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Safety evaluation of the food enzyme xylanase from the genetically modified *Aspergillus luchuensis* Inui strain RF7398

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Riviere, Inger-Lise Steffensen, Christina Tlustos, Henk van Loveren, Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Magdalena Andryszkiewicz, Davide Arcella, Ana Gomes, Natália Kovalkovičová, Yi Liu, Kim René Rygaard Nielsen[†], Karl-Heinz Engel* and Andrew Chesson

Abstract

The food enzyme xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) is produced with the genetically modified *Aspergillus luchuensis* Inui strain RF7398 by AB Enzymes GmbH. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. The food enzyme is intended to be used in baking and cereal-based processes. Based on the maximum use levels, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.008 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,000 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a high margin of exposure of at least 125,000. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood of this occurring is considered to be low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, xylanase, endo-14-β-xylanase, EC 3.2.1.8, 4-β-D-xylan xylanohydrolase, *Aspergillus luchuensis* Inui, genetically modified microorganism

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

* Member of the Working Group on Enzymes of the EFSA Panel Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) until 3-7-2018.

† Deceased.

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Riviere, Vittorio Silano, Inger-Lise Steffensen, Christina Tlustos, Henk van Loveren, Laurence Vernis and Holger Zorn.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

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

Three applications have been introduced by the companies "Paninkret Chem. Pharm. Werk GmbH" and "AB Enzymes GmbH" for the authorisation of the food enzymes trypsine and chymotrypsine from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF 6199) and endo-1,4-beta-xylanase from a genetically modified strain of *Aspergillus acidus* (strain RF 7398).

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

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes trypsine and chymotrypsine from pig pancreas, pectin lyase from a genetically modified strain of *Trichoderma reesei* (strain RF 6199) and endo-1,4-beta-xylanase from a

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

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

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

genetically modified strain of *Aspergillus acidus* (strain RF 7398) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme xylanase from a genetically modified *Aspergillus luchuensis* Inui (initially indicated as *A. acidus*; strain RF 7398).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme xylanase from a genetically modified *A. luchuensis* Inui (strain RF 7398).

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 21 January 2015, 19 May 2015 and 24 May 2019 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance of EFSA Scientific Committee.

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

3. Assessment

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

Xylanases catalyse the random hydrolysis of 1,4-ß-D-xylose linkages in xylans (including arabinoxylans) resulting in the generation of (1→4)-β-D-xylan oligosaccharides of different lengths. The enzyme is intended to be used in baking and cereal-based processes.

3.1. Source of the food enzyme

The xylanase is produced with the genetically modified filamentous fungus *A. luchuensis* Inui strain RF 7398 ([redacted]), which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with accession number [redacted].[4]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental and recipient strain is *A. luchuensis* Inui [redacted]).[5] Strain [redacted], initially indicated in the technical dossier as *A. acidus* (previously named *A. foetidus* var. *acidus*), was identified as *A. luchuensis* Inui on the basis of [redacted].[6]

[4] Technical dossier/Additional information November 2019/Enclosure 1.
[5] Technical dossier/Part 2/Appendix 1 and Additional data November 2019/Enclosure 2.
[6] Technical dossier/Additional data November 2019/Enclosure 2.

3.1.2. Characteristics of the introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise xylanase [redacted][9] [redacted][10]

3.1.4. Safety aspects of the genetic modification

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

The production strain *A. luchuensis* Inui RF7398 [redacted][11]

The genetic stability of the *A. luchuensis* Inui RF7398 strain was demonstrated [redacted][12]

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004[13], 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 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.[14]

[7] Technical dossier/Missing information June 2014/Reply to EFSA.
[8] Technical dossier/Part 2/Appendix 7.
[9] Technical dossier/Part 2 and Additional data March 2015.
[10] Technical dossier/Part 2/Appendix 9.
[11] Technical dossier/Part 2/Appendix 11.
[12] Technical dossier/Part 2/Appendix 12.
[13] Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[14] Technical dossier/Annex 10.

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The xylanase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[15] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. [redacted][16] No other relevant enzymatic side activities were reported.

The xylanase activity is determined [redacted] The enzyme activity is expressed in xylanase units/g (BXU/g). One BXU unit is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to 1 nmol xylose from birch xylan in 1 second under the assay conditions.[17]

The food enzyme has a temperature optimum around 50°C (pH 4.5) and a pH optimum around 4.0 (temperature 30°C). Thermostability was tested at different temperatures at pH 4.8. No residual activity was detected after 10 minutes at 65°C, or after 5 minutes at 95°C.[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for five food enzyme batches, three batches used for commercialisation and two batches produced for the toxicological tests (Table 1).[19] The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 45.8%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 4,669 BXU/mg TOS.

The three food enzyme batches used for commercialisation were dried concentrates [redacted][19] The two food enzyme batches used for the toxicological tests were concentrates without any added diluents.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches				
		1	2	3	4[(a)]	5[(b)]
Xylanase activity	BXU/g batch[(c)]	1,966,800	1,652,200	2,882,000	4,312,000	3,674,000
Protein	%	36.9	29.4	40.3	67.0	66.4
Ash	%	50.4	59.0	43.2	6.6	7.5
Water	%	3.9	2.9	3.1	4.6	7.8
Total Organic Solids (TOS)[(d)]	%	45.7	38.1	53.7	88.8	84.7
Xylanase activity/mg TOS	BXU/mg TOS	4,303	4,336	5,367	4,856	4,338

(a): Batch used for the bacterial reverse mutation test and *in vitro* mammalian chromosomal aberration test.
(b): Batch used for the repeated dose 90-day oral toxicity study in rodents.
(c): BXU/g: xylanase units/g (see Section 3.3.1).
(d): TOS calculated as 100% - % water - % ash.

[15] Technical dossier/Additional data June 2015.
[16] Technical dossier/Annex 4.
[17] Technical dossier/Annex 3.
[18] Technical dossier/Annex 6.
[19] Technical dossier/Annexes 16 and 17, and Additional data June 2015.

3.3.3. Purity

The lead content in the three commercial batches[20] and in the two batches used for toxicological studies[21] was below 0.08 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium and mercury were below the limits of detection of the employed methodologies. For arsenic, the concentration determined in the commercial batches was up to 0.6 mg/kg.[15,22] Taking account of the proposed use levels and the concentrations detected, the Panel considered these concentrations as not of concern.

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[15]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of mycotoxins (aflatoxin B1, B2, G1 and G2, deoxynivalenol, fumonisin B1 and B2, ochratoxin A, sterigmatocystin, T2-toxin and HT2-toxin, and zearalenone) was examined in all five food enzyme batches. All were below the limit of quantifications (LOQs) of the applied analytical methods, except for deoxynivalenol in all batches attributed to the use of [redacted] in the fermentation medium, and fumonisin B1[23] in the batches used for toxicological studies.[24] Taking account of the proposed use levels and the results of the toxicological examination of the food enzyme-TOS, the concentrations of the detected mycotoxins in the food enzyme were not considered to be of concern.

The applicant did not provide information on other secondary metabolites potentially produced under the conditions of fermentation which might contribute to the food enzyme–TOS. This issue is further addressed by the toxicological examination of the food enzyme–TOS.

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted][25]

A first set of polymerase chain reaction (PCR) data was provided for three batches analysed in triplicate [redacted] No DNA was detected, [redacted][26] A second test for recombinant DNA in the food enzyme was performed in two batches in triplicate. No DNA was detected [redacted][27]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. Batches 4 and 5 (Table 1) used in these studies were considered suitable as test items. Batch 4 has a slightly higher activity/mg TOS than two of batches for commercialisation. However, this value was still comparable to those of the commercial batches and thus was considered suitable for toxicological testing.

[20] LOD: Pb = 0.05 mg/kg; Technical dossier/Annexes 1 and 2; Additional data June 2015.
[21] LOD: Pb = 0.05 mg/kg; Additional data June 2015.
[22] LOQ: As = 0.5 mg/kg; Cd = 0.05 mg/kg; Hg = 0.1 mg/kg.
[23] Deoxynivalenol 110-502 μg/kg and below 10 μg/kg for fumonisin.
[24] LOQ: aflatoxins (B1, B2, G1 and G2) = 0.05 μg/kg each; deoxynivalenol = 40 μg/kg; fumonisin (B1 and B2) = 50 μg/kg; ochratoxin A = 2 or 0.5 μg/kg; sterigmatocystin = 10 μg/kg; T2-toxin = 8 μg/kg; HT2-toxin = 8 μg/kg; zearalenone = 10 μg/kg; Technical dossier/Annex 2; Additional data June 2015.
[25] Technical dossier/Additional data March and June 2015.
[26] Technical dossier/Additional data March 2015/Attachment 3.
[27] Technical dossier/Additional data November 2019/Enclosure 3.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in five strains of *Salmonella* Typhimurium (TA 1535, TA 1537, TA 98, TA 100, and TA 102), in the presence or absence of metabolic activation (S9 mix).[28] A direct plate incorporation (experiment 1) assay was carried out at different concentrations of the food enzyme (3, 10, 33, 100, 333, 1,000, 2,500 and 5,000 μg/plate, corresponding to 2.7, 8.9, 29.3, 88.8, 295.7, 888, 2,220 and 4,440 μg TOS/plate. A pre-incubation method (experiment 2) was applied at 33, 100, 333, 1,000, 2,500 and 5,000 μg food enzyme/plate, corresponding to 29.3, 88.8, 295.7, 888, 2,220 and 4,440 μg TOS/plate) (batch 4). No evidence of toxicity or precipitate was observed under any of the conditions tested. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9 mix.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in Chinese hamster V79 cells.[29] Two experiments were performed. In the first experiment, applying 4 h treatment + 14 h recovery, the cells were exposed to concentrations of 156.3, 312.5 and 625 μg food enzyme/mL (corresponding to 139, 278 and 555 μg TOS/mL) in the absence of S9 and 312.5, 625 and 1,250 μg food enzyme/mL (corresponding to 278, 555 and 1,110 μg TOS/mL) in the presence of S9. In the second experiment, applying 18 h treatment + 0 h recovery, the cells were exposed to concentrations of 312.5, 625 and 1,250 μg food enzyme/mL (corresponding to 278, 555 and 1,110 μg TOS/mL) in the absence of S9, and applying 4 h treatment + 14 h recovery, the cells were exposed to concentrations of 312.5, 625 and 1,250 μg food enzyme/mL (corresponding to 278, 555 and 1,110 μg TOS/mL) in the presence of S9 (batch 4). In the presence of S9 mix, the cell numbers were reduced to 56% of controls at 1,250 ug/mL. At higher concentrations precipitation was observed.

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 structural and numerical chromosomal aberrations in Chinese hamster V79 cells when tested up to 1,250 μg/mL (corresponding to 1,110 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.

3.4.2. 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, 1998) and following GLP.[30] Four groups of 10 male and 10 female Wistar RccHanTM: WIST(SPF) rats received by gavage the food enzyme in doses of 100, 300 and 1,000 mg TOS/kg body weight (bw) per day. Controls received the vehicle (bi-distilled water).

One high-dose female died on day 17 due to mis-dosing (at necropsy, lesions in lungs and trachea were recorded).

Clinical observation revealed that one mid-dose male had moderate to slight hair loss at the left cheek (week 7–13) and slight hair loss at the right ear (week 7). One high-dose female had slightly ruffled fur and dyspnoea (day 16), and another female of this group had also slightly ruffled fur in one single treatment day (week 5). The Panel considered these clinical signs not to be treatment-related.

In ophthalmological examination, a high incidence of corneal opacity was seen across all test groups. No statistically significant differences were observed. The Panel noted that corneal opacity is recognised as a spontaneous change in Wistar Hannover rats (Okamura et al., 2011; Hashimoto et al., 2013).

Among functional observation battery parameters, the only statistically significant differences to controls were higher forelimb grip strength in low-dose females, and an increase in the mean

[28] Technical dossier/Annex 18.
[29] Technical dossier/Annex 19.
[30] Technical dossier/Annex 20.

locomotor activity in all-dose females in the interval 0–10 min. This latter finding was not observed in other time intervals. Consequently, the Panel considered both findings as incidental.

Haematological examination revealed in males a statistically significant increase in haemoglobin (low-dose), red cell distribution width (mid-dose) and mean cell haemoglobin concentration (mid-dose), decreased absolute lymphocytes count (low- and high dose), and a dose-dependent increase in relative neutrophil count and a dose-dependent decrease in relative lymphocyte and absolute basophil counts. In females, a statistically significant decrease in absolute eosinophil count in low-dose was recorded. Reticulocyte maturity index (low fluorescence) was statistically significantly increased, while reticulocyte maturity indexes (medium and high fluorescence) were significantly decreased in the high-dose female group. The Panel noted that the mean values of all haematological parameters were within the range of the historical control data and therefore these values were considered not to be toxicologically significant.

Clinical chemistry investigation revealed that the glucose concentration was statistically significantly increased in all-dose males (not dose-dependent) and in low-dose females. In addition, statistically significantly increased sodium levels were observed in mid- and high-dose males and in all-dose females. Statistically significantly increased chloride levels were observed in mid- and high-dose males and females. In males, statistically significantly increased potassium (high-dose) and protein concentration (low-dose) and a decreased phosphorus concentration (low-dose) and total bilirubin levels (mid-dose) were recorded. In high-dose females, a statistically significant increase in phosphorus concentration and triglycerides and a decrease in bile acid concentration were observed. All mean values of clinical biochemistry parameters were without dose response and/or without consistency between sexes and were within the range of the historical control data and therefore considered not to be toxicologically significant.

There was a statistically significant increase in liver to body weight ratio in low- and mid-dose males and a decrease in mid-dose females. A significant increase in the epididymis to body weight ratio and epididymis to brain weight ratio were observed in low-dose males. A statistically significant increase in brain weight was recorded in mid-dose females. In all treated female groups, a statistically significant decrease in heart to body weight ratio and in heart to brain weight ratio in mid-dose females were recorded. The Panel considered these small changes in organ weights as incidental because there was no apparent dose–response relationship and the changes were not accompanied by histopathological findings.

No other statistically significant differences to controls were observed.

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

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of the xylanase produced with the genetically modified *A. luchuensis* Inui strain RF7398 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, one match was found.[31] The matching allergen was CPA63, a pollen allergen from *Cryptomeria japonica*.

No information is available on oral sensitisation or elicitation reactions of this xylanase.

Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al., 1998; Merget et al., 2001). However, several studies have shown that adults with occupational asthma to an enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Such information is not reported for xylanase. Overall, the likelihood of an allergic reaction upon oral ingestion of this xylanase, produced with the genetically modified *A. luchuensis* Inui strain RF7398 in individuals respiratory sensitised to xylanase cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

[31] Technical dossier/Annex 21.

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

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded but the likelihood of such reactions 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.[32]

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	Up to 0.4 mg TOS/kg flour
Cereal-based processes	Flour	Up to 1 mg TOS/kg flour

TOS: total organic solids.

In baking and cereal-based processes, the food enzyme is added to the raw materials during the preparation of the dough. It is used to hydrolyse (arabino)xylans, which interact with gluten and water, thus contributing to reduce the viscosity of the dough. The decrease in dough viscosity facilitates the handling of the dough, gives improved crumb structure and increases the volume during baking, 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 expected that the xylanase is inactivated during baking processes, and during drying, boiling or steaming steps in cereal-based processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[33] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the average 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).

[32] Technical dossier/Additional data November 2019.
[33] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 Months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.000–0.002 (10)	0.002–0.005 (14)	0.002–0.004 (19)	0.001–0.002 (18)	0.001–0.002 (19)	0.000–0.001 (18)
Min–max 95th percentile (number of surveys)	0.002–0.005 (8)	0.003–0.008 (12)	0.003–0.007 (19)	0.002–0.005 (17)	0.001–0.003 (19)	0.001–0.003 (18)

TOS: total organic solids.

3.5.3. Uncertainty analysis

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

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

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

TOS: total organic solids.
+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0–0.005 mg TOS/kg bw per day at the mean and 0.001–0.008 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 125,000.

4. Conclusions

Based on the data provided, the intended food production processes and the derived margin of exposure, the Panel concluded that the food enzyme xylanase produced with the genetically modified *A. luchuensis* Inui strain RF7398 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 its recombinant DNA.

Documentation provided to EFSA

1) Dossier "Application for authorisation of an endo-1,4-beta-xylanase from a genetically modified strain of *Aspergillus acidus* in accordance with Regulation (EC) No 1331/2008", March 2014. Submitted by AB Enzymes GmbH.
2) Additional information was received from AB Enzymes GmbH in March 2015.
3) Additional information was received from AB Enzymes GmbH in June 2015.
4) Additional information was received from AB Enzymes GmbH in November 2019.
5) Summary report on genotoxicity, subchronic toxicity study and allergenicity. January 2015. Delivered by FoBiG (Freiburg, Germany).
6) Summary report on technical data and dietary exposure. February 2015. Delivered by Hylobates Consulting and BiCT (Roma, Italy).

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Abbreviations

BXU	Xylanase activity units
bw	body weight
CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute
CEF	EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
FAO	Food and Agriculture Organization of the United Nations
GMO	genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
LOQ	limit of quantification
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization

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

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.5971).

The file contains two sheets, corresponding to two tables.

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

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

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