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Safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Trichoderma reesei* strain RF5427

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Abstract

The food enzyme endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase; EC 3.2.1.8) is produced with the genetically modified *Trichoderma reesei* strain RF5427 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. It is intended to be used in baking, brewing and cereal-based processes, distilled alcohol production and grain treatment for the production of starch and gluten fractions. Since residual amounts of the food enzyme are removed by distillation and during grain treatment, dietary exposure was only calculated for baking, brewing 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 0.119 mg TOS/kg body weight (bw) per day. Genotoxicity tests did not raise a safety concern. The Panel identified a no observed adverse effect level at the highest dose tested of 939 mg TOS/kg bw per day in a repeated dose 90-day oral toxicity study in rats, resulting in a margin of exposure of at least 7,890. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and no matches were found. The Panel considered that allergenicity can be excluded for distilled alcohol production. The risk of allergic sensitisation and elicitation reactions cannot be excluded for baking, brewing and cereal-based processes, and for grain treatment for the production of starch and gluten fractions, but the likelihood of such reactions to occur is considered to be low. Based on the data provided, the removal of TOS during the production of distilled alcohol and grain treatment, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or 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 came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 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 in 2013

Only food enzymes included in the European Union (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 Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

Two applications have been introduced by the companies AB enzymes GmbH and Novozymes A/S for the authorisation of the food enzymes endo 1,4-β xylanase from a genetically modified strain of *Trichoderma reesei* (strain RF5427), and glucan 1,4-α-glucosidase (principal activity) and α-amylase (subsidiary activity) from a genetically modified strain of *Aspergillus niger* (strain NZYM-BX), respectively.

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

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 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, pp. 1–6.

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

1.1.2. Terms of Reference in 2013

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes endo 1,4-β xylanase from a genetically modified strain of *Trichoderma reesei* (strain RF5427), and glucan 1,4-α-glucosidase (principal activity) and α-amylase (subsidiary activity) from a genetically modified strain of *Aspergillus niger* (strain NZYM-BX), in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.3. Background as provided by the European Commission in 2014

Only food enzymes included in the European Union (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 Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

An application was introduced by the AB enzymes GmbH for the authorisation of the food enzyme endo 1,4-beta xylanase from a genetically modified strain of *Trichoderma reesei* (strain RF5427) in baking process and other cereal-based process. The mandate to carry out the risk assessment was made on 25 October 2013 (Ref. Ares(2013)3340723). An extension of use has been submitted by AB enzymes GmbH to the Commission for the following uses: brewing, grain processing and beverage alcohol processing.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application for an extension of the above uses falls within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.4. Terms of Reference in 2014

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes endo 1,4-β xylanase from a genetically modified strain of *Trichoderma reesei* (strain RF5427) as part of the mandate established on 25 October 2013, 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 requests in 2013 and 2014 to carry out the safety assessment of food enzyme endo 1,4-β xylanase from a genetically modified *Trichoderma reesei* strain RF5427.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Trichoderma reesei* (*T. reesei*) strain RF5427.

Additional information was requested from the applicant on 8 July 2014, 19 November 2014, 20 February 2015 and 26 November 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 guidances of EFSA Scientific Committees.

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 with 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:	endo-(1→4)-β-xylan 4-xylanohydrolase; xylanase; β-1,4-xylanase; β-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-xylosidic 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 brewing processes, cereal-based processes, distilled alcohol production and grain treatment for the production of starch and gluten fractions.

3.1. Source of the food enzyme

The endo-1,4-β-xylanase is produced with the genetically modified filamentous fungus *Trichoderma reesei* strain RF5427, which is deposited at the Westerdijk Fungal Biodiversity Institute (previously Centraalbureau voor Schimmelcultures, CBS, the Netherlands) with deposit number CBS114044.[4]

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient strain, *T. reesei* RF4847, is a [redacted] derived [redacted] from the parental strain QM6a (Seidl et al., 2008; Peterson and Nevalainen, 2012). Taxonomic identification of *T. reesei* QM6a (ATCC 13631) was performed by polymerase chain reaction (PCR) fingerprinting and by sequence analysis of the internal transcribed spacers (ITS-1 and ITS-2) of the nuclear ribosomal DNA region and the 5.8 S rRNA gene (Kuhls et al., 1996). [redacted][5]

3.1.2. Characteristics of introduced sequences

[redacted][6]

3.1.3. Description of the genetic modification process

[redacted][7]

[4] Technical dossier/Volume III/Part2_Appendices/Appendix 9.3.
[5] Technical dossier/Additional data September 2014/pp. 2–4.
[6] Technical dossier/Volume III/Part2_Appendices/Appendix 7.
[7] Technical dossier/Volume III/Part2_Appendices/Appendix 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 *T. reesei* RF5427 differs from the recipient strain RF4847 in [redacted]

The traits introduced or removed do not raise safety concerns. [redacted]

Therefore, the enzyme endo-1,4-β-xylanase produced with *T. reesei* strain RF5427 does not raise safety concern regarding the genetic modification of the production strain.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular weight material passes the filtration membrane and is discarded.[9] Finally, the food enzyme was spray-dried prior to analysis.[10] 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.[11]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The endo-1,4-β-xylanase is a single polypeptide chain of [redacted] amino acids. The molecular mass, derived from the amino acid sequence, was calculated to be [redacted] kDa ([redacted]).[12] The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches examined. The gels showed [redacted] major protein bands corresponding to the [redacted] endo-1,4-β-xylanase [redacted][13] Beta-glucanase side activities were also found in the three commercial batches.[14]

The in-house determination of endo-1,4-β-xylanase activity is based on hydrolysis of birch xylan, (reaction conditions: pH 5.3, 50°C, 5 min), and measuring the release of reducing carbohydrates, using dinitrosalicylic acid. The colour produced is measured spectrophotometrically at 540 nm. One xylanase unit (BXU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one nmol xylose from birch xylan in one-second under the assay conditions.[15]

The food enzyme has a temperature optimum around 80°C (pH 3.6 and pH 5.3) and a pH optimum between pH 5.3 and 7.0 (40°C).[16] Thermostability was tested after a pre-incubation of the food enzyme for 60 min at different temperatures. Under the conditions (pH 5.3) of the applied

[8] 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.
[9] Technical dossier/Volume I/Section 3.2.1.2.5 and Additional data March 2020/pp. 2-4.
[10] Technical dossier/Volume I/Section 3.2.1.2.5.
[11] Technical dossier/Volume II/Annex 10, Annex 12 and Additional data March 2020/p. 2.
[12] Technical dossier/Volume I/p. 34.
[13] Technical dossier/Volume II/Annex 3 and Additional data May 2015/Enclosure 4.
[14] Technical dossier/Additional data March 2020/Enclosure 2.
[15] Technical dossier/Volume II/Annex 2.
[16] Technical dossier/Volume I/p. 36 and Volume II/Annex 5.

temperature stability assay, endo-1,4-β-xylanase activity decreased above 70°C showing no residual activity above 90°C.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch used for the toxicological studies (Table 1).[18] The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 93.5%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation was 4,994 BXU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Endo-1,4-β-xylanase activity	BXU/g batch[(b)]	4,880,000	4,440,000	4,690,000	4,095,000
Protein	%	75.4	74.8	74.7	75.7
Ash	%	1.39	1.55	1.6	1.97
Water	%	4.81	5.2	5.01	4.1
Total organic solids (TOS)[(c)]	%	93.8	93.3	93.4	93.9
Activity/mg TOS	BXU/mg TOS	5,203	4,759	5,021	4,361

(a): Batch used for the toxicological studies.
(b): BXU: Xylanase unit (see Section 3.3.1 and additional data May 2015, March 2020).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The average lead content in the three commercial batches[19] was below 0.05 mg/kg and in the batch used for toxicological studies[20] was 0.074 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, mercury and arsenic were below the limits of quantification (LOQs) of the employed methodologies. For cadmium and arsenic, the concentrations determined in the batch used for toxicological studies were 0.085 and 0.13 mg/kg, respectively. The Panel considered these concentrations as not of concern.[21]

The food enzyme concentrates comply 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 the tested batches (FAO/WHO, 2006).[22]

Strains of *Trichoderma*, 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, ochratoxin A, sterigmatocystin, T2 toxin, HT2 toxin, fumonisin B1 and B2) was examined in the four food enzyme batches[23] and were below the LOQs of the applied analytical methods and of no concern.[24] The applicant did not provide information on other secondary metabolites possibly produced under the conditions of fermentation which might contribute to the food enzyme–TOS. This issue is addressed by the toxicological examination of the food enzyme–TOS.

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

[17] Technical dossier/Volume II/Annex 5 and Additional data March 2020/p. 4.
[18] Technical dossier/Volume I/p. 65; Volume II/Annex 1Ac and Additional data March 2020/Enclosure 2.
[19] Technical dossier/Additional data March 2020/p. 5 and Enclosures 2 and 3, LOQ: Pb = 0.05 mg/kg.
[20] Technical dossier/Volume II/Annex 1Ac and Additional data March 2020/p. 5 and Enclosure 3.
[21] Technical dossier/Volume II/Annex 1Ac and Additional data March 2020/p. 5 and Enclosures 2 and 3, LOQ: As = 0.5 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg (for batch 1–3 in Table 1); Hg = 0.1 mg/kg (for batch 4 in Table 1).
[22] Technical dossier/Volume II/Annex 1Ac and Additional data March 2020/Enclosure 2.
[23] Technical dossier/Volume II/Annex 1Ac and Additional data March 2020/p. 5 and Enclosures 2 and 4.
[24] LOQ: Aflatoxins (B1, B2, G1 and G2) = 0.1 μg/kg each and 0.05 μg/kg each; Ochratoxin A = 2 and 20 μg/kg; Sterigmatocystin = 10 μg/kg; T2 Toxin = 20 μg/kg and 10 μg/kg; HT2 toxin = 20 μg/kg and 10 μg/kg; Fumonisin B1 = 20 μg/kg; Fumonisin B2 = 20 μg/kg.

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 commercial batches of [redacted][25]

The absence of recombinant DNA in the food enzyme was demonstrated in three batches of the enzyme concentrate, each analysed [redacted][26]

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. The commercial batch 4 (Table 1) used in these studies is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was made according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[27] Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9-mix), applying the 'treat and plate' assay. Two separate experiments were carried out in triplicate using six concentrations of the food enzyme (17, 50, 167, 500, 1,667 and 5,000 μg/plate, corresponding to 16.0, 46.9, 156.8, 469.5, 1,667.1 and 4,694.5 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. In the first experiment, a statistically significant increase in revertant colony numbers was observed in strain TA100 in the presence of S9 at 5,000 μg/plate (corresponding to 4,694.5 μg TOS/plate). This increase was not reproduced in the second experiment. In all the other strains, the numbers of the revertant colonies were comparable to the values observed in the vehicle control groups, in both the experiments in the presence and absence of metabolic activation.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test

An *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP in Chinese Hamster Ovary (CHO) cell cultures with and without metabolic activation (S9-mix).[28] Two separate experiments were carried out in duplicate.

In a first experiment, the cultures were exposed to concentrations of 1,250, 2,500 and 5,000 μg of food enzyme/mL (corresponding to 1,174, 2,347 and 4,695 μg TOS/mL) in the presence and absence of S9-mix, applying 6 h treatment + 24 h recovery. In a second experiment, the same concentrations were applied for 6 h treatment + 24 h recovery with S9-mix and for 22 h treatment + 24 h recovery without metabolic activation, whereas cultures were exposed to 625, 1,250 and 2,500 μg of food enzyme/mL (corresponding to 587, 1,174 and 2,347 μg TOS/mL) for 22 h followed by 48 h recovery without S9-mix. Reduced cell counts (below 50% of the control values) were observed at the two highest concentrations (2,500 and 5,000 μg of food enzyme/mL) after 22 h treatment + 48 h recovery without S9-mix. For all food enzyme concentrations tested, the frequency of cells with structural chromosomal aberrations was similar to that of negative controls. No significant increases in the frequency of polyploid cells were observed in cultures harvested 48 h after treatment.

[25] Technical dossier/Additional data March 2020/p. 6 and Enclosures 5 and 6.
[26] Technical dossier/Volume III/Part2_Appendices/Appendix 19; Additional data September 2014/p. 5 and Additional data December 2014.
[27] Technical dossier/Volume II/Annex 18.
[28] Technical dossier/Volume II/Annex 19.

The Panel concluded that the food enzyme did not induce structural and numerical chromosomal aberrations in CHO cells, under the test conditions employed for this study.

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

The repeated dose 90-day oral toxicity study in rats was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[29] Groups of 10 male and 10 female Sprague-Dawley (Crl:CD(SD) IGS BR) rats received by gavage the food enzyme at doses of 250, 500 and 1,000 mg/kg per day corresponding to 235, 470 and 939 mg TOS/kg bw per day. Controls received the vehicle (water).

One low-dose female was found dead on day 74 and one mid-dose male was sacrificed on day 90. Both cases were related to misdosing (a gavage error).

Body weight of mid-dose males was statistically significantly lower than in the control group on days 7, 14 and 21, but this did not affect the final mean body weight of this group compared to that of the concurrent control group.

In mid- and high-dose males, slightly reduced food consumption was observed throughout the treatment period. The difference to the control group achieved statistical significance during days 7–35 in mid-dose males and on days 28 and 84 in high-dose males. In all treated females, statistically significant reduction in food consumption was recorded from day 7 to 14.

The findings on body weight and food consumption were considered not to be toxicologically significant, as they were transitory and final body weights in the treated groups were comparable with those of the control group.

Regarding haematology, high-dose females showed a statistically significant reduction in prothrombin time, and low- and mid-dose females elicited a statistically significant reduction in large unclassified cells.

In clinical chemistry, mid-dose females showed a statistically significant reduction in alanine aminotransferase levels. A statistically significant increase in phosphate levels was observed in low- and high-dose females.

In urinalysis, urinary pH was statistically significantly decreased in all treated males. In mid-dose females, a slight but statistically significant increase of specific gravity was recorded.

As all changes in haematology, clinical chemistry and urinalysis were slight, without dose–response relationship or supporting histopathological findings, and limited to one sex, they were not considered treatment-related.

No other statistically significant findings were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 939 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the endo-1,4-β-xylanase produced with the genetically modified *T. reesei* strain RF5427 was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific Opinion on the assessment of allergenicity of 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 sliding window of 80 amino acids as the criterion, no match was found.

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-1,4-β-xylanase.

Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al., 1998; Mergets et al., 2001). However, several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any xylanase have been reported in the literature. Therefore, it can be concluded that an allergic reaction upon oral ingestion of xylanase produced with the genetically

[29] Technical dossier/Volume II/Annex 20.

modified *T. reesei* strain RF5427, in individuals respiratory sensitised to xylanase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[30]) are used as raw materials ([redacted]) in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

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 such as is the case for distilled alcohol production. For grain treatment for the production of starch and gluten fractions, experimental data showed a significant removal (> 99%) of protein. However, traces of protein could be present in starch and gluten. The food enzyme remains in foods including beer, baked products, breakfast cereals and pasta/noodles (Section 3.5.1). 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 can be excluded for distilled alcohol production. The risk cannot be excluded for baking, brewing and cereal-based processes, and for grain treatment for the production of starch and gluten fractions, 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 five food processes. Intended uses and the recommended use levels are summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[(a)]

Food manufacturing process[(b)]	Raw material	Recommended dosage of the food enzyme
Baking processes	Flour	Up to 10 mg TOS/kg flour
Brewing processes	Cereals	Up to 5 mg TOS/kg cereal
Cereal-based processes	Flour	Up to 10 mg TOS/kg flour
Distilled alcohol production	Cereals	Up to 5 mg TOS/kg cereal
Grain treatment for the production of starch and gluten fractions	Cereals, Flour	Up to 10 mg TOS/kg cereal Up to 10 mg TOS/kg flour

(a): Technical dossier EFSA-Q-2014-00753/pp. 8-12 and Additional data March 2020/pp. 6-7.
(b): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

In baking and cereal-based processes,[31] the xylanase is added during the preparation of the dough. It hydrolyses (arabino)xylans, which interact with gluten and bind water, thus reducing the dough viscosity and shortening the processing time. The decrease in viscosity facilitates the handling of the dough, results in more uniform products with better properties (increased firmness, reduced oil absorption and less stockiness).

In brewing process,[31] the food enzyme is added at the beginning of the mashing step. The food enzyme has the capacity to degrade the cell walls, promoting the release of starch and protein and increasing the brewing yield. The food enzyme is also added during fermentation to aid beer filtration.

[30] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.
[31] Technical dossier EFSA-Q-2014-00753/Section 3.3.1.

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

In distilled alcohol production,[31] the food enzyme is applied during liquefaction and fermentation and may also be added during slurry mixing and pre-saccharification.

In grain treatment,[32] the food enzyme can be added to the grain to obtain flour, or to the dough to obtain starch and gluten fractions.

Concerning distilled alcohol production and grain treatment for the production of starch and gluten fractions, technical information and experimental data provided on the removal of food enzyme–TOS was considered by the Panel as sufficient to exclude these processes from the exposure assessment (Annex B in EFSA CEF Panel, 2016).

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by distillation, during purification steps and by repeated washing (see Section 3.5.1), foods/ingredients derived by these three processes, i.e. distilled alcohols, starch and gluten were excluded from the estimation.

For the remaining three food manufacturing processes (baking, brewing and cereal-based processes), chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the 'EFSA Comprehensive European Food Consumption Database 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 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 European countries (Appendix B).

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)	0.006–0.035 (12)	0.031–0.074 (16)	0.035–0.065 (19)	0.018–0.040 (20)	0.013–0.029 (22)	0.011–0.025 (21)
Min–max 95th percentile (number of surveys)	0.026–0.119 (10)	0.069–0.109 (14)	0.059–0.118 (19)	0.034–0.080 (19)	0.027–0.061 (22)	0.023–0.043 (21)

TOS: total organic solid.

[32] Technical dossier/Additional data March 2020/p. 7.

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	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure estimate: – distilled alcohol production – grain treatment for the production of starch and gluten fractions	 – –

+: 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 baking and brewing and cereal-based processes, 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.

The exclusion of two food manufacturing processes (distilled alcohol production and grain treatment for the production of starch and gluten fractions) from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (939 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.006–0.074 mg TOS/kg bw per day at the mean and 0.023–0.119 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure of at least 7,891.

4. Conclusions

Based on the data provided, removal of TOS during distilled alcohol production and grain treatment for the production of starch and gluten fractions and the derived margin of exposure for baking, brewing and cereal-based processes, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *T. reesei* strain RF5427 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 "Application for authorisation of an endo-1-4, β-xylanase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008". September 2013. Submitted by AB Enzymes GmbH.

2) Summary report on GMM part related to endo 1,4-beta xylanase from *Trichoderma reesei* RF5427 by AB Enzymes GmbH. December 2013. Delivered by Technical University of Denmark, National Food Institute (Lyngby, Denmark).
3) Summary report on genotoxicity, subchronic toxicity study and allergenicity related to endo-1,4-beta-xylanase from *Trichoderma reesei* (strain RF5427) by AB Enzymes GmbH. December 2013. Delivered by FoBiG (Freiburg, Germany).
4) Additional information. September 2014. Submitted by AB Enzymes GmbH.
5) Dossier "Application for authorisation of an endo-1-4,β-xylanase from a genetically modified strain of *Trichoderma reesei* RF5427 (EFSA-Q-2013-00876). Updated list of food applications where the enzyme is intended to be used". October 2014. Submitted by AB Enzymes GmbH.
6) Additional information. December 2014. Submitted by AB Enzymes GmbH.
7) Summary report on technical data and dietary exposure related to endo-1,4-ß-xylanase from a genetically modified strain of *Trichoderma reseei* (strain RF5427) by AB Enzymes. March 2015. Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy).
8) Additional information. May 2015. Submitted by AB Enzymes GmbH.
9) Additional information. March 2020. Submitted by AB Enzymes GmbH.

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Abbreviations

ATCC	American Type Culture Collection
bp	base pair
bw	body weight
BXU	Xylanase unit
CAS	Chemical Abstracts Service
[redacted]	[redacted]
CBS	Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
CHO	Chinese hamster ovary
DNA	deoxyribonucleic acid
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	food classification system
GLP	Good Laboratory Practice
GM	genetically modified
GMM	genetically modified microorganism
GMO	genetically modified organism
ITS	internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOQ	limit of quantification

NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
rRNA	ribosomal Ribonucleic Acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

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

Information provided in this appendix is shown in an Excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6127#support-information-section).

The file contains two sheets, corresponding to two tables.

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

Table 2: 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, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, 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, Greece, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, 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).