SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 8 July 2022

doi: 10.2903/j.efsa.2022.7471

Safety evaluation of a food enzyme containing aspergillopepsin I and II from the *Aspergillus niger* var. *macrosporus* strain PTG8398

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*, Magdalena Andryszkiewicz, Ana Gomes, Yi Liu, Joaquim Maia, Sandra Rainieri and Andrew Chesson

Abstract

The food enzyme with aspergillopepsin I (EC 3.4.23.18) and aspergillopepsin II (EC 3.4.23.19) activities is produced with a non-genetically modified *Aspergillus niger* var. *macrosporus* strain PTG8398 by Meiji Seika Pharma Co., Ltd. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in wine production. Based on the maximum use levels, dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.14 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 919 mg TOS/kg bw per day, the highest dose tested which, when compared with the estimated dietary exposure, results in a margin of exposure above 6,700. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and four matches with respiratory allergens 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, but the likelihood for this to occur is considered low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

Keywords: food enzyme, aspergillopepsin I and II, EC 3.4.23.18, EC 3.4.23.19, *Aspergillus niger* var. *macrosporus*, non-genetically modified microorganism

Requestor: European Commission

Correspondence: fip@efsa.europa.eu

Question number: EFSA-Q-2015-00623

* Member of the WG on Enzymes from July 2018 until March 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, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and 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.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Chesson A, 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, Andryszkiewicz M, Gomes A, Liu Y, Maia J, Rainieri S and Chesson A, 2022. Scientific Opinion on the safety evaluation of a food enzyme containing aspergillopepsin I and II from the *Aspergillus niger* var. *macrosporus* strain PTG8398. EFSA Journal 2022;20(8):7471, 15 pp. https://doi.org/10.2903/j.efsa.2022.7471

ISSN: 1831-4732

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

Table of contents

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

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme consisting of Protease, Leucyl amino-peptidase, Orysin and Aspergillopepsin I from *Aspergillus oryzae* and the companies "BENEO-Palatinit GmbH" for the authorisation of the food enzyme Isomaltulose synthase from *Protaminobacter rubrum* (strain Z12A), "Nagase (Europa) GmbH" for the authorisation of the food enzyme Chitinase from a genetically modified strain of *Streptomyces violaceoruber* (strain pChi), "Clasado Ingredients Ltd." for the authorisation of the food enzyme Beta-galactosidase from a genetically modified strain of *Escherichia coli* (strain BglA MCB3) and "Meiji Seika Pharma Co., Ltd" for the authorisation of the food enzyme consisting of Aspergillopepsin I and II from *Aspergillus niger* var. *macrosporus* (strain DBD0406).

Following the requirements of Article 12.1 of Commission 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] 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 assessment on the food enzymes Protease, Leucyl amino-peptidase, Orysin and Aspergillopepsin I from *Aspergillus oryzae*, Isomaltulose synthase from *Protaminobacter rubrum* (strain Z12A), Chitinase from a genetically modified strain of *Streptomyces violaceoruber* (strain pChi), Beta-galactosidase from a genetically modified strain of *Escherichia coli* (strain BglA MCB3) and Aspergillopepsin I and II from *Aspergillus niger* var. *macrosporus* (strain DBD0406) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme containing aspergillopepsin I and II from *Aspergillus niger* var. *macrosporus* strain PTG8398.

Recent information provided by the applicant identified the production stain as PTG8398 (Section 3.1). Therefore, this designation will be used in this opinion instead of DBD0406.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme containing aspergillopepsin I and II from a non-genetically modified microorganism *Aspergillus niger* var. *macrosporus* (strain PTG8398).

Additional information was requested from the applicant during the assessment process on 25 August 2020 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 25 August 2020, the applicant requested a clarification teleconference, which was held on 3 December 2020.

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 of EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

The food enzyme contains two declared activities.

IUBMB nomenclature:	Aspergillopepsin I
Systematic name:	Not assigned
Synonyms:	*Aspergillus* aspartic proteinase, carboxyl proteinase, pepsin-type aspartic proteinase,
IUBMB No:	EC 3.4.23.18
CAS No:	9025-49-4

Aspergillopepsin I is an aspartic endopeptidase that catalyses the hydrolysis of peptide bonds in proteins, with broad specificity. Generally, it favours hydrophobic residues in P1 and P1', but also accepts Lys in P1, which leads to activation of trypsinogen.

IUBMB nomenclature:	Aspergillopepsin II
Systematic name:	Not assigned
Synonyms:	Proteinase A; *Aspergillus niger* var. *macrosporus* aspartic proteinase
IUBMB No:	EC 3.4.23.19
CAS No:	9025-49-4

Aspergillopepsin II is an aspartic endopeptidase with primary specificity at the P1 position for Tyr, Phe, His, Asn, Asp, Gln and Glu.

The food enzyme under this assessment is intended to be used in wine production.

3.1. Source of the food enzyme

The food enzyme with the two activities, aspergillopepsin I and aspergillopepsin II, is produced with a non-genetically modified microorganism *Aspergillus niger* var. *macrosporus* strain PTG8398.

The parental strain is a [redacted].[4] The production strain PTG8398 was derived from the parental strain by classical mutagenesis and was [redacted].[5] The production strain PTG8398 is deposited at the National Institute of Technology and Evaluation (NITE, Japan) with accession number [redacted].[6] Strain PTG8398 was identified as *A. niger* by [redacted].[7]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Sanitation Act in Japan. In addition, the production plant is licensed by the Ministry of Health, Labour and Welfare Government of Japan, which requires implementation of a hazard analysis and critical control point plan and is considered equivalent to the European standard and legislation, in accordance with current good manufacturing practice.[8]

The production strain is grown as a pure culture using a typical industrial medium in a [redacted] fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth [redacted]. Finally, the food enzyme is [redacted].[9] 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.[10]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The aspergillopepsin I is a single polypeptide chain of [redacted] amino acids.[11] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[12] The aspergillopepsin II consists of [redacted] with a total of [redacted] amino acids.[13] The molecular mass of the mature aspergillopepsin II, derived from the amino acid sequences, was calculated to be [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed the target proteins migrating between the marker proteins of 20 and 45 kDa in all batches, consistent with the expected mass of the enzymes.[15]

The in-house determination of the overall protease activity (i.e. aspergillopepsin I and aspergillopepsin II activities) is based on hydrolysis of casein (reaction conditions: pH 2.6, 37°C,

[4] Technical dossier/Document 4, Annex F6.
[5] Technical dossier/Additional information July 2021/AI2.
[6] Technical dossier/Additional information July 2021/AI3.
[7] Technical dossier/Additional information July 2021/AI4.
[8] Technical dossier/Document 4, Annex A20 and Additional information July 2021/AI6-AI10.
[9] Technical dossier/p. 24–25 and Annex A19.
[10] Technical dossier/Additional information July 2021/AI11 and AI54.
[11] Technical dossier/Document 4/p. 15/Annex A3 and Additional information July 2021/AI26.
[12] Technical dossier/Additional information July 2021/AI26 and AI30.
[13] Technical dossier/Document 4/p. 14/Annex A2 and Additional information July 2021/AI26 and AI27.
[14] Technical dossier/Additional information July 2021/AI26 and AI28.
[15] Technical dossier/Additional information July 2021/AI12 and AI26.

10 min). The enzymatic activity is determined by measuring the released tyrosine spectrophotometrically at 660 nm. The enzyme activity is expressed in proteinase Units (U)/g. One Unit is defined as the amount of enzyme that produces 1 μg/min of tyrosine equivalent under the conditions of the assay.[16]

The protease activity of the food enzyme has a temperature optimum at around 55°C (pH 2.6) and a pH optimum between pH 2.0 and 3.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme at 55°C at different times (pH 2.6). Protease activity decreased to around 10% after 60 min of pre-incubation.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[18] The mean total organic solids (TOS) of the three food enzyme batches is 91.4% and the mean enzyme activity/TOS ratio is 1,272 U/mg TOS. Prior to drying, the food enzyme is stabilised with calcium lactate (~ 4.8%).

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3[(a)]
Protease activity	U/g batch[(b)]	1,186,000	1,153,000	1,147,000
Protein	%	74.4	74.4	73.8
Ash	%	2.3	2.3	2.3
Water	%	1.2	1.9	1.6
[redacted]	%	4.7	4.8	4.8
Total organic solids (TOS)[(c)]	%	91.8	91.0	91.3
Activity/mg TOS	U/mg TOS	1,292	1,267	1,256

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

3.3.3. Purity

The lead content in all batches was below 0.05 mg/kg[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of mercury were below the limit of detection (LOD) of the employed method. For arsenic and cadmium, the concentrations determined in two batches were 0.1 and 0.01 mg/kg, respectively. The Panel considered those concentrations as not of concern.[20,21]

The food enzyme 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).[22] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[23]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of mycotoxins (aflatoxin B1, nivalenol, deoxynivalenol, ochratoxin A, sterigmatocystin, and zearalenone in two batches, and fumonisin B1, B2 and B3 in two other batches) was examined in four food enzyme batches and was below the LOD of the applied method and of no concern. The potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.[24,25]

[16] Technical dossier/Document 4/p. 19-20/Annex 11.
[17] Technical dossier/Document 4/p. 20/Annex A14.
[18] Technical dossier/Document 4/p. 18, Annexes A4, F5 and Additional information July 2021/AI13, AI14, AI15, AI17.
[19] Technical dossier/ Document 4/p. 15/Annexes A4, A5 and Additional information July 2021/AI13, AI14 and AI16.
[20] LOD: Pb = 0.05 mg/kg; As = 0.1 mg/kg; Cd = 0.01 mg/kg; Hg = 0.01 mg/kg.
[21] Technical dossier/ Document 4/Annex A5 and Additional information July 2021/AI13, AI14 and AI16.
[22] Technical dossier/ Document 4/p. 15/Annex 6 and Additional information July 2021/AI13, AI14, AI18 and AI19.
[23] Technical dossier/ Document 4/Annex A4 and Additional information July 2021/AI13, AI14 and AI15.
[24] LOD: aflatoxins B1 = 5 μg/kg; nivalenol, deoxynivalenol, ochratoxin A, sterigmatocystin, zearalenone = 50 μg/kg each; fumonisin B1, B2 and B3 = 0.5 mg/kg each.
[25] Technical dossier/ Document 4/p. 16/Annexes A9, A10.

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

3.3.4. Viable cells of the production strain

The absence of viable cells of the production strain in the product was demonstrated in three independent batches analysed in triplicate. One gram of product was incubated on selective medium at 28°C for 7 days. No colonies were produced.[26]

3.4. Toxicological data

A battery of toxicological tests has been provided, 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. The batch 3 (Table 1)[27] used in these studies was one of the three batches used for commercialisation and, thus, is 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] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. A dose-range study was carried out using six concentrations of the food enzyme (4.88–5,000 μg dry matter/plate, corresponding to 5, 18, 72, 290, 1,160 and 4,639 μg TOS/plate). Growth inhibition was detected at the highest concentration tested for all the *S*. Typhimurium strains with and without metabolic activation, but not for *E. coli* strain WP2uvrA (pKM101). Therefore, the concentrations of the food enzyme tested subsequently in two separate main experiments in duplicate were the following: 156–5,000 μg dry matter/plate (corresponding to 145, 290, 580, 1,160, 2,320 and 4,639 μg TOS/plate) for *S*. Typhimurium strains and 313–5,000 μg dry matter/plate (corresponding to 290, 580, 1,160, 2,320 and 4,639 μg TOS/plate) for the *E. coli* strain with and without metabolic activation.

Growth inhibition was observed in the presence of metabolic activation for strains TA98 and TA1535 from 2,500 μg dry matter/plate and for strains TA100 and TA1535 at the highest concentration tested. In the absence of metabolic activation, a growth inhibition was observed in strains TA98, TA100, TA1535 and TA1537 only at the highest concentration tested.

Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out with Chinese hamster lung cells (CHL/IU) according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[29]

Two separate experiments were performed in duplicate cultures. In the first experiment, cells were exposed to the food enzyme in a short treatment (6 h + 18 h recovery) in the presence and absence of S9-mix. In the second experiment, a short-term treatment with S9-mix and a continuous treatment for 24 h without S9-mix were applied. Based on the results of a preliminary cell-growth inhibition test, the concentrations for the main experiments were set as follows: 625, 1,250, 2,500 and 5,000 μg/mL food enzyme (corresponding to 570.6, 1,141.2, 2,282.5 and 4,565 μg TOS/mL) for the short-term treatment without S9-mix; 1,250, 2,500 and 5,000 μg/mL food enzyme (corresponding to 1,141.2, 2,282.5 and 4,565 μg TOS/mL) for the short-term treatment with S9-mix and 313, 625, 1,250, 2,500 and 5,000 μg/mL food enzyme (corresponding to 285.8, 570.6, 1,141.2, 2,282.5 and 4,565 μg TOS/mL) for the continuous treatment without S9-mix. Chromosome aberrations were scored at 1,141.2, 2,282.5 and 4,565 μg TOS/mL for all the experimental conditions tested.

[26] Technical dossier/Additional information July 2021/AI5.
[27] Technical dossier/Annex F5 and Additional information July 2021/AI13, AI14, AI15, AI17.
[28] Technical dossier/Annex S2_J14067.
[29] Technical dossier/Annex S4_J14068.

The cell proliferation rate at the highest concentration tested was 72.4% in the short-term treatment without S9-mix, 83.9% and 99.4 in the short-term treatment with S9-mix, in the first and second experiment, respectively, and 49.7% in the continuous treatment without S9-mix.

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 chromosomal aberrations in mammalian cells under the conditions employed.

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 Sprague–Dawley rats received the food enzyme in three doses corresponding to 92, 276 and 919 mg TOS/kg bw per day by gavage. Controls received the vehicle (water for injection). Additionally, two groups of 5 male and 5 female rats each received either the vehicle (recovery control) or the food enzyme in the high dose by gavage and were terminated 28-days after the end of the treatment (recovery groups).

No mortality was observed.

In the functional observations, statistically significant decreases were observed in ambulatory counts in low- and high-dose males (−49% and −53%, respectively) in the 20–30-min interval, and in the vertical counts in high-dose males (−70%) in the 40–50-min interval. In the recovery period, statistically significant decreases were observed in high-dose-recovery males in vertical counts (−74%) in the 50–60-min interval, in high-dose-recovery females in ambulatory counts (−57%) in the 40–50-min interval, in vertical counts in the 30–40-min interval (−59%) and in the 50–60-min interval (−67%), and in the total vertical count (−39%). The Panel considered these changes as not toxicologically relevant as they were recorded sporadically (both parameters), they were only observed in one sex (both parameters in the dosing period, ambulatory counts in the recovery period) and there was no consistency between sexes regarding intervals in which the changes were recorded (both parameters).

The haematological investigation revealed a statistically significant decrease in the platelet count (−13%) and in the differential relative monocyte count (−29%) in high-dose-recovery males. The Panel considered these changes as not toxicologically relevant as they were only recorded at the end of the recovery period (both parameters), they were only observed in one sex (both parameters), the changes were small (both parameters) and there were no changes in other relevant parameters (for platelet count no changes in prothrombin time and in activated partial thromboplastin time).

The clinical chemistry investigation revealed a statistically significant decrease in aspartate aminotransferase activity in the high-dose-recovery males (−41%). The Panel considered the change as not toxicologically relevant as it was only recorded at the end of the recovery period, it was only observed in one sex and the change was small.

Statistically significant changes in organ weights included an increase in the absolute (+22%) and relative (+30%) epididymis weights and in the relative testes weight (+12%) in the high-dose-recovery males, a decrease in the absolute (−8%) and relative (−14%) kidney weights in the high-dose-recovery females. The Panel considered these changes as not toxicologically relevant as they were only recorded at the end of the recovery period (all parameters), they were only observed in one sex (kidney), the changes were small (kidney) and there were no histopathological findings in the organs (all parameters).

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

The Panel identified the no observed adverse effect level (NOAEL) of 919 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 aspergillopepsin I and II produced with the non-genetically modified *Aspergillus niger* var. *macrosporus* strain PTG8398 was assessed by comparing its amino acids sequences with those of known allergens according to the 'Scientific opinion on the assessment of

[30] Technical dossier/Annex S7_J14066.

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, four matches were found.[31] The matching allergens were [redacted].

No information is available on oral and respiratory sensitisation or elicitation reactions of the aspergillopepsin I or aspergillopepsin II.

[redacted] is a mould that can be inhaled and cause allergic reactions. It is not known as an oral allergen. [redacted] is associated with occupational asthma and rhinitis (Cartier et al., 1984; Añíbarro Bausela and Fontela, 1996). 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). [redacted] are associated with allergic reactions to insects, but allergic reactions after oral exposure have not been reported (Cantillo et al., 2017).

[redacted] products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[18]), as well as [redacted] are used as raw materials. 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 foods employed as protein sources are not expected to be present.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in wine production at the recommended use level up to 17,400 U/L of grape juice, corresponding to 13.65 mg TOS/L of grape juice.[32]

This food enzyme is used mainly for [redacted].[33] During wine production, [redacted].[34] The two declared endopeptidases hydrolyse the haze-forming proteins, such as chitinases and thaumatin-like proteins, into small peptides, thus preventing the formation of haze during wine storage.

The food enzyme remains in the finished wine. Considering the maximum temperature (30°C) that is reached during wine production, it is expected that the aspergillopepsin complex is not inactivated during wine production.

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 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 mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as

[31] Technical dossier/Annex VIII and Additional information July 2021/AI37-AI52.
[32] Technical dossier/p. 35.
[33] Technical dossir/Additional information July 2021/AI24.
[34] Technical dossier/Additional information July 2021/AI23.

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 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 0.136 mg TOS/kg bw per day in elderly people at the 95th percentile.

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)	0–0.001 (11)	0–0.002 (15)	0–0.003 (19)	0–0.002 (21)	0.003–0.024 (22)	0.001–0.036 (22)
Min–max 95th percentile (number of surveys)	0–0.002 (9)	0–0.010 (13)	0.001–0.011 (19)	0.001–0.008 (20)	0.013–0.099 (22)	0.003–0.136 (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 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	+
The applicants indicated only wine as the relevant FoodEx categories,[35] however the calculation included not only wines but also wine vinegars.	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

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 overestimation of the exposure.

[35] Additional data July 2021/AI24.

3.6. Margin of exposure

A comparison of the NOAEL (919 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.036 mg TOS/kg bw per day at the mean and from 0 to 0.136 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 6,757.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme containing aspergillopepsin I and II produced with *Aspergillus niger* var. *macrosporus* strain PTG8398 does not give rise to safety concerns under the intended conditions of use.

5. Documentation provided to EFSA

1) Dossier "Meiji Protease Powder", October 2015. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
2) Additional information. July 2021. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.

References

Añíbarro Bausela B and Fontela JL, 1996. Occupational asthma in a cheese worker. Allergy, 51, 960–961.

Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204. https://doi.org/10.1016/j.aller.2009.05.001

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502. quiz 502, 426

Cantillo JF, Puerta L, Puchalska P, Lafosse-Marin S, Subiza JL and Fernández-Caldas E, 2017. Allergenome characterization of the mosquito *Aedes aegypti*. Allergy, 72, 1499–1509.

Cartier A, Malo J, Pineau L and Dolovich J, 1984. Occupational asthma due to pepsin. The Journal of Allergy and Clinical Immunology, 73, 574–577.

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

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, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

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, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: http://www.fao.org/3/a-a0675e.pdf

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus Niger, Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology, 102, 9481–9515. https://doi.org/10.1007/s00253-018-9354-1

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: bacterial reverse mutation test. 21 July 1997, 11 pp. Available online: https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1639400502&id=id&accname=guest&checksum=D2E89C16911BE1BEB13BBC206B402098

OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: in vitro mammalian chromosomal aberration test. 21 July 1997, 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: repeated dose 90-day oral toxicity study in rodents. 21 September 1998, 10 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition and Food Research, 48, 413–423. https://doi.org/10.1002/mnfr.200400029

Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
kDa	kiloDalton
LOD	limit of detection
MOE	margin of exposure
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.2022.7471#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).