossier has not been evaluated in the EU.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase produced with a genetically modified *T. reesei* (strain DP-Nzd22).

Additional information was sought from the applicant during the assessment process in response to requests from EFSA sent on 13 July 2017 and 27 June 2018 and subsequently provided (see section 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 existing Guidance from the EFSA Scientific Committee (EFSA, 2007).

This application has been evaluated following the current guidance on the submission of a dossier for safety evaluation of a food enzyme (EFSA CEF Panel, 2009), and the Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011). The exposure assessment was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

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

Endo-1,4-β-xylanase catalyses the hydrolysis of 1,4-glycosidic linkages in xylans (including arabinoxylans in which the xylan chain is substituted with arabinose residues) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths. It is intended to be used in baking processes, brewing processes and distilled alcohol production.

3.1. Source of the food enzyme

The endo-1,4-β-xylanase is produced with the genetically modified filamentous fungus *T. reesei* strain DP-Nzd22, which is deposited in the Westerdijk Fungal Biodiversity Institute with accession number [redacted][4]

[4] Technical dossier/Additional data September 2018/Annex AA.

3.1.1. Characteristics of the parental and recipient microorganisms[5]

The parental strain is *T. reesei* RL-P37, which has been derived from QM6a (ATCC 13631) by classical mutagenesis and selection for high cellulase activity (Sheir-Neiss and Montenecourt, 1984). The genome sequence and annotation data for QM6a are deposited in GenBank (accession number AAIL00000000, Martinez et al., 2008). Taxonomic identification of *T. reesei* QM6a (ATCC 13631) was performed by polymerase chain reaction (PCR) fingerprinting and by sequence analysis of the nuclear ribiosomal DNA region containing the internal transcribed spacers (ITS-1 and ITS-2) and the 5.8S rRNA gene (Kuhls et al., 1996).

[redacted]

3.1.2. Characteristics of the introduced sequences[5]

[redacted]

3.1.3. Description of the genetic modification process[5]

[redacted]

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 DP-Nzd22 differs from the recipient strain [redacted] only in its capacity to produce xylanase [redacted] The genetic stability of the production strain was demonstrated by Southern analysis, [redacted][6] The consistency of enzyme activity observed in three batches intended for commercialisation (Table 1) indicates that the production strain is phenotypically stable.

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

[5] Technical dossier/1st submission/Annex R.
[6] Technical dossier/2nd submission/Annex Y.
[7] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, p. 3–21.

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 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. 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 327 amino acids.[8] The molecular mass of the mature protein derived from the amino acid sequence (without the signal peptide) was calculated to be 35.5 kDa. The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Gels showed a major band with an apparent molecular mass of about 35 kDa which is consistent with the calculated value for the endo-1,4-β-xylanase. No other enzymatic side activities were reported.

The in-house determination of endo-1,4-β-xylanase activity is based on hydrolysis of the substrate *o*-nitrophenyl β-xylotrioside that results in the releasing *o*-nitrophenyl (reaction conditions: pH 5.0, 30°C, 10 min). The enzymatic activity is determined by measuring the release of *o*-nitrophenyl. The endo-1,4-β-xylanase activity is quantified relative to an enzyme standard and expressed in Xylanase Units/g (XBU/g).[9]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 55°C (pH 5.0) and a pH optimum between pH 4.0 and 5.0 (temperature 30°C). Thermostability was tested after a pre-incubation of the food enzyme for 60 min at different temperatures. Under the conditions (pH 5.0) of the applied temperature stability assay, the endo-1,4-β-xylanase activity decreased rapidly above 55°C showing no residual activity after pre-incubation at temperatures above 65°C.[10]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 18.3% (range 18.1–18.5%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 1,464.16.

Table 1: Compositional data of the food enzyme[(e)]

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Xylanase activity	XBU/g batch[(b)]	256,391	271,352	265,151	268,873
Protein	%	17.81	17.69	17.39	NA[(c)]
Ash	%	0.11	0.24	0.19	0.16
Water	%	81.61	81.24	81.73	84.59
Total organic solids (TOS)[(d)]	%	18.28	18.52	18.08	15.25
Activity/mg TOS	XBU/mg TOS	1,402.58	1,465.18	1,524.73	1,763

(a): Batch used for the toxicological studies.
(b): XBU/g: Xylanase Units/g (see Section 3.1.3).
(c): NA: not analysed.
(d): TOS calculated as 100% - % water - % ash.
(e): Technical dossier/1st submission /Annex F and Additional data September 2017.

[8] Technical dossier/1st submission/Annex H.
[9] Technical dossier/1st submission/Annex D.
[10] Technical dossier/Additional data September 2017.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 mg/kg which complies with the specification for lead (< 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[11]

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[12]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to elaborate a range of secondary metabolites (Blumenthal, 2004) including trichodermin (Watts et al., 1988). The applicant did not provide information on possible secondary metabolites produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in nine independent batches; [redacted][13]

The absence of recombinant DNA in the food enzyme was demonstrated by (PCR) analysis of three batches in triplicate. [redacted]

3.4. Toxicological data

The food enzyme batch 4 used for the toxicological assays has a similar activity/mg TOS than the three batches for commercialisation (Table 1). This indicates a similar purity as the commercial batches and thus batch 4 was considered suitable for the toxicological testing.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test[14]

A bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline No. 471 (OECD, 1997a,b) and following Good Laboratory Practice (GLP). Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation, applying the 'treat and plate' assay. Two separate experiments were carried out in duplicate and triplicate, respectively, using concentrations of the food enzyme of 1.5 (only for the first assay), 5.0, 15, 50, 150, 500, 1,500 and 5,000 μg/plate (corresponding to 0.2, 0.8, 2.3, 7.7, 22.9, 76.2, 228.8 and 762.5 μg TOS/plate). Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* Mammalian Chromosome Aberration test

An in vitro mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in human peripheral blood lymphocytes. Based on the results obtained in a dose-range finding test, the cells were treated with 1,000, 2,500 and 5,000 μg food enzyme/mL (corresponding to 152.5, 381.25 and 762.5 μg TOS/mL), applying a short-term treatment (4 h followed by 16 h recovery) in the presence and absence of S9-mix, and a continuous treatment (20+0 h) in the absence of S9-mix. In all the tested conditions, no statistically significant increases in the frequency of structural and numerical chromosomal aberrations and endoreplicated cells were observed in the treated cultures compared to the negative controls.

[11] LOD (Pb) = 0.5 ppm. Technical dossier/2nd submission/Annex G.
[12] Technical dossier/1st submission /Annex F.
[13] Technical dossier/1st submission /Annex U.
[14] Technical dossier/1st submission /Annex M.

The Panel concluded that the food enzyme did not induce chromosomal damage under the experimental conditions employed for this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents[15]

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD Test Guideline 408 (OECD, 1998) and following GLP. Three groups of 10 male and 10 female CD® [Crl:CD (SD)] rats were administered the food enzyme by oral gavage at the doses of 100, 300, and 1,000 mg TOS/kg body weight (bw) per day for 91 days. A control group received the vehicle (distilled water) at 10 mL/kg.

All animals survived to the scheduled termination.

Body weight and body weight gain of the test groups were similar to those of the control, except for statistically significant reduction in the high-dose males during the last week (days 84–91). This effect appeared to be driven by 2 out of 10 animals. Therefore, the Panel considered this difference as of no toxicological concern.

Statistically significant intermittent differences to controls were recorded in feed intake in all male and female groups, and in feed efficiency in all male groups. Overall feed intake in 300 and 1,000 mg TOS/kg per day females was statistically significantly lower than in controls. However, these differences were not considered treatment related since there was no test article-related differences in final body weight or overall body weight change of females and of males except in the last week of the study for high-dose males, and there was no difference in overall feed efficiency in either sex.

Measurement of locomotor activity showed some statistically significant differences in males but not in females; however they were intermittent and not dose related. Therefore, they were considered incidental.

Similarly, scattered differences in haematology and clinical chemistry parameters and urinalysis were observed, which were also considered incidental.

In macroscopic examination, one low-dose male had mild tan foci in the median lobe of the liver that correlated with microscopic finding of extensive, irregular areas of necrosis characterised by a central area of liquefaction surrounded by areas of coagulative necrosis of hepatocytes, and immature fibrous capsule. This finding was considered by the Panel as not treatment-related because of the single isolated incidence and a lack of a dose response.

The Panel identified a no observed adverse effect level (NOAEL) of 1,000 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[16]

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 this xylanase produced with the genetically modified *T. reesei* strain DP-Nzd22 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a window of 80 amino acids as the criterion, no matches were found.

No information is available on oral sensitisation or elicitation reactions of this endo-1,4-β-xylanase. However, 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 *T. reesei* strain DP-Nzd22 in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur 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.

[15] Technical dossier/1st submission /Annex O.
[16] Technical dossier/1st submission/Annex P.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food manufacturing 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(a)	Raw materials	Recommended dosage of the food enzyme
Baking processes	Flour	0.5–35 mg TOS/kg flour
Brewing processes	Cereals	0.5–35 mg TOS/kg cereals (brewing); 0.1–6 mg TOS/kg cereals (cereal beverages production)
Distilled alcohol production	Cereals	0.1–11 mg TOS/kg cereals

TOS: total organic solids.
(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.
(b): Technical dossier/p. 58–61 and p. 80–91.

In distilled alcohol production, the xylanase food enzyme is applied during liquefaction and fermentation, may also be added during slurry mixing and pre-saccharification. Endo-1,4-β-xylanase catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylan. The reaction products are (1→4)-β-D-xylan oligosaccharides of different lengths.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production (Documentation provided to EFSA No 4). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by distillation.

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

In brewing processes, the xylanase food enzyme is added to the mash and may also be added during the fermentation step.

The food enzyme remains in the dough and wort. Based on data provided on thermostability (see Section 3.3.1), it is expected that the endo-1,4-β-xylanase will be inactivated during baking and brewing processes.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by distillation (by > 99%), foods/ingredients derived through this process, i.e. distilled alcohol, were excluded from the estimation.

For the baking and brewing processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[17] 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 bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day per subject were excluded and high-level

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

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 food enzyme–TOS in six population groups

Population group	Estimated exposure (mg/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)	0.007–0.097 (10)	0.074–0.209 (14)	0.085–0.202 (19)	0.048–0.131 (18)	0.041–0.116 (19)	0.039–0.077 (18)
Min–max 95th percentile (number of surveys)	0.038–0.416 (8)	0.184–0.356 (12)	0.165–0.380 (19)	0.103–0.267 (17)	0.091–0.280 (19)	0.079–0.146 (18)

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.

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	+
Exposure from brewing processes, including cereal based beverages, was calculated using the TOS indicated for beer brewing	+
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	+/–

+: 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

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.007–0.209 mg/kg bw per day at the mean and from 0.038 to 0.416 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) of at least 2,400.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production and the MOE calculated when used in baking and brewing, the Panel concludes that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *T. reesei* strain DP-Nzd22 does not give rise to safety concerns under the intended conditions of use.

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

Documentation provided to EFSA

1) Technical dossier: Application for authorisation of Endo-1,4-beta-xylanase from *Aspergillus niger* expressed in a genetically modified strain of *Trichoderma reesei* DP-Nzd22 in accordance with Regulation (EC) No 1331/2008. October 2014. Submitted by Genencor International B.V.
2) Additional information. September 2017. Genencor International B.V.
3) Additional information. September 2018. Genencor International B.V.
4) Additional information on 'Food enzyme removal during the production of cereal-based distilled alcoholic beverages'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.
5) Summary report on GMM part for 1,4-beta-xylanase, produced by *Trichoderma reesei* strain DP-Nzd22, EFSA-Q-2014-00667. Delivered by Technical University of Denmark (Lyngby, Denmark).

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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 units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
[redacted]	[redacted]
GLP	Good Laboratory Practice
GM	genetically modified
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
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 (see Supporting document 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: The contribution of FoodEx categories to the food enzyme–TOS dietary exposure.

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, 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).