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Safety evaluation of the food enzyme endo-polygalacturonase from the genetically modified *Aspergillus luchuensis* strain FLYSC

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, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Jaime Aguilera, Magdalena Andryszkiewicz, Davide Arcella, Natalia Kovalkovicova, Yi Liu and Andrew Chesson

Abstract

The food enzyme endo-polygalacturonase ((1→4)-α-D-galacturonan glycanohydrolase; EC 2.3.1.15), is produced with the genetically modified *Aspergillus luchuensis* strain FLYSC by Advanced Enzyme Technologies Ltd. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in fruit and vegetable processing for juice production. Based on the maximum use level, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.138 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 800 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 5,800. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and six matches were 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 for individuals sensitised to cedar or grass pollen or maize. 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.

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Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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† Deceased.

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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 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 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 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

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

Five applications have been introduced by the companies "Roquette", "Novozymes A/S", "DSM Food Specialities B.V." and "Advanced Enzyme Technologies Ltd." for the authorization of the food enzymes Beta-amylase from wheat (*Triticum* spp), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AN), Chymosin from a genetically modified stain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC), and Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain FLZSC).

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 five applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out a safety assessments of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus*

[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, p. 15–24.

licheniformis (strain NZYM-AN), Chymosin from a genetically modified stain of *Kluyveromyces lactis* (strain CIN), Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain FLYSC), and Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain FLZSC) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out of the safety assessment of the food enzyme polygalacturonase from a genetically modified strain of *A. niger* (strain FLYSC).

Recent data identified the production microorganism as *Aspergillus luchuensis* (strain FLYSC) (Section 3.1). Therefore, this name will be used in this opinion instead of *Aspergillus niger* (strain FLYSC).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme from a genetically modified strain of *A. niger* (strain FLYSC). The dossier was submitted on 19 December 2014.

Additional information was requested from the applicant during the assessment process on 24 March 2021 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) and following the relevant existing guidance's 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 updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

3. Assessment

IUBMB nomenclature	Endo-polygalacturonase
Systematic name	(1→4)-α-D-galacturonan glycanohydrolase
Synonyms	Pectinase, pectin hydrolase, endo-D-galacturonase
IUBMB No	EC 3.2.1.15
CAS No	9032-75-1
EINECS No	232-885-6

Endo-polygalacturonases catalyse the hydrolysis of 1,4-α-D-galactosiduronic linkages of pectin and other galacturonans, resulting in the generation of partially hydrolysed galacturonans. The food enzyme is intended to be used in fruit and vegetable processing for juice production.

3.1. Source of the food enzyme

The endo-polygalacturonase is produced with the genetically modified filamentous fungus *A. luchuensis* (formerly *A. acidus*), which is deposited at the American Type Culture Collection (ATCC, USA), with deposit number [redacted][4] The production strain was identified as *A. luchuensis* [redacted][5]

[4] Technical dossier/Additional data May 2021/Annex 1.
[5] Technical dossier/3.2. Risk Assessment Data/Annex I1.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted][6]

3.1.2. Characteristics of introduced sequences

The sequence encoding the endo-polygalacturonase is [redacted]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain [redacted]

3.1.4. Safety aspects of the genetic modification

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

The production strain *A. luchensis* FLYSC differs from the recipient strain [redacted] The absence of [redacted]

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[8], with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, 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. 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 mass material passes the filtration membrane and is discarded. 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.

[6] Technical dossier/Additional data May 2021.
[7] Technical dossier/3.2. Risk Assessment Data /Annex M/Additional data May 2021/Annexure A.
[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/3.2. Risk Assessment Data/Annex F.
[10] Technical dossier/3.2. Risk Assessment Data/pg. 30-35/Annex G.
[11] Technical dossier/3.2. Risk Assessment Data/Annex G_Appendix 2.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The endo-polygalacturonase is a single polypeptide chain of [redacted] amino acids.[12] The molecular mass of the mature protein, derived from the amino acid sequence, is [redacted][6] The food enzyme was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a single major protein band of about [redacted] kDa, consistent with the expected mass of the enzyme, accompanied by some minor bands.[13] No other enzymatic activities were reported.[14]

The in-house determination of endo-polygalacturonase activity is based on the hydrolysis of polygalacturonic acid with a consequent increase in reducing groups (reaction conditions: pH 4.5, 40°C, 10 min). 3,5-Dinitrosalicylic acid (DNS) is added, which complexes with the reducing group, producing a colour. The enzymatic activity is then determined by spectrophotometry at 540 nm. Enzyme activity is expressed as Polygalacturonase Units (PGU)/g. One PGU is the quantity of enzyme which releases 2 milligrams of reducing sugars from polygalacturonic acid under the conditions of the assay.[15]

The food enzyme has a temperature optimum around 50°C (pH 4.5) and a pH optimum around pH 4.5 (40°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.5). Enzyme activity decreased above 40°C showing no residual activity above 70°C.[16]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for three commercial batches of a dried preparation, one of which (batch 3) was used for the toxicological testing (Table 1)[17]. The average total organic solids (TOS) of the three food enzyme batches is 80.0% and the average enzyme activity/TOS ratio is 298.0 PGU/mg TOS. Prior to drying the food enzyme is stabilised with [redacted].

Table 1: Compositional data of the food enzyme preparation

Parameters	Unit	Batches		
		1	2	3[(a)]
Endo-polygalacturonase activity	PGU/g[(b)] batch	228,642	235,124	251,156
Protein	%	43.5	45.4	48.9
Ash	%	8.9	8.5	7.2
Water	%	6.3	6.2	6.2
[redacted]		4.9	5.6	6.3
Total organic solids (TOS)[(c)]	%	79.9	79.7	80.3
Endo-polygalacturonase activity/mg TOS	PGU/mg TOS	286.1	295.0	312.8

(a): Batch used for the toxicological studies.
(b): PGU/g: see Section 3.3.1.
(c): TOS calculated as 100% – % water – % ash – % [redacted].

3.3.3. Purity

The lead content in two batches was below 0.25 mg/kg and in the batch used for toxicological testing was below 0.1 mg/kg[18] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the

[12] Technical dossier/3.2. Risk Assessment Data/pg. 5.
[13] Technical dossier/3.2. Risk Assessment Data/pg. 4/Annex B/Additional data May 2021.
[14] Technical dossier/3.2. Risk Assessment Data/pg. 10.
[15] Technical dossier/3.2. Risk Assessment Data/pg. 9; Annexes: C and A2.
[16] Technical dossier/3.2. Risk Assessment Data/pg. 10-12; Annex C.
[17] Technical dossier/3.2. Risk Assessment Data/pg. 4, 6, 43, 53; Annexes: A3 and J/Additional data May 2021.
[18] Technical dossier/3.2. Risk Assessment Data/pg. 6, 9; Annex D.

levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[19,20]

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[21] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[22]

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 aflatoxins, fumonisin B1, ochratoxin A, T-2 toxin, HT2-toxin, zearalenone, deoxynivalenol, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine was examined in the three food enzyme batches, and all were below the limit of detection (LoD) of the applied method.[23,24] The potential presence of other secondary metabolites 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.

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted] No colonies were produced.[25]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][26]

3.4. Toxicological data[27]

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 batch 3 (Table 1) used in these studies was 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 performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[28] Five strains of *Salmonella* Typhimurium (TA100, TA102, TA97a, TA98 and TA1535) were used in the presence or absence of metabolic activation (S9-mix), applying the pre-incubation method. Two separate experiments in triplicate were carried out using five different concentrations of the food enzyme 50, 150, 500, 1,500 and 5,000 μg/plate (corresponding to 40, 120, 400, 1,200 and 4,000 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

[19] Technical dossier/3.2. Risk Assessment Data/Annex D.
[20] LoDs: Pb, As, Cd = 0.25 mg/kg and 0.1 mg/kg; Hg = 0.025 mg/kg.
[21] Technical dossier/3.2. Risk Assessment Data/pg. 6, 9; Annexes: A1, A3.
[22] Technical dossier/3.2. Risk Assessment Data/pg. 6, 9; Annexes: A1, A3, E2.
[23] Technical dossier/3.2. Risk Assessment Data/Annexes: A1, E1, I1.
[24] LoDs: aflatoxins (B1, B2, G1, G2, M1) = 1 μg/kg each; fumonisin B1 = 100 μg/kg; ochratoxin A = 1 μg/kg; T-2 toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; zearalenone = 5 μg/kg; deoxynivalenol = 25 μg/kg; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine = 100 μg/kg each.
[25] Technical dossier/3.2. Risk Assessment Data/Annex M/Appendix 1.3.
[26] Technical dossier/ 3.2. Risk Assessment Data/Annex M/Appendix 1.4 and appendix 1.5/Additional data May 2021/Annexure B and C.
[27] Technical dossier/3.2 Risk Assessment Data_ANPG_Dec2014/p. 43–52; Technical dossier/Annex J.
[28] Technical dossier/Annex J/p. 1–50/Additional data May 2021.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[29]

A cytotoxicity test was performed at concentrations of food enzyme ranging from 125–5,000 μg/mL, and no inhibition of mitotic index by 50% or more was observed. Based on these results, duplicate cultures were exposed to the food enzyme at 500, 1,500 and 5,000 μg/mL (corresponding to 40, 1,200 and 4,000 μg TOS/mL) in the short-term treatment (3 h followed by 21 h recovery period) with and without metabolic activation (S9-mix), and in the continuous treatment (24 h) in the absence of S9-mix. No cytotoxicity was observed at any concentration level of the test substance. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the food enzyme did not induce chromosome aberrations 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 was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[30] Groups of 10 male and 10 female Wistar rats received by gavage the food enzyme at 250, 500 and 1,000 mg/kg bw per day, corresponding to 200, 400 and 800 mg TOS/kg bw per day. Controls received the vehicle (analytical grade water). Two recovery groups of 5 male and 5 female Wistar rats each were treated with 0 or 800 mg TOS/kg bw per day for 90 days followed by a 4-week recovery period.

No mortality was observed.

Functional observations revealed a statistically significantly increased grip strength of fore limb in low- and high-dose males. As this change was not dose-dependent, the Panel considered it not to be toxicologically relevant.

Haematological investigation revealed a statistically significant increase in prothrombin time (PT) in mid-dose males (+7%), total white blood cells (WBC) (+49%) in mid-dose females and a statistically significant decrease in haemoglobin (Hb) (−11%) and packed cell volume (PCV) (−11%) in male recovery group. All the changes in haematological parameters were considered by the Panel as not toxicologically relevant because the differences were small (PT, Hb, PCV), were not dose dependent (PT, WBC) observed in only one sex (all parameters) or not seen at the end of the treatment with the test item (Hb, PCV).

Clinical chemistry investigation revealed a statistically significant decrease in albumin (−8%) in mid-dose males. The Panel considered this change as not toxicologically relevant because it was only observed in one sex, the low magnitude of the change and the absence of a dose–response relationship.

There were statistically significant increases in the following organ weights: relative testes weight (+9%) in mid-dose males, absolute spleen weight (+33%) in the male recovery group and relative liver weight (+8%) in the female recovery group. The Panel considered these changes as not toxicologically relevant because of a low magnitude of the changes (relative testes weight, relative liver weight), in the absence of a dose–response relationship (relative testes weight), as they were not accompanied by gross pathological and histopathological findings (all organs) or were not present at the end of the treatment with the test item (absolute spleen weight, relative liver weight).

No other statistically significant or biologically relevant differences to controls were reported during treatment and recovery periods.

The Panel identified the no observed adverse effect level (NOAEL) of 800 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-polygalacturonase produced with the genetically modified *A. luchuensis* strain FLYSC was assessed by comparing its amino acid sequence with those of known

[29] Technical dossier/Annex J/p. 51–107/Additional data May 2021.
[30] Technical dossier/Annex J/p. 108–282/Additional data May 2021.

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, six matches were found. The matching allergens were Pla a 2, a polygalacturonase from London plane tree; Jun a 2, a polygalacturonase from Mountain cedar; Phl p 13, a polygalacturonase from Timothy grass; Cha o 2.0101, a polygalacturonase from Japanese cypress; Cry j 2, a polygalacturonase from Japanese cedar all known as respiratory allergens; and Zea m 13, a non-specific lipid-transfer protein 1 from maize known as food allergen.[31]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-polygalacturonase.

The Panel notes that oral allergy syndrome, i.e., allergic reactions mainly in the mouth, and seldomly leading to anaphylaxis, is associated with sensitisation to cedar pollen (Midoro-Horiuti et al., 2003), as well as, with grass pollen (Muluk and Cingi, 2018). As there are matches with Mountain cedar and with Timothy grass pollen allergens in the food enzyme that is subject of this application, oral allergy may not be excluded after consumption.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[32]) are used as raw materials ([redacted]) in the media fed to the microorganisms. In addition, [redacted], known allergen, is also present 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 fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

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 in particular due to the potential presence of homology sequence with the cedar pollen and grass pollen allergens. In addition, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded in individuals sensitised to maize proteins, but this risk will not exceed that of maize consumption.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in fruit and vegetable processing for juice production[33] at the recommended use level of up to 3.208 mg TOS/kg fruit/vegetable mash.[34]

In fruit and vegetable processing for juice production, the food enzyme is added to a mash of fruits/vegetables (with or without peels)[35], where the polygalacturonase hydrolyses galacturonan-rich cell wall components to facilitate the release of juice. The enzymatic treatment can lead to higher yields. By using this food enzyme several types of juices can be produced, ready to drink, concentrated and dehydrated juices.[33]

The food enzyme remains in the processed juices. The survival of the activity will depend on the food process conditions.[36]

3.5.2. Dietary exposure estimation

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical

[31] Technical dossier/3.2. Risk Assessment Data/pg. 54/Annex L/Additional data May 2021/Annex 2.
[32] 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.
[33] Additional data May 2021.
[34] Technical dossier/Risk Assessment Data/pg. 39.
[35] Technical dossier/Risk Assessment Data/Figure 3.2.1.4-1.
[36] Technical dossier/Risk Assessment Data/Figure 3.2.1.4-1 & Risk Management Data.

conversion factors (EFSA CEP Panel, 2021). Exposure from all 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 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 2 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 B – Tables 1 and 2. For the present assessment, food consumption data were available from 41 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix A). The highest dietary exposure at the 95th percentile to the food enzyme-TOS was estimated to be 0.138 mg TOS/kg bw per day in children 3–9 years of age.

Table 2: 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)	0.001–0.020 (11)	0.005–0.077 (15)	0–0.044 (19)	0–0.024 (21)	0.001–0.014 (22)	0–0.009 (22)
Min–max 95th percentile (number of surveys)	0–0.081 (9)	0.035–0.130 (13)	0.001–0.138 (19)	0.001–0.083 (20)	0.008–0.060 (22)	0.002–0.040 (21)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

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

Sources of uncertainties	Direction of impact
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	+/–

TOS: total organic solid.
+: 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 an overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (800 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.077 mg TOS/kg bw per day at the mean and from 0–0.138 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 5,797.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme endo-polygalacturonase produced with the genetically modified *A. luchuensis* strain FLYSC does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Dossier for polygalacturonase produced from genetically modified *Aspergillus niger* agg. (strain FLYSC). December 2014. Submitted by Advanced Enzyme Technologies Ltd.

Additional information. May 2021. Submitted by Advanced Enzyme Technologies Ltd.

Summary report on genotoxicity and subchronic toxicity study/allergenicity report. October 2015. Delivered by FoBiG, Freiburg, Germany.

Summary report on technical data and dietary exposure. October 2015. Delivered by Hylobates Consulting and BiCT, Rome, Italy.

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Abbreviations

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 Materials, Enzymes and Processing Aids
CFU	colony forming units
DNS	3,5-dinitrosalicylic acid
DRF	dose-range finding
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MOE	margin of exposure
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.2022.7236#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
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
Children	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
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, Slovenia, Spain, Sweden
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
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

(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).