SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 25 January 2023

doi: 10.2903/j.efsa.2023.7837

Safety evaluation of the food enzyme endo-polygalacturonase from the genetically modified *Aspergillus niger* strain EPG

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, 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, Holger Zorn, Boet Glandorf*, Lieve Herman, Yrjö Roos, Jaime Aguilera, Magdalena Andryszkiewicz, Natália Kovalkovičová, Yi Liu and Andrew Chesson

Abstract

The food enzyme endo-polygalacturonase ((1–4)-α-D-galacturonan glycanohydrolase; EC 3.2.1.15) is produced with the genetically modified *Aspergillus niger* strain EPG by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in fruit and vegetable processing for juice production. The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.122 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate 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 1,014 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, resulted in a margin of exposure at least 8,311. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and 38 matches were found, two of which are food allergens. The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, in particular for individuals sensitised to papaya or maize, but that the risk will not exceed that of consumption of papaya or maize. In addition, oral allergy reactions cannot be excluded in pollen-sensitised individuals. 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, endo-polygalacturonase, (1–4)-α-D-galacturonan glycanohydrolase; EC 3.2.1.15, *Aspergillus niger*, genetically modified microorganism

Requestor: European Commission

Question numbers: EFSA-Q-2014-00402

Correspondence: fip@efsa.europa.eu

* Member of the Working Group on Enzymes of the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) until 30.3.2022.

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, Holger Zorn.

Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Erik Boinowitz, Ana Gomes, Simone Lunardi, Kim Rygaard Nielsen†.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Roos Y, Aguilera J, Andryszkiewicz M, Kovalkovičová N, Liu Y and Chesson A, 2023. Scientific Opinion on the safety evaluation of the food enzyme endo-polygalacturonase from the genetically modified *Aspergillus niger* strain EPG. EFSA Journal 2023;21(3):7837, 18 pp. https://doi.org/10.2903/j.efsa.2023.7837

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

† 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[1] 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 CEF Panel, 2009) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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 in Article 7(2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Two applications have introduced by the 'DSM Food Specialties B.V.' for the authorisation of the food enzymes Asparaginase from a genetically modified strain of *Aspergillus niger* (strain AGN) and Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG).

The mandate to EFSA to carry out the risk assessment of the food enzyme polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG) was made on 3 June 2014 (Ref. Ares (2014)1811865). In 2015, an extension of use has been submitted by the 'DSM Food Specialties B.V.' to the Commission for the following use: flavouring production (EFSA-Q-2015-00178).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008,[2] the Commission has verified that the two applications fall within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, 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

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Asparaginase from a genetically modified strain of *Aspergillus niger* (strain AGN) and Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

The EC provided clarification to the Terms of Reference regarding an extension of use of the food enzyme Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG) on 17 March 2015.

'DSM Food Specialties B.V.' has sent a letter to DG SANTE, registered on 26 October 2022, requesting the withdrawal of the extension of use for the food enzyme Endo-polygalacturonase from *Aspergillus niger* (strain EPG). Therefore, on 8 November 2022 the European Commission requested EFSA to stop the evaluation of the food enzyme Polygalacturonase from a genetically modified strain of *Aspergillus niger* (strain EPG) in flavouring production (EFSA-Q-2015-00178) and to continue the evaluation of the enzyme for the intended uses mentioned in the original dossier under evaluation with EFSA Question number: EFSA-Q-2014-00402.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request in 2014 to carry out the safety assessment of food enzyme endo-polygalacturonase from the genetically modified *A. niger* strain EPG.

2. Data and methodologies

2.1. Data

The applicant has submitted dossier in support of the application for authorisation of the food enzyme endo-polygalacturonase from a genetically modified *A. niger* (strain EPG).

Additional information was requested from the applicant during the assessment process on 10 March 2015, 17 June 2015, 16 September 2021 and 10 October 2022, and received on 11 May 2015, 27 August 2015, 29 June 2022 and 8 November 2022 respectively (see 'Documentation provided to EFSA'). The applicant also submitted spontaneous data on 19 March 2019 and 20 March 2020 (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, 2009) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have 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, 2021a).

3. Assessment[4]

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

[4] Technical dossier/p. 5–6, 11, 18, 47, 50, 93, 102–103.

Endo-polygalacturonases catalyse the random hydrolysis of α-(1–4) glycosidic bonds between galacturonic acid residues in pectin and other polygalacturonans, resulting in their progressive depolymerisation.[5] The food enzyme under assessment is intended to be used in fruit and vegetable processing for juice production.[6]

3.1. Source of the food enzyme[7]

The food enzyme endo-polygalacturonase is produced with the genetically modified filamentous fungus *A. niger* strain EPG ([redacted]), which is deposited in [redacted], with the deposit number [redacted].[8]

The production strain EPG has been identified as *A. niger* [redacted].[9]

3.1.1. Characteristics of the parental and recipient microorganisms

The recipient strain [redacted].[10]

3.1.2. Characteristics of introduced sequences

[redacted].[11] [redacted].[12]

3.1.3. Description of the genetic modification process

[redacted].[13]

[5] Technical dossier/p. 12, 18, 50, 94.
[6] Technical dossier/p. 71.
[7] Technical dossier/Annexes Part II/Annex II-2; Technical dossier/Spontaneous data submission, 20 May 2020; Technical dossier/Additional data, 29 June 2022/Annex 1.
[8] Technical dossier/Spontaneous data submission, 20 May 2020.
[9] Technical dossier/Annexes Part II/Annex II-2; Technical dossier/Additional data, 29 June 2022/Annex 1.
[10] Technical dossier/Annexes Part II/Annex II-3.
[11] Technical dossier/Annexes Part II/Annex II-6; Annex II-8.
[12] Technical dossier/Annexes Part II/Annex II-7; Annex II-9.
[13] Technical dossier/Annexes Part II/Annex II-12.

[redacted][14]

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. niger* EPG differs from the recipient strain only in its capacity to overproduce endo-polygalacturonase. [redacted][15]

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

3.2. Production of the food enzyme[16]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[17] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[18]

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 filtrate 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 mass material passes the filtration membrane and discarded.[19] 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.[20]

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-polygalacturonase is a single polypeptide chain of [redacted] amino acids.[21] The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[22] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[23] A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 50 kDa.[24] Other than [redacted][25] no enzymatic activities were reported.[26]

The in-house determination of endo-polygalacturonase activity[27] is based on the hydrolysis of polygalacturonic acid (pH 4.5–4.7, room temperature). The enzymatic activity is measured based on the reduction in viscosity of the substrate and is expressed in polygalacturonase units (PGU)/g. One PGU is defined as the amount of enzyme that reduces the viscosity of the substrate so that the ratio of the viscosity between the blank and the test solution increases with the incubation time with a slope of 0.068 under the conditions of the test.[28]

[14] Technical dossier/Annexes Part II/Annex II-13.
[15] Technical dossier/Additional data, 11 May 2015/Annex 3; Technical dossier/Additional data, 29 June 2022/Annex 1.
[16] Technical dossier/p. 13–14; 21, 60–67; Technical dossier/Annexes Part I/Annex I-5; Annex I-6; Annex I-7.
[17] 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.
[18] Technical dossier/p. 13, 60; Technical dossier/Annexes Part I/Annex I-5.
[19] Technical dossier/p. 60–67; Technical dossier/Annexes Part I/Annex I-6.
[20] Technical dossier/Annexes Part I/Annex I-7.
[21] Technical dossier/p. 49.
[22] Technical dossier/p. 50.
[23] Technical dossier/p. 48; Technical dossier/Additional data, 11 May 2015/p. 4–5.
[24] Technical dossier/p. 48.
[25] Technical dossier/Additional data, 11 May 2015/p. 6–7.
[26] Technical dossier/p. 12, 50–51.
[27] Technical dossier/p. 50; Technical dossier/Annexes Part I/Annex I-2.
[28] Technical dossier/ p. 50; Technical dossier/Annexes Part I/Annex I-2.

The food enzyme has a temperature optimum around 50°C (pH 3.5) and a pH optimum around pH 3.5–4.2 (50°C).[29] Enzyme activity decreased above 55°C, showing no residual activity at 100°C after 15 min pre-incubation.[30]

3.3.2. Chemical parameters[31]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 23.2% and the mean enzyme activity/TOS ratio was 263.7 PGU/mg TOS.[32]

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Polygalacturonase activity	PGU/g[(b)]	59,200	66,130	57,780	45,013
Protein	%	19.5	19.8	17.0	19.1
Ash	%	1.59	1.43	1.27	0.9
Water	%	74.4	73.8	77.9	76.8
Total organic solids (TOS)[(c)]	%	24.01	24.77	20.83	22.3
Activity/TOS ratio	PGU/mg TOS	246.6	267.0	277.4	201.9

(a): Batch used for the toxicological studies.
(b): PGU: Polygalacturonase Unit (see Section 3.3.1).
(c): TOS calculated as 100% - % water - % ash.

3.3.3. Purity[33]

The lead content in the three commercial batches and in the batch used for toxicological studies was below 2 mg/kg[34,35] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria for total coliforms, *E. coli* and *Salmonella*,[36] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[37]

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 ochratoxin A and fumonisins was examined in the three commercial food enzyme batches and in the batch[38] used for toxicological studies. All were below the limit of detection (LoD) of the applied method.[39,40] Adverse effects caused by the possible 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.

[29] Technical dossier/ p. 12, 51–52.
[30] Technical dossier/Additional data, 11 May 2015/p. 4.
[31] Technical dossier/p. 48, 79; Technical dossier/Annexes Part I/Annex I-1, Annex I-2, Annex I-3; Technical dossier/Annexes Part II/Annex II-1; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[32] Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[33] Technical dossier/p. 12, 49, 79, 93, 103; Technical dossier/Annexes Part I/Annex I-4; Annex I-3; Technical dossier/Additional data, 11 May 2015/Annex 4; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[34] Technical dossier/p. 49, 79; Technical dossier/Annexes Part I/Annex I-3; Annex I-4; Technical dossier/Additional data, 11 May 2015; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[35] Technical dossier/Additional data, 11 May 2015: LoD: Pb = 0.006 mg/L sample solution.
[36] Technical dossier/p. 49, 79; Technical dossier/Annexes Part I/Annex I-3; Annex I-4; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[37] Technical dossier/p. 49, 93, 103; Technical dossier/Annexes Part I/Annex I-3; Annex I-4; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[38] Technical dossier/p. 49, 79.
[39] Technical dossier/Annexes Part I/Annex I-4; Technical dossier/Additional data, 11 May 2015; Technical dossier/Additional data, 29 June 2022/Annex 4; Annex 7.
[40] Technical dossier/Additional data, 11 May 2015; Technical dossier/Annexes Part I/Annex I-4/p. 88: LoD: fumonisin B1, B2 and B3 = 10 μg/kg for each toxin; ochratoxin A = 0.1 μg/kg.

3.3.4. Viable cells and DNA of the production strain[41]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches in triplicate.[42] [redacted]. No colonies were produced. A positive control was included [redacted].

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] three industrial batches [redacted], each tested in triplicate. No DNA was detected [redacted], with a LoD of 10 ng spiked DNA/mL food enzyme.

3.4. Toxicological data[43]

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 4 (Table 1) used in these studies has similar protein pattern as the batches used for commercialisation, but has lower chemical purity, and thus 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 performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[44]

Four strains of *Salmonella* Typhimurium (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* WP2*uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the standard plate incorporation method. Two separate experiments were carried out using eight concentrations of the food enzyme (3, 10, 33, 100, 333, 1,000, 3,330 and 5,000 μg TOS/plate) in the presence or absence of 5% S9-mix in the first experiment and using five concentrations of the food enzyme (100, 333, 1,000, 3,330 and 5,000 μg TOS/plate) in the presence or absence of 10% S9-mix in the second experiment.

No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no biologically relevant increase in the number of revertant colonies above the control values, in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-polygalacturonase did not induce gene mutations under the test conditions applied in this study.

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.[45]

A dose range-finding study was performed at concentrations ranging from 100–5,000 μg/mL, and no decrease in the mitotic index by 50% or more was observed. Based on these results, in the first experiment, the cells were exposed to the food enzyme at 1,000, 3,330 and 5,000 μg TOS/mL in the short-term treatment (3 h exposure and 21 h recovery period) with and without metabolic activation (S9-mix). In the second experiment, the cells were exposed to the food enzyme at 1,000, 3,330 and 5,000 μg TOS/mL in the short-term treatment (3 h exposure and 45 h recovery period) with S9-mix and in the continuous treatment (24 and 48 h exposure) in the absence of S9-mix.

No cytotoxicity was seen either in the short-term (with or without S9-mix) or in the long-term treatment. The frequency of structural and numerical chromosomal aberrations was not statistically significantly different to the negative controls at any concentrations tested.

[41] Technical dossier/Additional data, 29 June 2022/Annex 2; Annex 3.
[42] Technical dossier/Additional data, 11 May 2015; Technical dossier/Spontaneous data submission, 19 March 2019.
[43] Technical dossier/p. 16, 22; 75–80; Technical dossier/Annexes Part I/Annex I-13; Annex I-14; Annex I-15.
[44] Technical dossier/Annexes Part 1/Annex I-13.
[45] Technical dossier/Annexes Part 1/Annex I-14.

The Panel concluded that food enzyme endo-polygalacturonase did not induce an increase in the frequency of structural and numerical aberrations under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study followed OECD Test Guideline 408 (OECD, 1998) and GLP.[46] Groups of 10 male and 10 female Wistar rats received by gavage the food enzyme in doses of 455, 1,364 and 4,545 mg/kg body weight (bw) per day, corresponding to 101, 304 and 1,014 mg TOS/kg bw per day. Controls received the vehicle (double distilled water).

No mortality was observed.

The feed consumption was statistically significantly decreased in weeks 11 and 12 in mid-dose females (−9% and −9%). The Panel considered the change as not toxicologically relevant as it was only recorded sporadically and there was no dose–response relationship.

In the functional observations, a statistically significant decrease in body temperature (−2% and −1.5%) and motor activity score (−13% and −17%) in low- and mid-dose females, respectively, and an increase in hind limb foot splay values (+14%) in mid-dose females were observed. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex and there was no dose–response relationship.

The haematological investigation showed a statistically significant increase in mean corpuscular haemoglobin (MCH) (+6%) in low-dose males. The Panel considered this change as not toxicologically relevant as it was only observed in one sex, the change was small, there was no dose–response relationship and there were no changes in other relevant parameters (haemoglobin and red blood cells).

The clinical chemistry investigation showed a statistically significant decrease in sodium level (−4%) in high-dose males and an increase in chloride level (+8%) in low-dose males and in mid-dose females (+6%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (sodium), there was no dose–response relationship (chloride), the changes were small and there were no histopathological changes in kidneys.

Statistically significant changes in organ weights included a decrease in the relative kidney weight in low-dose males (−8%) and an increase in the relative uterus weight (+65%) in the mid-dose females. The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (kidneys), there was no dose–response relationship (both parameters) and there were no histopathological changes in kidneys and uterus.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 1,014 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[47]

The allergenicity assessment considered 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. niger* strain EPG 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, 38 matches were found, of which one, namely the endo-polygalacturonase [redacted] from *Carica papaya* (papaya), is a known food allergen.

The other 37 are pollen allergens: [redacted]

[46] Technical dossier/Annexes Part 1/Annex I-15.
[47] Technical dossier/Additional data, 29 June 2022/Annex 5; Annex 6.

[redacted].[48]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-polygalacturonase.[47]

Papaya is both a food and a respiratory allergen (Sarkar et al., 2018; Bhowmik et al., 2021). Allergens present in papaya are chitinase, protease (papain), lysozyme, lipid transfer proteins, and recently, a polygalacturonase (Cari p 1) was identified. Cari p 1 cross-reacts with the same protein in papaya pollen (Poncet et al., 2020). Several studies reported occupational rhinitis and asthma in workers of industries where papain is handled (Baur and Fruhmann, 1979; Baur et al., 1982; Niinimaki et al., 1993; Soto-Mera et al., 2000; Van Kampen et al., 2005). In other studies, allergy to papaya-derived products unrelated to occupational exposure has also been described. García-Ortega et al. (1991) showed that administration of chymopapain for chemonucleolysis resulted in sensitisation in some patients. Mansfield and Bowers (1983) reported severe systemic allergic reactions mediated by papain-specific IgE in some individuals that ingested papain-containing meat tenderiser. Sensitisation to papaya does not typically occur from eating papaya fruit. However, once sensitised, individuals may suffer allergic reactions following any type of exposure to papaya or papaya-derived products (Morton, 1987). Sensitisation to papaya has been regularly found in people with oral allergy syndrome (OAS) and/or in latex-allergic patients (Isola et al., 2003); however, reports of clinically relevant allergic reactions are scarce (Mandal et al., 2009; Sharda et al., 2010; Vlaicu et al., 2011; Wan and Chiu, 2012; Bedolla-Barajas et al., 2014; Dey et al., 2014).

Maize is a food allergen, although not frequently leading to allergic reactions in Europe (Scibilia et al., 2008).

The Panel noted that OAS is associated with sensitisation to many pollen allergens, including those from Japanese cedar (Midoro-Horiuti et al., 2003; Bonds et al., 2019), timothy grass (Ibarrola et al., 2004; Chiang et al., 2006) and olives (Palomares et al., 2008; Unsel et al., 2009). However, in case of OAS, the inflammation is usually restricted to the buccal cavity since the allergens are rapidly degraded by gastric enzymes upon ingestion and seldomly leads to anaphylaxis (Sarkar et al., 2018).

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product 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 no potentially allergenic residues are present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, in particular for individuals sensitised to papaya or maize, but that the risk will not exceed that of consumption of papaya or maize. In addition, oral allergy reactions cannot be excluded in pollen-sensitised individuals.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in processing fruit and vegetables at the recommended use levels summarised in Table 2.[6]

[48] Technical dossier/p. 16, 80–82; Technical dossier/Annexes Part I/Annex I-16; Technical dossier/Additional data, 29 June 2022/ Annex 5; Annex 6.

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

Food manufacturing process[(a)]		Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]	
Fruit and vegetable processing for juice production	Extraction	Fruit and vegetable mash	1.14–**2.84**	0.76–2.27
	Clarification	Raw juice		0.38–0.57

TOS: total organic solids.
(a): The description 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.
(b): The number in bold was used for calculation.

In fruit and vegetable processing for juice production, the food enzyme is added to the mash of fruit and vegetables (with or without peels), where the endo-polygalacturonase hydrolyses galacturonan-rich cell wall components (e.g. pectin) to facilitate the release of juice. The food enzyme is applied also to the extracted juice in a lower dose to clarify the juice. The enzymatic treatment can lead to higher yields of juice and/or a reduced processing time.[49]

Based on data provided on thermostability (see Section 3.3.1), it is expected that the endo-polygalacturonase may remain active in juices, depending on the pasteurisation conditions.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) 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 1 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 mean 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 41 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 0.122 mg TOS/kg bw per day in children of 3–9 years of age at the 95th percentile.

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)	0.001–0.018 (11)	0.005–0.068 (15)	0–0.039 (19)	0–0.021 (21)	0.001–0.013 (22)	0–0.008 (22)
Min–max 95th (number of surveys)	0–0.072 (9)	0.031–0.115 (13)	0.001–0.122 (19)	0.001–0.073 (20)	0.007–0.053 (22)	0.002–0.035 (21)

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, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

[49] Technical dossier/p. 69–71.

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	
The sum of the maximum use level used in two steps during the juice production was used to calculate the dietary exposure	+
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 an overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (1,014 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.068 mg TOS/kg bw per day at the mean and from 0 to 0.122 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure of at least 8,311.

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. niger* strain EPG 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

Technical dossier 'Application for authorisation of polygalacturonase from a genetically modified strain of *Aspergillus niger* in accordance with Regulation (EC) No 1331/2008'. 13 May 2014. Submitted by DSM Food Specialties B.V.

Additional information. 11 May 2015. Submitted by DSM Food Specialties B.V.

Additional information. 27 August 2015. Submitted by DSM Food Specialties B.V.

Additional information. 29 June 2022. Submitted by DSM Food Specialties B.V.

Spontaneous data submission. 19 March 2019. Submitted by DSM Food Specialties B.V.

Spontaneous data submission. 20 March 2020. Submitted by DSM Food Specialties B.V.

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Abbreviations

bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FoodEx	standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
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
MCH	mean corpuscular haemoglobin
NOAEL	no observed adverse effect level
OAS	oral allergy syndrome
OECD	Organisation for Economic Cooperation and Development
[redacted]	[redacted]
PGU	polygalacturonase unit
RM	raw material
TOS	total organic solids
[redacted]	[redacted]
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.2023.7837#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 1 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, Hungary, 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, Hungary, 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, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, 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).