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## Safety evaluation of the food enzyme endo-1,4- $\beta$ -xylanase from a genetically modified *Aspergillus oryzae* (strain NZYM-FA)

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### Abstract

The food enzyme is an endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) produced with a genetically modified strain of *Aspergillus oryzae* 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 proposed maximum use levels, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.027 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. Subchronic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. In this study, effects were seen in the control group on reproductive parameters (particularly the seminiferous epithelium atrophy in the testes) at incidences that far exceeded the background range. Consequently, a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, including haematological parameters and the immunology cohort, was conducted. The Panel identified a no observed adverse effect level at the highest dose tested of 1,101.3 mg TOS/kg bw per day. When compared with the dietary exposure, resulted in a sufficiently high margin of exposure (at least 40,000). 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 for allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered low. Based on the data provided, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

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**Keywords:** food enzyme, xylanase, endo-1, 4- $\beta$ -xylanase, EC 3.2.1.8, 1,4- $\beta$ -D-xylan xylanohydrolase, *Aspergillus oryzae*, genetically modified microorganism

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## Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background as provided by the European Commission.....	4
1.1.2. Terms of Reference.....	5
1.2. Interpretation of the Terms of Reference.....	5
1.3. Information on existing authorisation and evaluations.....	5
2. Data and methodologies.....	5
2.1. Data.....	5
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Source of the food enzyme.....	6
3.1.1. Characteristics of the parental and recipient microorganisms.....	6
3.1.2. Characteristics of the introduced sequences.....	6
3.1.3. Description of the genetic modification process.....	6
3.1.4. Safety aspects of the genetic modification.....	7
3.2. Production of the food enzyme.....	7
3.3. Characteristics of the food enzyme.....	7
3.3.1. Properties of the food enzyme.....	7
3.3.2. Chemical parameters.....	8
3.3.3. Purity.....	8
3.3.4. Viable cells and DNA of the production strain.....	9
3.4. Toxicological data.....	9
3.4.1. Genotoxicity.....	9
3.4.1.1. Bacterial reverse mutation test.....	9
3.4.1.2. In vitro Mammalian Chromosome Aberration test.....	9
3.4.2. Repeated dose 90-day oral toxicity study in rodents.....	10
3.4.3. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test... ..	10
3.4.4. Allergenicity.....	11
3.5. Dietary exposure.....	11
3.5.1. Intended use of the food enzyme.....	11
3.5.2. Dietary exposure estimation.....	12
3.5.3. Uncertainty analysis.....	13
3.6. Margin of exposure.....	13
4. Conclusions.....	13
Documentation provided to EFSA.....	13
References.....	14
Abbreviations.....	15

## 1. Introduction

Article 3 of the Regulation (EC) No 1332/2008<sup>1</sup> 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<sup>1</sup> 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<sup>2</sup> established European Union 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; and
- iii) its use does not mislead the consumer.

All food enzymes currently on the European Union (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 food enzymes for safety evaluation' (EFSA CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

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

#### 1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list 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<sup>1</sup> on food enzymes. According to Regulation (EC) No 1332/2008<sup>1</sup> on food enzymes, a food enzyme which falls within the scope of Regulation (EC) No 1829/2003<sup>3</sup> on genetically modified food and feed should be authorised in accordance with that Regulation as well as under this Regulation.

An application has been introduced by the company Novozymes A/S for the authorisation of the food enzyme xylanase from a genetically modified *Aspergillus oryzae* strain NZYM-FA.

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011<sup>4</sup> implementing Regulation (EC) No 1331/2008<sup>2</sup>, 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.

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

<sup>4</sup> 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 xylanase from a genetically modified strain of *Aspergillus oryzae* (NZYM-FA) in accordance with the article 17.3 of Regulation (EC) No 1332/2008<sup>1</sup> 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 xylanase from a genetically modified strain of *A. oryzae* (strain NZYM-FA).

## 1.3. Information on existing authorisation and evaluations<sup>5</sup>

The applicant reports that the Danish and French food authorities have evaluated and authorised the use of the food enzyme from the genetically modified *A. oryzae* strain NZYM-FA in a number of food manufacturing processes. Only the Danish authority specifies the conditions of use, which are for flour and bread up to 400 Fungal Xylanase Units (FXU)(W)/kg flour.

## 2. Data and methodologies

### 2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme xylanase produced with the genetically modified *A. oryzae* strain NZYM-FA. The food enzyme is intended to be used in the following food manufacturing process: baking and other cereal based processes.

Additional information was sought from the applicant during the assessment process in response to a request from EFSA sent on 3 December 2014; 24 March 2015, 20 January 2017, 31 January 2017 and 12 April 2017 and was consequently provided (see 'Documentation provided to EFSA'). Consequently, the Panel concluded this assessment on the basis of the available data.

### 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), '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 guidances from the EFSA Scientific Committee.

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

## 3. Assessment<sup>6</sup>

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

The xylanase catalyses the endo-hydrolysis of 1,4- $\beta$ -D-xylosidic linkages in xylan (including arabinoxylan, which is xylan branched with arabinose), resulting in the generation of (1 $\rightarrow$ 4)- $\beta$ -D-xylan oligosaccharides of different chain lengths. The xylanase of *A. oryzae* strain NZYM-FA does not require co-factors<sup>7</sup>. It is intended to be used in the following food manufacturing process: baking and other cereal based processes.

<sup>5</sup> Technical dossier/p. 56.

<sup>6</sup> Technical dossier/p. 30.

<sup>7</sup> Technical dossier/p. 35.



### 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 recipient strain [REDACTED] differs from the parental strain [REDACTED] by its inability to produce cyclopiazonic acid and its reduced production of kojic acid.<sup>17</sup> [REDACTED]

The production strain NZYM-FA differs from the recipient strain [REDACTED] by increased yield of xylanase [REDACTED].

Southern analysis of the production strain NZYM-FA from three independent batches at the end of pilot scale fermentation confirmed the stability of the genetic modifications.<sup>19</sup> 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<sup>20</sup>

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004<sup>21</sup> and with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

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.

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 xylanase is a single polypeptide chain of 194 amino acids.<sup>22</sup> The molecular mass derived from the amino acid sequence was calculated to be 21.3 kDa.<sup>22</sup> The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.<sup>23</sup> The apparent molecular mass based on this technique is about 24 kDa.<sup>6</sup> The gels presented for four batches were comparable. The food enzyme was tested for alpha-amylase, lipase and protease and no relevant activities were detected.<sup>24</sup> No other enzymatic side activities were reported.

The in-house method for the determination of enzymatic activity<sup>25</sup> is based on the hydrolysis of wheat arabinoxylan and is expressed in Fungal Xylanase Units/g (FXU(W)/g). Hydrolysis of the arabinoxylan results in the release of reducing carbohydrates (reaction conditions: pH = 6.0, T = 50°C, incubation time = 5 min). The reaction is stopped by adding *p*-hydroxybenzoic acid hydrazide

<sup>17</sup> GMM dossier/Annex A3.

<sup>18</sup> GMM dossier/p. 16 and GMM dossier/Appendix D1.

<sup>19</sup> GMM dossier/Annex D2.

<sup>20</sup> Technical dossier/p. 45–52, p. 48 (the main process step), Annex 6 (raw materials).

<sup>21</sup> 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.

<sup>22</sup> Technical dossier/p. 30 and Annex 1.

<sup>23</sup> Technical dossier/p. 31–32.

<sup>24</sup> Technical dossier/p. 39 and Annex 3.02, 3.03 and 3.04 (analytical methods), Additional information received on 30 March 2017.

<sup>25</sup> Technical dossier/p. 36 and Annex 3.01 (analytical method).



(PAHBAH) and bismuth (III)-tartrate, forming complexes with the reducing carbohydrates, which are quantified spectrophotometrically at 405 nm. The xylanase activity is measured relative to an internal enzyme standard.

The food enzyme xylanase has been characterised regarding its temperature and pH profiles.<sup>26</sup> It has a temperature optimum around 60°C (pH 5) and a pH optimum between 5 and 6 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures. Under the conditions (pH 6.0) of the applied temperature stability assay, enzyme activity decreased above 60°C. The food enzyme showed 50% residual activity at 65–70°C; above 90°C no enzyme activity remained after 30 min.<sup>27</sup>

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three commercial batches and two batches used for the toxicological tests (Table 1).<sup>28</sup>

The average total organic solids (TOS) of the three commercial food enzyme batches was 10.4% (range 7.8–11.8%, Table 1). The enzyme activity/TOS ratio of the three commercial food enzyme batches ranged from 170.3 to 194.9 FXU(W)/mg TOS (Table 1). The average enzyme activity/mg TOS ratio of 179.6 FXU(W)/mg TOS was used for subsequent calculations.

**Table 1:** Compositional data of the food enzyme<sup>(e)</sup>

Parameter	Unit	Batches				
		1	2	3	4 <sup>(a)</sup>	5 <sup>(b),(f)</sup>
Xylanase activity	FXU(W)/g batch <sup>(c)</sup>	20,300	15,200	20,100	17,830	23,200
Protein	%	9.1	7.8	10.0	9.1	9.8
Ash	%	0.5	0.5	0.5	0.3	0.5
Water	%	87.8	91.7	87.7	89.6	88.9
Total organic solids (TOS) <sup>(d)</sup>	%	11.7	7.8	11.8	10.1	10.6
Activity/mg TOS	FXU(W)/mg TOS	173.5	194.9	170.3	176.5	219.0

(a): Batch used for the genotoxicity testing and a repeated dose 90-day oral toxicity study.

(b): Batch used for combined repeated dose toxicity study with the reproduction/developmental toxicity screening test.

(c): FXU(W): Fungal Xylanase Units (relative to an internal enzyme standard 'W') (see Section 3.3.1).

(d): TOS calculated as 100% - % water - % ash.

(e): Technical dossier/p. 31 and 57; Certificates of analysis for all parameters: Additional information received on 30 March 2017; Technical dossier/Annex 2 (analytical methods).

(f): Additional information received on 30 March 2017 (data on heavy metals, antifoam, mycotoxins and compliance with the JECFA specification).

### 3.3.3. Purity<sup>29</sup>

The food enzyme complies with the specification for lead (not more than 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). Furthermore, the levels of arsenic, cadmium and mercury were below their respective levels of detection<sup>30</sup> (LODs) of the employed methodologies.

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 units (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Blumenthal, 2004). The presence of aflatoxin B1 (AFB1), cyclopiazonic acid,  $\beta$ -nitropropionic acid and kojic acid was examined by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in the three commercial food enzyme batches as well as in batch 4 used for

<sup>26</sup> Technical dossier/p. 37–38 and Annex 9 (analytical method).

<sup>27</sup> Technical dossier/p. 38 and Annex 9 (analytical method).

<sup>28</sup> Technical dossier/p. 31 and Annex 2 (analytical methods).

<sup>29</sup> Technical dossier/p. 32–35, Annex 2 (analytical methods), Annex 4 (secondary metabolites).

<sup>30</sup> Technical dossier/p. 33, LODs: As: 0.1 mg/kg; Cd: 0.05 mg/kg; Hg: 0.03 mg/kg.



toxicological testing. None of these secondary metabolites were detected (LODs provided<sup>31</sup>). The potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

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

### 3.3.4. Viable cells and DNA of the production strain<sup>32</sup>

The absence of the production strain in the food enzyme was demonstrated in three independent liquid batches tested in triplicate. [REDACTED]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected [REDACTED].

## 3.4. Toxicological data

The toxicological assays were performed with food enzyme batches 4 and 5 (see Table 1) considered representative of the food enzyme.

### 3.4.1. Genotoxicity

#### 3.4.1.1. Bacterial reverse mutation test<sup>33</sup>

The Ames test was performed according to OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *Escherichia coli* WP2uvrA, in the presence or absence of metabolic activation (S9 mix). The treat and plate assay was applied with *S. Typhimurium* and the plate incorporation assay with *E. coli*. Two experiments were carried out using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 µg/mL or µg/plate, corresponding to 15.8, 31.6, 63.1, 126.3, 252.5, 505 µg TOS/mL or µg TOS /plate)(Batch 4). No evidence of toxicity was observed under any of the conditions tested. Upon treatment with the food enzyme there was no increase in revertant colony numbers. Therefore, the Panel concluded that the food enzyme xylanase did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

#### 3.4.1.2. *In vitro* mammalian chromosomal aberration test<sup>34</sup>

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in cultured human peripheral blood lymphocytes. Two experiments were performed. In the first experiment, applying 3 h treatment + 17 h recovery, the cultures were exposed to concentrations of 2,813, 3,750 and 5,000 µg food enzyme/mL (corresponding to 284, 379 and 505 µg TOS/mL) or 2,109, 3,750 and 5,000 µg food enzyme/mL (corresponding to 213, 379 and 505 µg TOS/mL), either in the presence or the absence of the S9 mix. In the second experiment, applying continuous 20 h treatment without metabolic activation and 3 h treatment + 17 h recovery with the S9 mix, the concentrations tested were 3,200, 4,000 and 5,000 µg food enzyme/mL (corresponding to 323, 404 and 505 µg TOS/mL) (Batch 4). For all food enzyme concentrations used, the frequency of cells with chromosomal aberrations was similar to that of negative controls. No significant increase in polyploid or endoreplicated cells was observed. The Panel concluded that the food enzyme xylanase did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested up to 5,000 µg food enzyme/mL (corresponding to 505 µg TOS/mL) under the experimental conditions employed for this study.

Therefore, the Panel concluded that on the basis of the *in vitro* studies there is no concern for genotoxicity for the xylanase tested.

<sup>31</sup> Technical dossier/p. 33.

<sup>32</sup> GMM dossier/Annex E1 and E2.

<sup>33</sup> Technical dossier/Annex 7.01 and Additional information received on 9 June 2017 (historical control data).

<sup>34</sup> Technical dossier/Annex 7.02.

### 3.4.2. Repeated dose 90-day oral toxicity study in rodents<sup>35</sup>

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD Test Guideline 408 (OECD, 1998) and following GLP. Four groups of 10 male and 10 female Crl:CD<sup>®</sup> (SD) IGS BR rats were given by gavage for 13 weeks a dose of 10 mL/kg body weight (bw) per day of purified water (control), or the food enzyme (Batch 4) at the doses of 105; 346.5 and 1,050 mg TOS/kg bw per day.

One low-dose male died in week 12 without *ante mortem* signs and histopathological abnormalities, and 1 high-dose male died in week 13 under anaesthesia during blood sampling.

The food consumption and body weight gain of low-dose males was slightly lower (7–8%) than in controls, but no similar observation was made in males at higher dosages or in females at any dose level.

Statistically significant decreases in a few parameters in haematology were observed in both mid-dose males (haemoglobin) and females (haemoglobin, white blood cells, lymphocytes, and mean corpuscular haemoglobin concentration (MCHC)) and in blood chemistry (phosphate levels) in mid-dose females only. As these effects did not exhibit a dose dependency, they were considered by the Panel as incidental.

No other significant effects were observed.

A relatively high proportion of male and female animals exhibited changes in reproductive organs, e.g. at necropsy small epididymis and testes and fluid uterine distension, and seminal tubular epithelial atrophy in testes and hypospermia in epididymides by microscopy. Although, similar incidences occurred in control and treated animals the Panel asked the applicant to perform a combined repeated dose toxicity study with the reproduction/developmental toxicity screening (28-day oral) test, including haematological parameters and the immunology cohort (see below) in order to confirm that there were no treatment-related effects of the food enzyme.

### 3.4.3. Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test<sup>36</sup>

A combined repeated dose toxicity study with the reproduction/developmental/toxicity screening test was performed in accordance with OECD Test Guideline 422 (OECD, 1996) and following GLP. The objective of the study was an assessment of general systemic toxic potential of the food enzyme (see Table 1, batch 5) in rats including a screening for reproductive/developmental effects, after daily oral (gavage) administration for at least 5 weeks. Three groups, each comprising 10 males and 10 females of Sprague–Dawley Crl:CD(SD) rats, received the food enzyme at concentrations of 10, 33 or 100% (corresponding to 110.13; 363.43; 1,101.3 mg TOS/kg bw per day). Males were treated daily for 2 weeks before pairing, throughout pairing and up to necropsy thus for a minimum of 5 consecutive weeks. Females were treated daily for 2 weeks before pairing, throughout pairing, gestation and until Day 6 of lactation. Females were allowed to litter, rear their offspring and both females and F1 offspring were killed on Day 7 of lactation. The F1 generation received no direct administration of the test substance, meaning that any exposure if present was *in utero* or via the milk. A similarly constituted control group of both sexes received the vehicle (reverse osmosis water) at the same volume as treated groups, 10 mL/kg bw.

No mortality was observed.

In the high-dose males, haematological investigation in week 2 before pairing showed a statistically significant decrease in the number of lymphocytes and large unstained cells, which also resulted in a reduction of the total white blood cell count (WBC). The Panel noted that this effect was transitory and limited to one sex. In the high-dose females a small but statistically significant increase of erythrocyte count and in the mid- and high-dose females a small but statistically significant decrease in mean cell volume (MCV) was observed. The Panel noted that individual erythrocyte counts of high-dose females were either in the concurrent or historical control range, except one, and that several individual erythrocyte counts in the concurrent control were below the historical control range. The Panel further noted that only two individual MCV values in the mid- and high-dose groups were below the concurrent control range and that 1/3 of the concurrent control MCV values were below the historical control range. Consequently, the Panel considered the differences to control in erythrocyte count and MCV values as not adverse. Prothrombin time and activated partial thromboplastin time were statistically significantly reduced in males and increased in females. The Panel noted lack of correlation of the changes between the sexes and lack of dose response. Furthermore, the Panel noted that

<sup>35</sup> Technical dossier/Annex 7.03, Annex 7.04 and Additional information received on 22 December 2014 (historical control data).

<sup>36</sup> Additional information received on 18 January 2016.

examination of bone marrow did not reveal any statistically significant differences between the high-dose and the control groups.

Clinical chemistry examination showed a small reduction of creatinine concentration in the high-dose males and females, and lower potassium concentrations in high-dose females as compared to controls. Concerning the creatinine concentration the Panel noted that 9/10 males and 7/10 females from the high-dose group had values within the concurrent control range and that values for all males and all females were within the historical control ranges. The Panel further noted that the difference to controls in potassium concentration was small, and limited to one sex and that the values were within the historical control range. Therefore, the Panel considered that these findings were not of toxicological significance.

No other significant effects were observed.

No treatment-related organ weight changes, macroscopic or histopathological findings were observed. Specifically, there was no evidence for any of the findings in the testes and epididymis and uterus that had been reported in the earlier repeated dose 90-day oral toxicity study in rodents.

Reproductive assessment demonstrated that there was no adverse effect of the food enzyme on oestrous cycles, mating performance, fertility or gestation length. There was no adverse effect of xylanase on litter size, and sex ratio and offspring survival and body weight up to day 7 of age. There were also no treatment-related macroscopic findings in the offspring.

The Panel concluded that oral administration of the food enzyme (batch 5) to Sprague–Dawley rats at doses up to 1,101.3 mg TOS/kg bw per day, did not cause any adverse change, and that this dose level represents the no observed adverse effect level (NOAEL) of the food enzyme for both males and females in this study.

#### 3.4.4. Allergenicity<sup>37</sup>

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 xylanase produced with the genetically modified *A. oryzae* strain NZYM-FA 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 (GM) plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a window of 80 amino acids as the criterion, no match was found.

Several cases of occupational allergy consecutive to inhalation of aerosols containing xylanase or other enzymes have been reported (Elms et al., 2003; 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 information is available on oral and respiratory sensitisation or elicitation reactions of this xylanase.

Xylanase from a genetically modified *A. oryzae* strain was also tested in the study of Bindslev-Jensen et al. (2006). The authors investigated 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, allergens of bee or wasp or drugs). 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.

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.

The Panel considers that under the intended condition 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.

### 3.5. Dietary exposure

#### 3.5.1. Intended use of the food enzyme

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

<sup>37</sup> Technical dossier/p. 62–64 and Annex 8.

**Table 2:** Intended uses and recommended use levels of the food enzyme as provided by the applicant<sup>(a)</sup>

Food-manufacturing process	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	Up to 2.23 mg TOS/kg flour (150–400 FXU(W)/kg of flour)
Cereal-based processes	Flour	Up to 2.23 mg TOS/kg flour (200–400 FXU(W)/kg of flour)

FXU(W): Fungal Xylanase Units (relative to an internal enzyme standard 'W'); TOS: Total organic solids.

(a): Technical dossier/p. 53–55 and Additional information received on 30 March 2017.

In baking and cereal-based processes,<sup>38</sup> the food enzyme is added to the raw materials during the preparation of the dough. It is used to hydrolyse arabinoxylans, which interact with gluten and water, thus contributing to the viscosity of the dough. The decrease in dough viscosity facilitates the handling of the dough, resulting in more uniform products.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the xylanase is inactivated during baking and cereal-based processes.<sup>39</sup>

**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 of means (number of surveys)	0.002–0.008 (10)	0.007–0.017 (14)	0.008–0.014 (19)	0.004–0.009 (18)	0.003–0.006 (19)	0.002–0.006 (18)
Min–max of 95th percentiles (number of surveys)	0.007–0.027 (8)	0.015–0.023 (12)	0.013–0.026 (19)	0.007–0.017 (17)	0.006–0.010 (19)	0.005–0.010 (18)

### 3.5.2. Dietary exposure estimation

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 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).

<sup>38</sup> Technical dossier/p. 73–75.

<sup>39</sup> Technical dossier/p. 52.

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

Sources of uncertainties	Direction of impact
<b>Model input data</b>	
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	+/-
<b>Model assumptions and factors</b>	
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	+/-

+: uncertainty with potential to cause over-estimation of exposure; -: uncertainty with potential to cause underestimation of exposure.

FoodEx: a standardised food classification and description system; TOS: Total Organic Solids.

### 3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

The conservative approach applied to the exposure estimate to food enzyme-TOS, in particular, assumptions made regarding 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 (1,101.3 mg TOS/kg bw per day) from the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, with the derived exposure estimates of 0.002–0.017 mg/kg bw per day at the mean and 0.005–0.027 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) of at least 40,000.

## 4. Conclusions

Based on the data provided, the margin of exposure calculated when used in baking and cereal based processes, the Panel concludes that the food enzyme xylanase produced with the genetically modified *A. oryzae* strain NZYM-FA 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) Dossier 'Xylanase produced by a genetically modified strain of *Aspergillus oryzae* (strain NZYM-FA)'. 12 July 2013. Submitted by Novozymes A/S.
- 2) Additional information. 22 December 2014. Submitted by Novozymes A/S.
- 3) Additional information. 18 January 2016. Submitted by Novozymes A/S.
- 4) Additional information. 30 March 2017. Submitted by Novozymes A/S.
- 5) Additional information received from Novozymes A/S on 9 June 2017 by the applicant.
- 6) Xylanase, Batch PPQ38584: Combined Repeat Dose Toxicity. Study and Reproductive/Developmental Toxicity Screening Study in Sprague-Dawley Rats by Oral Gavage Administration. Envigo study number: LKG0124. 08 January 2016. Unpublished report.



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## Abbreviations

AFB1	aflatoxin B1
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Material, Enzymes and Processing Aids
CFU	colony forming units
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO/WHO	Food and Agriculture Organization of the United States/World Health Organization
█	█
FoodEx	a standardised food classification and description system
FXU	Fungal Xylanase Units
GLP	Good Laboratory Practice
GM	genetically modified
GMM	genetically modified microorganism
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
█	█
IUBMB	International Union of Biochemistry and Molecular Biology
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LODs	limits of detection
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PAHBAH	<i>p</i> -hydroxybenzoic acid hydrazide
PCR	polymerase chain reaction
█	█
rRNA	ribosomal ribonucleic acid
S9	metabolic activation
SDS-PAGE	sodium dodecyl sulfate-poly acrylamide gel electrophoresis
TOS	total organic solids
█	█
W	internal enzyme standard
WBC	white blood cell count

## Appendix A – 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.2018.5480/supinfo>).

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 <sup>(a)</sup>	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 <sup>(a)</sup>	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).