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

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Abstract

The food enzyme considered in this opinion is an endo-1,4-β-xylanase (EC 3.2.1.8) produced with a genetically modified strain of *Aspergillus niger*. The genetic modifications do not give rise to safety concerns. The food enzyme contains neither the production organism nor recombinant DNA. The endo-1,4-β-xylanase is intended to be used in baking processes. Based on the maximum use levels recommended for the respective food process, dietary exposure to the food enzyme–total organic solids (TOS) was estimated on the basis of individual data from the EFSA Comprehensive European Food Consumption Database. This exposure estimate is below 0.013 mg TOS/kg body weight (bw) per day in European populations. No safety concerns were identified in relation to the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, allergenicity and exposure assessments. The allergenicity was evaluated by comparing the amino acid sequence to those of known allergens; no match was found. The Panel considered that the likelihood of allergic reactions to dietary intake of endo-1,4-β-xylanase is low and, therefore, does not give rise to safety concerns. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rodents. A no observed adverse effect level was derived (4,095 and 4,457 mg TOS/kg bw per day for males and females, respectively), which, compared with the dietary exposure, results in a sufficiently high margin of exposure. However, the genotoxicity data were incomplete. Due to the absence of the recommended combination of microbial strains used in the Ames test (i.e. lack of *Salmonella* Typhimurium TA102 and *Escherichia coli* WP2), no conclusions can be drawn on potential DNA oxidising or cross-linking mechanisms giving rise to gene mutations. Consequently, no final conclusions can be drawn on genotoxicity.

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

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Note: The full opinion will be published in accordance with Article 5 of Regulation (EC) No 1331/2008 once the decision on confidentiality, in line with Article 12(3) of the Regulation, will be received from the European Commission. The following information has been provided under the confidentiality framework and has been redacted awaiting the decision of the Commission: steps and control points during the processing, process chemicals, and genetic modifications.

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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 microorganisms 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 entered 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 an approval via a Union list.

The Guidance on submission of a dossier on a food enzyme for evaluation (EFSA, 2009b) 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 on food enzymes. According to Regulation (EC) No 1332/2008 on food enzymes, a food enzyme which falls within the scope of Regulation (EC) No 1829/2003[3] on genetically modified food and feed should be authorised in accordance with that Regulation as well as under this Regulation.

An application has been submitted by the company DSM Food Specialities for the authorisation of the food enzyme endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XYL).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[4] implementing 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 1493/199, 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] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003 p. 1–23.

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

1.1.2. Terms of Reference

The European Commission requests EFSA to carry out the safety assessment on the food enzyme endo-1,4-β-xylanase from a genetically modified strain of *Aspergillus niger* (strain XYL) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Information on existing authorisations and evaluations

The applicant reports that the Australian/New Zealand, Brazilian, Canadian, Chinese, Danish, French, Russian and Singaporean authorities have evaluated and authorised the use of endo-1, 4-ß-xylanase from genetically modified *Aspergillus niger* in baking processes. The Danish authority also sets out the conditions of use, including the dosages for specific foods, which is up to 248 endo-xylanase units (EDX)/kg flour.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme endo-1,4-β-xylanase obtained from a genetically modified microorganism and also in the Abbreviations respectively. (GMM) *Aspergillus niger* (strain XYL). The food enzyme is intended to be used in baking processes.

2.2. Methodologies

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

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA, 2009b) has been followed for the evaluation of this 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

3.1. Technical data

3.1.1. Identity of the food enzyme

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

3.1.2. Chemical parameters

The endo-1,4-β-xylanase produced with the genetically modified *Aspergillus niger* strain XYL is a single polypeptide chain of 211 amino acids, including a signal sequence of 27 amino acids. The molecular mass of the mature protein, with the signal sequence cleaved off, derived from the amino acid sequence, was calculated to be about 20 kDa. The protein homogeneity status of the food enzyme was investigated by sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) analysis. The apparent molecular mass based on this technique is about 22 kDa. The protein profile also included bands corresponding to 116 kDa, 66 kDa and 55 kDa.

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

The total organic solids (TOS) content is a calculated value derived as 100% minus % water minus % ash. The average TOS content of the three commercial enzyme batches was 23.5% (w/w); the values ranged from 21.1% to 26.1% (Table 1). The four food enzyme batches presented in Table 1

are concentrates without any added diluents. The batch used for toxicological tests was spray-dried after concentration. Consequently, the water content in the latter batch was lower.

The average enzyme activity/TOS ratio of the three commercial food enzyme batches was 212 EDX/mg TOS; the values ranged from 207 to 220 EDX/mg TOS (Table 1). Considering the low variability of the enzyme activity/TOS ratio in the three commercial food enzyme batches, the average activity/TOS ratio of 212 EDX/mg TOS was used for subsequent calculations.

Other arabinoxylan-degrading enzymes (xylan-1,4-β-xylosidase and α-L-arabinofuranosidase), which play a subsidiary role in the application, are known to be present in the food enzyme. No other enzymatic side activities have been reported by the applicant.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4[(a)]
Endo-1,4-β-xylanase activity	EDX/g batch[(b)]	57,550	47,965	44,050	143,000
Protein	%	19.4	17.7	17.2	76.2
Ash	%	0.7	0.4	0.5	2.0
Water	%	73.2	76.4	78.4	3.4
Total organic solids (TOS)[(c)]	%	26.1	23.2	21.1	94.6
Endo-1,4-β-xylanase activity/mg TOS	EDX/mg TOS	220	207	209	151

(a): Batch used for the toxicological tests. At the time, the tox-batch was produced, the activity was expressed in so-called EXU units. The activity of the batch 4 was 937,500 EXU/g, corresponding to 143,000 EDX/g.
(b): EDX: Endo-Xylanase units (see Section 3.1.3).
(c): TOS calculated as 100%-% water-% ash.

The food enzyme 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).

The presence of mycotoxins (aflatoxins (B1, B2, G1 and G2), ochratoxin A, fumonisins (B1, B2 and B3), nivalenol (NIV), 3-acetyl-deoxynivalenol (3AcDON), 15-acetyl-deoxynivalenol (15AcDON), fusarenone, T-2 toxin, zearalenone, deoxynivalenol (DON), HT2 toxin (HT2), diacetoxyscirpenol (DAS) and neosolaniol (NEO)) was examined in the four food enzyme batches and these mycotoxins were found not to be present at detectable levels in the food enzyme.

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 are not more than 30 colony forming units (CFU) per gram.

The applicant has provided information on the identity of the antifoam agent used. Taking into account the nature and properties of the antifoam agent, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers its use as of no safety concern.

The data provided regarding compositional batch-to-batch-variability are considered sufficient. Table 1 shows that the food enzyme batch used for the toxicological assays has a lower activity/TOS ratio and higher ash concentration compared with the three commercial food enzyme batches. Consequently, this food enzyme batch is cruder than the three commercial food enzyme batches used and is therefore considered suitable for the toxicological testing.

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

3.1.3. Properties of the food enzyme

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

The endo-1,4-β-xylanase activity is quantified based on the hydrolysis of arabinoxylan and is expressed in Endo-Xylanase units/g (EDX/g). The enzyme activity is measured relative to an internal enzyme standard. The analytical principle is based on the hydrolysis of the substrate arabinoxylan, resulting in a decrease of the viscosity at 47°C and pH 2.75. One EDX unit is defined as the enzyme activity that creates a certain viscosity change in one millilitre of the reaction mixture under the conditions of the assay.

Endo-1,4-β-xylanase has been characterised regarding its activity depending on temperature and pH. The temperature profile has been measured from 0 up to 75°C and the xylanase. The xylanase shows the temperature optimum of 60°C. The food enzyme is rapidly inactivated at temperatures above 60°C during baking. The pH profile has been measured within a pH range of 1.5–8.5 (with an optimum of pH 3).

3.1.4. Information on the source material

3.1.4.1. Information related to the genetically modified microorganism

According to the CEF Guidance, the certificate of deposit of the strain in a public validated culture collection should be provided. The applicant deposited the endo-1,4-β-xylanase production strain *Aspergillus niger* XYL only in the DSM internal culture collection under code DS 26538. The Panel noted that this would not allow a verification of the strain independently of the company.

The production strain was taxonomically identified based on morphology by the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). The taxonomic identification is supported by whole genome sequence (see Section 3.1.4.2).

3.1.4.2. Characteristics of the recipient or parental microorganisms

The parental microorganism is a filamentous fungus *A. niger*. *A. niger* has a long history of use in the production of food enzymes (Schuster et al., 2002; van Dijck et al., 2003). *A. niger* strains are not qualified for Qualified Presumption of Safety (QPS) status because of the potential of toxin production (EFSA BIOHAZ Panel, 2017). The mycotoxins of concern are ochratoxin A (OTA) and fumonisins (FUM) (Frisvad et al., 2007); Pel et al. 2007.

[redacted]

3.1.4.3. Characteristics of the donor organisms

[redacted]

3.1.4.4. Description of the genetic modification process

[redacted]

3.1.4.5. Safety aspects of the genetic modifications

[redacted]

[redacted]

3.1.5. Manufacturing process

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[5] and in accordance with current Good Manufacturing Practice (GMP). The manufacturing process is certified according to Food Safety Systems Certification 22000 (FSSC 22000).

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

The downstream processing includes recovery, purification and concentration. The food enzyme produced is recovered from the fermentation broth after biomass separation using filtration. Further purification and concentration involve a series of filtration steps, including ultrafiltration and sterile filtration.

[redacted]

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

3.1.6. Safety for the environment

Accordingly, as the food enzyme belongs to Category 2 of the guidance on risk assessment of genetically modified microorganisms and their products (EFSA GMO Panel, 2011), environmental exposure to the genetically modified microorganism is negligible and hence no environmental risk assessment is required.

3.1.7. Reaction and fate in food

The enzyme endo-1,4-β-xylanase catalyses the hydrolysis of 1,4-β-D-xylosidic linkages in xylan (including arabinoxylan, i.e. xylan branched with arabinose) resulting in the production of (1→4)-β-D-xylan and (1→4)-β-D-arabinoxylan oligosaccharides of different lengths.

Endo-1,4-β-xylanase is specific in its action, not known to catalyse other reactions than this endo-hydrolysis of xylans to shorter xylan chains, xylo-oligosaccharides and xylose. These reaction products are naturally present in xylan-containing foods. Owing to the substrate specificity of the xylanase, no unintended reaction products in foods are to be expected.

The food enzyme has not been tested for other enzyme activities, however and according to the applicant it contains side activities such as xylan-1,4-β-xylosidase and α-L-arabinofuranosidase that are not relevant for food applications.

Therefore, no unintended products resulting from the food enzyme are to be expected.

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

3.1.8. Case of need and intended conditions of use

The endo-1,4-β-xylanase is intended to be used in baking processes[6] at a recommended use level of 0.16–1.17 mg TOS/kg flour.

In baking processes, endo-1,4-β-xylanase is used to facilitate handling of the dough, to improve its structure and behaviour during baking as well as to reduce batter viscosity. The food enzyme is added during the preparation of the dough at the beginning of this process.

[5] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, p. 3–21.

[6] 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 adoption of this opinion.

According to the applicant, the food enzyme is used at the minimum dosage necessary to achieve the desired reaction in line with GMP. The use level applied by a food manufacturer in practice depends on the particular process.

3.2. Dietary exposure

Exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment of the food processes covered in this opinion involved selection of relevant food groups and application of process and technical conversion factors (Appendix B). These input data were subject to a stakeholder consultation through open calls,[7] and adjusted in accordance with feedback received.

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (hereafter the EFSA Comprehensive Database[8]) has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011a). New consumption surveys recently added to the Comprehensive Database were also taken into account in this assessment.

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to possible subjects' underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Appendix A).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

3.2.2. Exposure assessment methodology

Chronic exposure was calculated based on individual consumption from the Comprehensive Database, averaged over the total survey period, excluding surveys with only 1 day per subject. High-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011a).

The exposure per FoodEx category was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of survey days and normalised for individual body weight (bw), resulting in an individual average exposure/day per kg bw for the survey period. This was done for all individuals in the survey and per age class, resulting in distributions of individual average exposure per survey and age class. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class.

3.2.3. Exposure to food enzyme–TOS according to the intended use proposed by the applicant

Exposure to the food enzyme–TOS was based on intended use and the recommended maximum use levels of the food enzyme–TOS provided by the applicant (Table 2). Food enzyme–TOS exposure was calculated from foods produced involving a baking process.

Relevant food groups and/or individual foods were selected from the Comprehensive Database and were assumed to always contain the food enzyme–TOS at the maximum recommended use level. This will result in an overestimation of exposure to food enzyme–TOS.

To facilitate matching of the reported use levels for baking processes with foods identified in the Comprehensive Database, the selected foods were disaggregated to ingredient level as appropriate,

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

and converted into the corresponding raw material, i.e. flour, via the application of conversion factors (Appendix B). For example, consumption of 100 g of bread was converted into an intake of 70 g flour (recipe fraction of 0.7) and then multiplied by 1.17 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 0.08 mg TOS/100 g bread.

Exposure to the food enzyme–TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight (kg bw) separately for each individual in the database. Table 2 provides an overview of the derived exposure estimates. The average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey are reported in Appendix C – Table 1. The contribution of the food enzyme–TOS from each FoodEx category to the total dietary exposure is indicated in Appendix C – Table 2.

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Estimated exposure (mg/kg bw 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)	0.000–0.004 (6)	0.003–0.007 (10)	0.003–0.007 (18)	0.002–0.005 (17)	0.001–0.003 (17)	0.001–0.002 (14)
Min–max 95th percentile (number of surveys)	0.004–0.010 (5)	0.007–0.012 (7)	0.006–0.013 (18)	0.003–0.009 (17)	0.003–0.006 (17)	0.002–0.004 (14)

bw: body weight.

3.2.4. Uncertainty analysis

Uncertainties in the exposure assessment of the food enzyme have been discussed above. 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 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
	Exposure to food enzyme–TOS
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
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 based on the description of the food process provided by the applicant (based on examples given by applicant)	+
Use of recipe fractions in disaggregation FoodEx categories likely to contain the food enzyme	+/–

+: 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.

3.3. Toxicological data

The toxicological assays were performed with the tox-batch which was produced according to the procedure used for commercial production. It was spray-dried after ultrafiltration (UF) concentration and the test material became more concentrated.

3.3.1. Bacterial reverse mutation test

In order to investigate the potential to induce gene mutations in bacteria, a bacterial reverse mutation assay (Ames test) was performed according to OECD Test Guideline 471 (OECD, 1983) and following Good Laboratory Practice (GLP) strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) in the presence or absence of metabolic activation (S9-mix) applying the 'treat and plate assay'. The food enzyme was not tested in *S.* Typhimurium TA102 or *E. coli* WP2 strains. Two independent experiments were carried out using five different concentrations of the food enzyme (100, 320, 1,000, 3,200 and 10,000 μg food enzyme/ml, corresponding to ca. 95, 303, 946, 3,027 and 9,460 μg TOS/ml), positive controls and purified water as negative control.

Neither a certificate on quality control and production for the post-mitochondrial fraction nor a statement clarifying whether each batch has been characterized with a mutagen that requires metabolic activation by microsomal enzymes, were provided. All positive controls induced significant increases in revertant colony numbers, while the negative controls were within the normal ranges. Upon treatment with the food enzyme, there was no increase in revertant colony numbers above control values in any strain either with or without metabolic activation. The Panel concluded that the food enzyme did not induce gene mutations in the four strains tested. However, based on literature (Wilcox et al., 1990) and on the most recent version of OECD Guideline No. 471 (OECD, 1997) these four *S.* Typhimurium strains may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E. coli* WP2 strains or *S.* Typhimurium TA102. The applicant was requested, but declined to provide these data. Therefore, no conclusions can be drawn on the possibility of the food enzyme to induce gene mutations by DNA oxidising or cross-linking mechanisms.

3.3.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1983) and following GLP. Human peripheral whole blood cultures were treated with physiological saline (negative control), the food enzyme or positive controls (chlorambucil and cyclophosphamide, in the absence and presence of S9-mix, respectively). Two experiments were performed in duplicate cultures. In the absence of S9-mix, the cells were exposed continuously to the food enzyme for 21 h (1,250, 2,500 and 5,000 μg/mL, corresponding to ca 1,183, 2,365 and 4,730 μg TOS/mL) and 45 h (5,000 μg/mL corresponding to ca 4,730 μg TOS/mL). In the presence of S9-mix, the cultures were exposed to the food enzyme for 3 h and harvested after 18 or 42 h of recovery (1,250, 2,500 and 5,000 μg/mL corresponding to ca 1,183, 2,365 and 4,730 μg TOS/mL). Two hundred metaphases were scored per experimental point. The reductions in mitotic index did not exceed 41% of negative control values at any concentration of food enzyme tested. The frequency of chromosomal aberrations in treated cultures was comparable to the values detected in negative controls. No significant increase in polyploid or endoreplicated cells was observed. Based on the biological relevance of the results, it was concluded that the food enzyme did not induce structural chromosome aberrations in cultured mammalian cells when tested up to 5,000 μg food enzyme/mL (corresponding to ca 4,730 μg TOS/mL) under the conditions of the study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1981) and following GLP. Groups of 10 male and 10 female CD strain rats, 4–5 weeks old, received the food enzyme as a powder mixed with their RM1 diet for 13 weeks at low-, mid- and high-dose levels of 2,000, 10,000 and 50,000 mg food enzyme/kg diet equivalent to 1,892, 9,460 and 47,300 mg TOS/kg diet. Average consumptions of food enzyme corresponded to 162, 811

and 4,095 mg TOS/kg bw per day for males and 171, 855 and 4,457 mg TOS/kg bw per day for females, respectively. Control groups received RM1 diet alone.

No treatment-related deaths or clinical signs or consistent effects on body weight, food consumption, food conversion efficiency, ophthalmic lesions or haematological parameters were observed. During week 13 of treatment, prothrombin time was marginally higher for all treated females (13.8 ± 0.8, 13.3 ± 0.6 and 13.2 ± 0.9 s for low, mid and high dose, respectively) in comparison to the control group (12.4 ± 0.6 s). However, these were within the range of the laboratory historical control data (13.9 ± 1.2 s for 744 animals) and no pathological or liver enzyme changes were observed.

Marginal, but statistically significant increases of urinary total protein and alpha-1-globulin levels observed in high-dose females were considered of no toxicological relevance.

Although increases of the absolute and relative weights of the adrenal glands (16% and 17%, respectively) and ovary (21% and 22%, respectively) were seen in the female group at the highest dose, histopathological examination showed no evidence of any changes related to treatment.

Overall, the Panel derived a no observed adverse effect level (NOAEL) based on the high dose level of 4,095 mg TOS/kg bw per day for males and 4,457 mg TOS/kg bw per day for females, respectively.

A comparison of the NOAEL (4,457 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.001–0.007 mg/kg bw per day at the mean and from 0.002–0.013 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) above 3 x 10^5, indicating that there is no safety concern.

3.4. Allergenicity

The potential allergenicity of the endo-1,4-β-xylanase (xylanase) from the genetically modified *A. niger* (strain XYL) has been assessed by comparison of its amino acid sequence with those of known allergens according to the EFSA Scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

Several cases of occupational allergy consecutive to inhalation of aerosols containing xylanase or other enzymes have been reported (Martel et al., 2010). However, several studies have shown that adults with occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no food allergic reactions to xylanase have been reported in the literature.

Xylanase from a genetically modified *Aspergillus oryzae* was also tested in the study of Bindslev-Jensen et al. (2006) to investigate the possible cross-reactivity of 19 different commercial enzymes used in the food industry in allergic patients (400 patients allergic to inhalation allergens, food allergens, bee or wasp). In a few patients, the tested xylanase from a genetically modified *A. oryzae* gave positive results in a skin prick test and a histamine release test; however, these positive reactions are without clinical relevance as oral exposure to even high doses of the xylanase did not result in allergic reactions.

Consequently, the CEF Panel considers that the likelihood of food allergic reactions to this endo-1, 4-β-xylanase produced with the genetically modified strain of *A. niger* (strain XYL) is low and therefore does not raise safety concerns.

Conclusions

No safety concerns were identified in relation to the genetic modifications performed, the manufacturing process, the compositional and biochemical data provided, allergenicity and exposure assessments. Regarding the toxicological studies, the repeated dose oral 90-day study also did not raise safety concerns. However, in the absence of the recommended combination of microbial strains used in the Ames test, no conclusions can be drawn on a DNA oxidising or cross-linking potential. Consequently, no final conclusion can be drawn on genotoxicity.

Documentation provided to EFSA

1) Dossier 'Application for authorisation of xylanase derived from a genetically modified strain of *Aspergillus niger* (strain XYL)'. November 2014. Submitted by DSM Food Specialities.
2) Additional information submitted on 17 June 2015 by the applicant.
3) Additional information submitted on 26 October 2016 by the applicant.

4) Summary report on GMM part for xylanase derived from a genetically modified strain of *Aspergillus niger* (strain XYL), EFSA-Q-2014-00305'. Delivered by Technical University of Denmark (DTU).
5) Summary report on technical data and dietary exposure for xylanase derived from a genetically modified strain of *Aspergillus niger* (strain XYL). Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy).
6) Summary report on genotoxicity and subchronic toxicity study related to endo-1,4-beta-xylanase produced with a strain of *Aspergillus niger* (strain XYL). Delivered by FoBiG GmbH, (Freiburg, Germany).

References

Andersen MR, Salazar MP, Schaap PJ, van de Vondervoort PJ, Culley D, Thykaer J, Frisvad JC, Nielsen KF, Albang R, Albermann K, Berka RM, Braus GH, Braus-Stromeyer SA, Corrochano LM, Dai Z, van Dijck PW, Hofmann G, Lasure LL, Magnuson JK, Menke H, Meijer M, Meijer SL, Nielsen JB, Nielsen ML, van Ooyen AJ, Pel HJ, Poulsen L, Samson RA, Stam H, Tsang A, van den Brink JM, Atkins A, Aerts A, Shapiro H, Pangilinan J, Salamov A, Lou Y, Lindquist E, Lucas S, Grimwood J, Grigoriev IV, Kubicek CP, Martinez D, van Peij NN, Roubos JA, Nielsen J and Baker SE, 2011. Comparative genomics of citric-acid-producing Aspergillus niger ATCC 1015 versus enzyme-producing CBS 513.88. Genome Research, 21, 885–897. https://doi.org/10.1101/gr.112169.110

Armentia A, Días-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204.

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P and Brinch DS, 2006. Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry. Food and Chemical Toxicology, 44, 1909–1915.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502; quiz 502, 426.

Corrick CM, Twomey AP and Hynes MJ, 1987. The nucleotide sequence of the amdS gene of *Aspergillus nidulans* and the molecular characterization of 5' mutations. Gene, 53, 63–71.

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. EFSA Journal 2007;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Committee on transparency in scientific aspects of risk assessments carried out by EFSA. Part 2: Generel principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2093/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2009b. Guidance of EFSA prepared by the Scientific panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the submission of a dossier on food enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):1970, 27 pp. https://doi.org/10.2903/j.efsa.2011.1970

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Klein G (deceased), Prieto Maradona M, Querol A, Peixe L, Suarez JE, Sundh I, Vlak JM, Aguilera-Gomez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C and Fernández Escámez PS, 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal 2017;15(3):4664, 177 pp. https://doi.org/10.2903/j.efsa.2017.4664

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi JP, Fowler P, Franz R, Grob K, Gürtler R, Husøy T, Kärenlampi S, Mennes W, Milana MR, Penninks A, Smith A, Tavares PM, Tlustos C, Wölfle D, Zorn H, Zugravu CA, Arcella D, Liu Y and Engel KH, 2016. Panel statement on the exposure assessment of food enzymes. EFSA Journal 2016;10 (10):4581, 11 pp. https://doi.org/10.2903/j.efsa.2016.4581

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion. Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. https://doi.org/10.2903/j.efsa.2011.2193

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: ftp://ftp.fao.org/docrep/fao/009/a0675e/a0675e00.pdf

Frisvad JC, Larsen TO, deVaries R, Meijer M, Houbraken J, Cabañes FJ, Ehrlich K and Samson RA, 2007. Secondary metabolite profiling, growth profiles and other tools for species recognition and important *Aspergillus* mycotoxins. Studies in Mycology, 59, 31–37. https://doi.org/10.3114/sim.2007.59.04

Martel C, Nielsen GD and Mari A, et al., 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitisation. European Food Safety Authority (EFSA) - external report, 2010. Question number: EFSA-q-2009-00789. Available online: http://www.efsa.europa.eu/fr/supporting/pub/75e

OECD (Organisation for Economic Co-operation and Development), 1981. Repeated Dose 90-Day Oral Toxicity Study in Rodents Guideline 408, adopted 12/05/1981.

OECD (Organisation for Economic Co-operation and Development), 1983. Genetic Toxicology: Salmonella typhimurium, Reverse Mutation Assay, adopted 26/05/1983.

OECD (Organisation for Economic Co-operation and Development), 1983. Genetic Toxicology: In vitro Mammalian Cytogenetic Test. Guideline 473, adopted 12/05/1983.

Pel HJ, de Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, de Vries RP, Albang R, Albermann K, Andersen MR, Bendtsen JD, Benen JAE, van den Berg M, Breestraat S, Caddick MX, Contreras R, Cornell M, Coutinho PM, Danchin EGJ, Debets AJM, Dekker P, van Dijck PWM, van Dijk A, Dijkhuizen L, Driessen AJM, d'Enfert C, Geysens S, Goosen C, Groot GSP, de Groot PWJ, Guillemette T, Henrissat B, Herweijer M, van den Hombergh JPTW, van den Hondel CAMJJ, van der Heijden RTJM, van der Kaaij RM, Klis FM, Kools HJ, Kubicek CP, van Kuyk PA, Lauber J, Lu X, van der Maarel MJEC, Meulenberg R, Menke H, Mortimer MA, Nielsen J, Oliver SG, Olsthoorn M, Pal K, van Peij NNME, Ram AFJ, Rinas U, Roubos JA, Sagt CMJ, Schmoll M, Sun J, Ussery D, Varga J, Vervecken W, van der Vondervoort PJJ, Wedler H, Wösten HAB, Zeng A-P, van Ooyen AJJ, Visser J and Stam H, 2007. Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. Nature Biotechnology, 25, 221–231.

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.

Schuster E, Dunn-Coleman N, Frisvad JC and van Dijck PWM, 2002. On the safety of *Aspergillus niger* — a review. Applied Microbiology and Biotechnology, 59, 426–435.

Van Dijck PWM, Selten GCM and Hempenius RX, 2003. On the safety of a new generation of DSM Aspergillus niger enzyme production strains. Regulatory Toxicology and Pharmacology, 38, 27–35.

Wilcox P, Naidoo A, Wedd DJ and Gatehouse DG, 1990. Comparison of Salmonella typhimurium TA 102 with Escherichia coli WP2 Tester strains. Mutagenesis, 5, 285–291.

Abbreviations

3AcDON	3-acetyl-deoxynivalenol
15AcDON	15-acetyl-deoxynivalenol
bw	body weight
CAS	Chemical Abstracts Service
CBS	Centraalbureau voor Schimmelcultures
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CFU	colony forming units
DAS	diacetoxyscirpenol
DON	deoxynivalenol
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EDX	endo-xylanase units
FAO	Food and Agricultural Organization
FSSC	Food Safety Systems Certification
FUM	fumonisin
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
NEO	neosolaniol
NIV	nivalenol
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OTA	ochratoxin A

RNA	ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
QPS	Qualified Presumption of Safety
TOS	total organic solids
UF	ultrafiltration
WHO	World Health Organization

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

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

Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material	Recipe fraction	mg TOS/kg flour
A.01	Grains and grain-based products (unspecified)	0.8	1	1.17
A.01.03	Grain milling products (unspecified)	1	1	1.17
A.01.03.001	Wheat milling products (unspecified)	1	1	1.17
A.01.03.001.001	Wheat flour, brown	1	1	1.17
A.01.03.001.002	Wheat flour, Durum	1	1	1.17
A.01.03.001.003	Wheat flour, white	1	1	1.17
A.01.03.001.004	Wheat flour, wholemeal	1	1	1.17
A.01.03.001.005	Graham flour	1	1	1.17
A.01.03.001.006	Wheat flour, gluten free	1	1	1.17
A.01.03.001.014	Wheat starch	1.2	1	1.17
A.01.03.002	Rye milling products (unspecified)	1	1	1.17
A.01.03.002.001	Rye flour, gluten free	1	1	1.17
A.01.03.002.002	Rye flour, light	1	1	1.17
A.01.03.002.003	Rye flour, medium	1	1	1.17
A.01.03.002.004	Rye flour, wholemeal	1	1	1.17
A.01.03.003	Buckwheat milling products (unspecified)	1	1	1.17
A.01.03.003.001	Buckwheat flour	1	1	1.17
A.01.03.004	Corn milling products (unspecified)	1	1	1.17
A.01.03.004.001	Corn flour	1	1	1.17
A.01.03.004.003	Corn starch	1.3	1	1.17
A.01.03.005	Oat milling products (unspecified)	1	1	1.17
A.01.03.005.002	Oat flour	1	1	1.17
A.01.03.005.004	Oat starch	1.2	1	1.17
A.01.03.006	Rice milling products (unspecified)	1	1	1.17
A.01.03.006.001	Rice flour	1	1	1.17
A.01.03.006.002	Rice flour white	1	1	1.17
A.01.03.006.003	Rice flour, instant	1	1	1.17
A.01.03.006.004	Rice starch	1.2	1	1.17
A.01.03.007	Spelt milling products	1	1	1.17
A.01.03.008	Other milling products (unspecified)	1	1	1.17
A.01.03.008.001	Amaranth flour	1	1	1.17
A.01.03.008.002	Barley flour	1	1	1.17
A.01.03.008.003	Chapatti flour	1	1	1.17
A.01.03.008.004	Flour mix, wheat/rye/barley/oats	1	1	1.17
A.01.03.008.005	Millet flour	1	1	1.17
A.01.03.008.007	Sorghum flour	1	1	1.17
A.01.04	Bread and rolls (unspecified)	1	0.7	1.17
A.01.04.001	Wheat bread and rolls	1	0.7	1.17
A.01.04.002	Rye bread and rolls	1	0.7	1.17
A.01.04.003	Mixed wheat and rye bread and rolls	1	0.7	1.17
A.01.04.004	Multigrain bread and rolls	1	0.7	1.17
A.01.04.005	Unleavened bread, crisp bread and rusk (unspecified)	1	0.8	1.17
A.01.04.005.001	Crisp bread, rye wholemeal	1	0.9	1.17
A.01.04.005.002	Crisp bread, rye, light	1	0.9	1.17

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material	Recipe fraction	mg TOS/kg flour
A.01.04.005.003	Crisp bread, wheat, wholemeal	1	0.9	1.17
A.01.04.005.004	Crisp bread, wheat, light	1	0.9	1.17
A.01.04.005.005	Rusk, light	1	0.9	1.17
A.01.04.005.006	Rusk, wholemeal	1	0.9	1.17
A.01.04.005.007	Pita bread	1	0.7	1.17
A.01.04.005.008	Matzo	1	0.9	1.17
A.01.04.005.009	Tortilla	1	0.7	1.17
A.01.04.006	Other bread	1	0.7	1.17
A.01.04.007	Bread products	1	0.7	1.17
A.01.07	Fine bakery wares (unspecified)	1	0.5	1.17
A.01.07.001	Pastries and cakes (unspecified)	1	0.5	1.17
A.01.07.001.001	Beignets	1	0.15	1.17
A.01.07.001.002	Buns	1	0.7	1.17
A.01.07.001.003	Cake from batter	1	0.25	1.17
A.01.07.001.004	Cheese cream cake	1	0.24	1.17
A.01.07.001.005	Cheese cream sponge cake	1	0.24	1.17
A.01.07.001.006	Chocolate cake	1	0.24	1.17
A.01.07.001.007	Chocolate cake with fruits	1	0.24	1.17
A.01.07.001.008	Cream cake	1	0.24	1.17
A.01.07.001.009	Cream cheese cake	1	0.24	1.17
A.01.07.001.010	Cream custard cake	1	0.24	1.17
A.01.07.001.011	Cream custard sponge cake	1	0.24	1.17
A.01.07.001.012	Croissant	1	0.5	1.17
A.01.07.001.013	Croissant, filled with chocolate	1	0.5	1.17
A.01.07.001.014	Croissant, filled with cream	1	0.5	1.17
A.01.07.001.015	Croissant, filled with jam	1	0.5	1.17
A.01.07.001.016	Croquembouche	1	0.15	1.17
A.01.07.001.017	Doughnuts	1	0.24	1.17
A.01.07.001.018	Clair	1	0.15	1.17
A.01.07.001.019	Flan	1	0.5	1.17
A.01.07.001.020	Fruit cake	1	0.6	1.17
A.01.07.001.021	Fruit pie	1	0.15	1.17
A.01.07.001.022	Cheese pie	1	0.15	1.17
A.01.07.001.023	Fruit tart	1	0.15	1.17
A.01.07.001.024	Gingerbread	1	0.6	1.17
A.01.07.001.025	Gougere	1	0.15	1.17
A.01.07.001.026	Kringles	1	0.25	1.17
A.01.07.001.027	Nut cream cake	1	0.24	1.17
A.01.07.001.028	Pancakes	1	0.25	1.17
A.01.07.001.029	Profiterole	1	0.15	1.17
A.01.07.001.030	Pyramid cake	1	0.25	1.17
A.01.07.001.031	Rhubarb flan	1	0.15	1.17
A.01.07.001.032	Scone	1	0.5	1.17
A.01.07.001.033	Sponge dough	1	0.25	1.17
A.01.07.001.034	Sponge cake	1	0.25	1.17
A.01.07.001.035	Sponge cake roll	1	0.25	1.17
A.01.07.001.036	Muffins	1	0.25	1.17

FoodEx code	FoodEx category	Conversion factor from FoodEx food group to raw material	Recipe fraction	mg TOS/kg flour
A.01.07.001.037	Waffles	1	0.25	1.17
A.01.07.001.038	Apple strudel	1	0.15	1.17
A.01.07.001.039	Cream-cheese strudel	1	0.24	1.17
A.01.07.001.040	Cheese pastry goods from puff pastry	1	0.15	1.17
A.01.07.001.041	Croissant from puff pastry	1	0.6	1.17
A.01.07.001.042	Brioche	1	0.5	1.17
A.01.07.001.044	Lebkuchen	1	0.6	1.17
A.01.07.001.045	Dumpling	1	0.5	1.17
A.01.07.001.046	Cake marbled, with chocolate	1	0.5	1.17
A.01.07.001.047	Marzipan pie	1	0.25	1.17
A.01.07.001.048	Baklava	1	0.15	1.17
A.01.07.002	Biscuits (cookies)	1	0.9	1.17
A.01.07.002.001	Biscuits, sweet, plain	1	0.9	1.17
A.01.07.002.002	Biscuits, chocolate filling	1	0.81	1.17
A.01.07.002.003	Biscuits, cream filling	1	0.81	1.17
A.01.07.002.004	Biscuits, fruit filling	1	0.81	1.17
A.01.07.002.005	Biscuits, vanilla filling	1	0.81	1.17
A.01.07.002.006	Butter biscuits	1	0.81	1.17
A.01.07.002.007	Biscuit, iced	1	0.81	1.17
A.01.07.002.008	Speculaas	1	0.9	1.17
A.01.07.002.009	Biscuits, sweet, wheat wholemeal	1	0.9	1.17
A.01.07.002.010	Biscuits, oat meal	1	0.9	1.17
A.01.07.002.011	Biscuits, spelt meal	1	0.9	1.17
A.01.07.002.012	Biscuits, salty	1	0.9	1.17
A.01.07.002.013	Biscuits, salty, with cheese	1	0.81	1.17
A.01.07.002.014	Sticks, salty	1	0.81	1.17
A.17.03.003	Biscuits, rusks and cookies for children	1	0.9	1.17
A.18.04.001	Find bakery products for diabetics	1	0.5	1.17
A.19.01.002	Pizza and pizza-like pies	1	0.3	1.17

TOS: total organic solids.

(a): Food and Agriculture Organization of the United Nations. Technical Conversion Factors for Agricultural Commodities. Available from: http://www.fao.org/economic/the-statistics-division-ess/methodology/methodology-systems/technical-conversion-factors-for-agricultural-commodities/en/

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

Information provided in this appendix is shown in an excel file (downloadable http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2017.4755/suppinfo).

The file contains two sheets, corresponding to two tables.

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

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