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SCIENTIFIC OPINION

Safety evaluation of the food enzyme endo-1,3(4)-β-glucanase from the non-genetically modified *Talaromyces versatilis* strain PF8

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Abstract

The food enzyme endo-1,3(4)-β-glucanase (3-(1–3;1–4)-β-D-glucan 3(4)-glucanohydrolase; EC 3.2.1.6) is produced with the non-genetically modified *Talaromyces versatilis* strain PF8 by Erbslöh Geisenheim AG. The food enzyme was free from viable cells of the production organism. It is intended to be used in four food manufacturing processes. Dietary exposure to the food enzyme–total organic solids (TOS) was calculated to be up to 0.110 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 2229 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure resulted in a margin of exposure of at least 20,264. A search for homology of the amino acid sequence of the food enzyme to known allergens was made and four matches with respiratory or contact allergens were found. The Panel considered that the risk of allergic reactions upon dietary exposure cannot be excluded, but the likelihood is 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

3-(1–3;1–4)-β-D-glucan 3(4)-glucanohydrolase; EC 3.2.1.6, EFSA-Q-2015-00663, endo-1,3(4)-β-glucanase, food enzyme, non-genetically modified microorganism, *Talaromyces versatilis*

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

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[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 EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEP 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.

Five applications have been introduced by the companies `Erbslőh Geisenheim AG' for the authorisation of the food enzyme endo-1,3(4)-beta-glucanase from *Talaromyces versatilis* (strain PF8), `Novozymes A/S' for the authorisation of the food enzyme lipase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-PH), and `Danisco US Inc.' for the authorisation of the food enzymes 4-phytase from a genetically modified strain of *Trichoderma reesei* (DP-Nzt55), alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54) and pullulanase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb39).

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

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzymes endo-1,3(4)-beta-glucanase from *Talaromyces versatilis* (strain PF8), lipase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-PH), 4-phytase from a genetically modified strain of *Trichoderma reesei* (DP-Nzt55), alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb54) and pullulanase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb39) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

[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.

1.2 | 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 endo-1,3(4)-β-glucanase from *T. versatilis* (strain PF8).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,3(4)-β-glucanase from *T. versatilis* strain PF8.

Additional information, requested from the applicant during the assessment process on 27 July 2022, were received on 2 May 2024 (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 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) has been followed for the evaluation of dietary exposure.

3 | ASSESSMENT[4]

IUBMB nomenclature	Endo-1,3(4)-β-glucanase
Systematic name	3-(1–3;1–4)-β-D-glucan 3(4)-glucanohydrolase
Synonyms	Endo-1,3-β-D-glucanase; laminarinase; β-1,3-glucanase
IUBMB no	EC 3.2.1.6
CAS no	62213-14-3
EINECS no	263-462-4

Endo-1,3(4)-β-glucanases catalyse the hydrolysis of 1,3- or 1–4-β-glycosidic linkages in β-D-glucans, resulting in the generation of partially hydrolysed β-D-glucans. The food enzyme under assessment is intended to be used in four food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) processing of cereals and other grains for the production of brewed products; processing of fruits and vegetables for the production of (2) juices and (3) wine and wine vinegar; and (4) processing of yeast and yeast products.

3.1 | Source of the food enzyme[5]

The food enzyme endo-1,3(4)-β-glucanase is produced with the non-genetically modified filamentous fungus *T. versatilis* strain PF8 (former designation *Penicillium funiculosum*), which is deposited at [redacted] with the deposit number [redacted].[6] The production strain was identified as *T. versatilis* by using [redacted].[7]

The production strain *T. versatilis* PF8 was obtained [redacted] Strain PF8 differs from the parental strain [redacted].[8]

[4]Technical dossier/p. 3–4, 6–7, 34, 58, 87–88, 90.
[5]Technical dossier/p. 4, 8, 42–43, 87; Technical dossier/Annex K; Annex L; Technical dossier/Additional data, 2 May 2024/Attachment 1; Attachment 2.
[6]Technical dossier/p. 22; Technical dossier/Additional data, 2 May 2024/Attachment 7.
[7]Technical dossier/Additional data, 2 May 2024/Attachment 2.
[8]Technical dossier/Annex K.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[9] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[10]

The production strain is grown as a pure culture using a typical industrial medium in a solid state fermentation system with conventional process controls in place. After completion of the fermentation, water is added and the biomass and other solids are removed from the fermentation broth by centrifugation, followed by microfiltration. 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.[11] 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.[12]

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

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The endo-1,3(4)-β-glucanase is a single polypeptide chain of [redacted] amino acids.[13] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[15] A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about 74 kDa,[16] consistent with the expected mass of the enzyme.

No other enzyme activities were reported.[17]

The applicant's in-house determination of endo-1,3(4)-β-glucanase activity is based on the hydrolysis of a fungal β-glucan (reaction conditions: pH 4.0, 40°C, 15 min) and determined by measuring the release of glucose by means of a colorimetric reaction detected spectrophotometrically at 515 nm. The enzyme activity is expressed in units of β-glucanase (β-Glu-U)/g. A unit is defined as the quantity of reducing sugars, expressed as glucose, released in by 1 g (or 1 mL) of enzyme per minute under the assay conditions.[18]

The food enzyme has a temperature optimum around 55°C (pH 4.0) and a pH optimum around pH 4.0 (40°C).[19] Thermostability was tested after a pre-incubation of the food enzyme for [redacted] min at different temperatures ranging from [redacted]°C to [redacted]°C (pH [redacted]).[20] The enzyme activity decreased above 55°C showing no residual activity above 70°C.[21]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation and one batch produced for the toxicological tests (Table 1).[22] The mean total organic solids (TOS) of the three food enzyme batches intended for commercialisation was 20.9% and the mean enzyme activity/TOS ratio was 4.0 β-glucanase-U/mg TOS.

[9]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.
[10]Technical dossier/p. 8, 47; Technical dossier/Annex F.
[11]Technical dossier/p. 8–9, 48–56; Technical dossier/Annex F; Annex G; Annex H.
[12]Technical dossier/Annex H; Technical dossier/Additional data, 2 May 2024/Attachment 3.
[13]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 4/Attachment 4.
[14]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 4/Attachment 4.
[15]Technical dossier/p. 36; Technical dossier/Annex C.
[16]Technical dossier/p. 36–37.
[17]Technical dossier/p. 7, 36, 38–39.
[18]Technical dossier/p. 7, 38; Technical dossier/Annex B.
[19]Technical dossier/p. 39–41.
[20]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 5.
[21]Technical dossier/p. 41.
[22]Technical dossier/p. 35; Technical dossier/Annex A; Annex B; Annex D; Annex E; Annex J.

TABLE 1 Composition of the food enzyme.

		Batches			
Parameters	**Unit**	**1**	**2**	**3**	**4**[a]
Endo-1,3(4)-β-glucanase activity	U/g[b]	827	883	830	1070
Protein	%	10.8	10.9	11.1	11.4
Ash	%	0.8	0.8	0.9	0.9
Water	%	78.6	78.2	77.9	77
Total organic solids (TOS)[c]	%	20.6	21.0	21.2	22.1
Activity/TOS ratio	β-glucanase-U/mg TOS	4.0	4.2	3.9	4.8

[a]Batch used for the toxicological studies.
[b]Unit of β-glucanase/g (see Section 3.3.1).
[c]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity[23]

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg[24] 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 concentrations of arsenic, cadmium and mercury were below the limit of detection (LoD) of the employed methods.[25,26]

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).[27] No antimicrobial activity was detected in any of the tested batches.[28]

Strains of *Talaromyces*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of aflatoxin B1, B2, G1, G2, ochratoxin A, deoxynivalenol, sterigmatocystin, zearalenone, T-2 toxin, HT-2 toxin was examined in all food enzyme batches and all were below the LoD of the applied methods.[29,30] Adverse effects caused by the possible presence of other secondary metabolites are addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4 | Viable cells of the production strain[31]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted]. No colonies were produced. A positive control was included.[32]

3.4 | Toxicological data[33]

A battery of toxicological tests including a bacterial reverse mutation test (Ames test), an in vitro mammalian chromosomal aberration, in vitro mammalian cell micronucleus test, in vivo micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. The batch 4 (Table 1)[34] used in these studies has similar composition and activity/TOS value as the batches intended for commercialisation and was considered suitable as a test item.

[23]Technical dossier/p. 7, 37, 87; Technical dossier/Annex D; Annex E; Annex J; Annex M; Annex N; Annex O; Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 6; Attachment 5; Attachment 6.
[24]Technical dossier/p. 7, 35; Technical dossier/Annex D; Annex E; Annex J; Technical dossier/Additional data, 2 May 2024/Attachment 5.
[25]Technical dossier/Annex D; Technical dossier/Additional data, 2 May 2024/Attachment 5.
[26]Technical dossier/Additional data, 2 May 2024/Attachment 5: LoDs: Pb = 0.05 mg/kg; As = 0.1 mg/kg; Cd = 0.01 mg/kg; Hg = 0.005 mg/kg.
[27]Technical dossier/p. 7, 35; Technical dossier/Annex D; Annex E; Annex J.
[28]Technical dossier/p. 7, 35, 75; Technical dossier/Annex O.
[29]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 6; Attachment 5.
[30]LoDs: aflatoxins B1, B2, G1 and G2 = 0.1 μg/kg each; ochratoxin A = 0.5 μg/kg; deoxynivalenol = 20 μg/kg; sterigmatocystin = 50 μg/kg; zearalenone = 10 μg/kg, T-2 toxin = 10 μg/kg; HT-2 toxin = 10 μg/kg.
[31]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 7; Attachment 6.
[32]Technical dossier/Additional data, 2 May 2024/Annex/Response to EFSA Question 7; Attachment 6.
[33]Technical dossier/p. 69–76; Technical dossier/Annex J; Annex Q.
[34]Technical dossier/p. 70, 75; Technical dossier/Annex J.

3.4.1 | Genotoxicity

3.4.1.1 | In vitro *studies*

3.4.1.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[35] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvrA* were used with or without metabolic activation (S9-mix). The pre-incubation method was used in a preliminary study, the dose-finding study and the first main study. The 'treat and wash' method was used in the second main study and a confirmation study.

The preliminary experiment was carried out with one plate per dose, using six concentrations of the food enzyme ranging from 0.00107 to 107 U/plate, corresponding to 0.223, 2.23, 22.3, 223, 2229 and 22,292 µg TOS/plate. Upon treatment with the food enzyme, an increase in the number of revertant colonies was observed at 107 U/plate in *S.* Typhimurium TA100 and at 10.7 U/plate in *S.* Typhimurium TA1537 without S9-mix, and at 107 U/plate in *S.* Typhimurium TA100 with S9-mix.

The dose-finding study was carried out in triplicate, using six concentrations of the food enzyme ranging from 0.44 to 107 U/plate, corresponding to 92, 275, 825, 2479, 7438 and 22,292 µg TOS/plate. Upon treatment with the food enzyme, an increase in the number of revertant colonies was observed at 107 U/plate in *S.* Typhimurium TA100 without S9-mix and in *S.* Typhimurium strains TA98, TA100 and TA1535 with S9-mix.

The first main study was carried out in triplicate using six concentrations of the food enzyme ranging from 3.34 to 107 U/plate, corresponding to 696, 1394, 2792, 5583, 11,146 and 22,292 µg TOS/plate and using *S.* Typhimurium strains TA98, TA1535 and TA1537 and *E. coli* WP2*uvrA* without S9-mix and *E. coli* WP2*uvrA* and *S.* Typhimurium TA1537 with S9-mix. Upon treatment with the food enzyme, an increase in the number of revertant colonies was observed at 107 U/plate in *S.* Typhimurium TA98 without S9-mix.

Growth stimulation, as indicated by the thickening of the background bacterial lawn, recorded in the preliminary, dose-finding and the first main experiment applying the pre-incubation method, was probably caused by free amino acids present in the test item.

The second main study applying the 'treat and wash' method was carried out in triplicate using six concentrations of the food enzyme ranging from 3.34 to 107 U/plate, corresponding to 696, 1394, 2792, 5583, 11,146 and 22,292 µg TOS/plate, and using *S.* Typhimurium strains TA98 and TA100 in the absence of S9-mix and *S.* Typhimurium strains TA98, TA100 and TA1535 in the presence of S9-mix. No cytotoxicity was observed at any concentration 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 confirmation study was carried out in triplicate using five concentrations of the food enzyme ranging from 6.69 to 107 U/plate, corresponding to 1394, 2792, 5583, 11,146 and 22,292 µg TOS/plate, and using *S.* Typhimurium strains TA98 and TA100 in the absence of S9-mix and *S.* Typhimurium strains TA98, TA100 and TA1535 in the presence of S9-mix. No cytotoxicity was observed at any concentration 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 strains tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase did not induce gene mutations under the test conditions applied in this study.

3.4.1.1.2 | In vitro *mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[36] Two separate experiments were performed with duplicate cultures of Chinese hamster lung fibroblasts cell line (CHL/IU). The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

In the cell growth inhibition test (dose-finding study), an inhibition of cell growth of 50% or more was seen at 22.1 mg TOS/mL (49.3%) in the long-term treatment without S9-mix.

Based on these results, in the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 26.8, 53.5 and 107 U/mL, corresponding to 5583, 11,146 and 22,292 µg TOS/mL in a short-term treatment (6 h exposure and 18 hours recovery period) either with or without S9-mix and in the long-term treatment (24 h exposure without recovery period) without S9-mix. Cytotoxicity of 41.4%, evaluated as relative cell growth inhibition, was observed at concentration of 22,292 µg TOS/mL in the long-term treatment without S9-mix. The frequency of structural chromosome aberrations was statistically significantly different to the negative controls at concentration of 22,292 µg TOS/mL in the short-term treatment without S9-mix (7.5% vs. 1.5%) and in the long-term treatment without S9-mix (7.5% vs. 1.5%).

[35]Technical dossier/Annex Q.
[36]Technical dossier/Annex Q.

In the second (confirmatory) experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 52.4, 74.9 and 107 U/mL, corresponding to 10,917, 15,604 and 22,292 µg TOS/mL in a short-term treatment (6 h exposure and 18 h recovery period) without S9-mix and at concentrations of 36.7, 52.4, 74.9 and 107 U/mL, corresponding to 7646, 10,917, 15,604 and 22,292 µg TOS/mL in the long-term treatment (24 hours exposure without recovery period) without S9-mix. Cytotoxicity of 35.7%, evaluated as relative cell growth inhibition, was observed at concentration of 22,292 µg TOS/mL in the long-term treatment without S9-mix. The frequency of structural chromosome aberrations was statistically significantly different to the negative controls at concentrations of 15,604 and 22,292 µg TOS/mL in the short-term treatment without S9-mix (7.0% and 9.5%, respectively vs. 1.0% in the control) and at concentrations of 15,604 and 22,292 µg TOS/mL in the long-term treatment without S9-mix (16% and 25.5%, respectively vs. 1.5% in the control), with concentration response, outside the historical control range.

The frequency of numerical aberrations was not statistically significantly different to the negative controls at all the other concentrations tested.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase induced an increase in the frequency of structural chromosome aberrations under the test conditions applied in this study.

3.4.1.1.3 | In vitro *mammalian cell micronucleus test*

The in vitro mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2023) and following GLP.[37] An experiment was performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

In a range-finding test no cytotoxicity above 50% (replication index) was seen at any concentration tested up to 5000 µg TOS/mL with and without metabolic activation (S9-mix). Based on these results, in the main experiment, cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 1000, 2000 and 5000 µg TOS/mL in a short-term treatment (3 h exposure and 21 h recovery period) either with or without S9-mix and in a long-term treatment (24 h exposure and 24 h recovery period) without S9-mix.

No notable cytotoxicity was seen either in the short-term treatment with and/or without S9-mix or in the long-term treatment. The frequency of MNBN was not statistically significantly different to the negative controls at all concentrations tested.

The Panel concluded that the food enzyme endo-1,3(4)-β-glucanase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

3.4.1.2 | In vivo *studies*

3.4.1.2.1 | In vivo *mammalian erythrocyte micronucleus test*

The in vivo mammalian erythrocyte micronucleus test in rats was carried out according to the OECD Test Guideline 474 (OECD, 1997c) and following GLP.[38]

Five CRL:CD (SD) [SPF] rats (males) were treated with a single oral administration (gavage) for 2 consecutive days by food enzyme dissolved in water for injection at doses of 2680, 5350 and 10,700 U/kg body weight (bw) per day, corresponding to 558, 1115 and 2229 mg TOS/kg bw per day (Batch 4). Rats were sacrificed 24 h after final dosing. Negative controls received water for injection and positive controls received mitomycin C (2 mg/kg bw, single *i.v.* administration).

No mortalities and clinical signs of toxicity were reported after treatment with the test item. No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes (MNPCE) and no substantial decrease in the proportion of immature erythrocytes were observed in animals treated with the food enzyme, compared with vehicle control values.

The food enzyme endo-1,3(4)-β-glucanase did not induce micronuclei in bone marrow when tested up to 10,700 U/kg bw per day (corresponding to 2229 mg TOS/kg bw per day) under the experimental conditions employed. The Panel considered the results of this study as inconclusive because no data on bone marrow exposure were provided.

Conclusions on genotoxicity

Based on the negative results obtained with the Ames test and with the in vitro mammalian cell micronucleus test in human peripheral lymphocytes, the Panel concluded that there is no concern for genotoxicity of the food enzyme endo-1,3(4)-β-glucanase. The Panel considered that the positive results reported in the in vitro mammalian chromosomal aberration test with a transformed rodent cell line were overruled by those obtained with the primary human cell culture.

[37]Technical dossier/Additional data, 2 May 2024/Attachment 7.

[38]Technical dossier/Annex Q.

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

The repeated dose 90-day oral toxicity study was performed under GLP and according to the Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives, Notification No. 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan (1996) and OECD Test Guideline 408 (OECD, 1998),[39] with the following deviations: detailed weekly clinical observations were not performed, blood urea nitrogen was not measured, weight of epididymides was not recorded and medulla/pons was not collected for histopathological examination. The Panel considered that these deviations are minor and do not impact on the evaluation of the study.

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) [SPF] rats received by gavage the food enzyme in doses of 107, 1070 and 10,700 U/kg bw per day, corresponding to 22.3, 223 and 2229 mg TOS/kg bw per day for 90 days. Controls received the vehicle (water for injection).

No mortality was observed.

The feed consumption was statistically significantly increased on days 85–90 of administration (+14%) in low-dose females. The Panel considered the change as not toxicologically relevant, as it was only recorded sporadically, it was only observed in one sex, there was no dose–response relationship and there was no statistically significant change in the final feed consumption.

Haematological investigations revealed a statistically significant increase in the relative neutrophil count (+32%) in low-dose males. The Panel considered the change as not toxicologically relevant, as it was only observed in one sex, there was no dose–response relationship and there were no changes in other relevant parameters (i.e. in total white blood cell count).

Clinical chemistry investigations revealed a statistically significant decrease in sodium (–1%) and chloride (–2%) in high-dose males and in total proteins (–5%) in high-dose females. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), the changes were small (all parameters), there were no changes in other relevant parameters (A/G ratio), there were no histopathological changes in kidneys and the changes were within the historical control values (sodium, chloride).

The urinalysis revealed a statistically significant increase in osmotic pressure (+22%) and sodium concentration (+31%) in low-dose females. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex, there was no dose–response relationship and there were no histopathological changes in kidneys.

Statistically significant changes in organ weights detected were an increase in absolute brain weight (+4%) in mid-dose males, absolute prostate weight (+15%) in mid-dose males, relative prostate weight (+20%) in low-dose males and a decrease in relative liver weight (–6%) in mid-dose females. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (absolute brain weight, relative liver weight), the changes were small (absolute brain weight, relative liver weight), there was no dose–response relationship (all parameters) and there were no histopathological changes in the respective organs.

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

The Panel identified a no observed adverse effect level (NOAEL) of 2229 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity[40]

The allergenicity assessment considered only the food enzyme and not additives, preservatives, carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the enzyme endo-1,3(4)-β-glucanase produced with *T. versatilis* strain PF8 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, four matches were found.[41] The matching allergens were Der f 23 and a 98 kDa protein from *Dermatophagoides farinae* (House dust mite); Asp f 7 from *Aspergillus fumigatus* and villin 2 from *Nicotiana tabacum*.

No information is available on oral and respiratory sensitisation or elicitation reactions of the enzyme under assessment.

O'Connor et al. (2001) reported the first case of occupational asthma in an agricultural worker attributed to the enzymes phytase and β-glucanase. In addition, mite allergens (Bessot & Pauli, 2011) and *A. fumigatus* allergens (Chaudhary & Marr, 2011) are well known allergens that can trigger respiratory sensitisation and asthma. However, several studies have shown that individuals respiratorily sensitised to an enzyme are usually able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Poulsen, 2004).

Some reports indicated that ß-glucanases in pollen from olive trees may cause allergic reactions (Huecas et al., 2001; Palomares et al., 2003; Trevino et al., 2008; Callero et al., 2012). Torres et al. (2015) identified Fra e 9 as a novel allergenic β-glucanase from ash pollen. A small number of five IgE-binding epitopic areas were identified in β-glucanase from bananas and thought to be a causative agent of latex-fruit syndrome (Barre et al., 2009). However, the Panel noted that

[39]Technical dossier/Annex Q.

[40]Technical dossier/p. 12–13, 77–78; Technical dossier/Additional data, 2 May 2024/Attachment 8.

[41]Technical dossier/p. 12–13; 77–78; Technical dossier/Additional data, 2 May 2024/Attachment 8.

none of these allergens have a homology match with the food enzyme endo-1,3(4)-ß-glucanase.[42] Also villin-related proteins represent cross-reactive plant allergens in pollens and plant food (Mittermann et al., 2005) and may be involved in the oral allergy syndrome. However, the enzyme that is subject of this opinion shares some homology with villin-related proteins from *Nicotiana tabacum*, but not other plants or pollen thereof. Occupational contact dermatitis caused by tobacco has been reported (Gonçalo et al., 1990), but allergic responses after ingestion have not been reported.

[redacted], a product that may cause allergies (listed in the Regulation (EU) No 1169/2011[43]) is used as raw material. In addition, [redacted], a known source of allergens, is also present in the media fed to the microorganisms. However, during the fermentation process, these products will mostly 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. The Panel, however, considered that residual amounts of potentially allergenic proteins could still be present in the food enzyme.

Overall, the Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in four food manufacturing processes at the recommended use levels summarised in Table 2.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[44]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
• Production of brewed products	Cereals	0.1–**2.0**
Processing of fruits and vegetables		
• Production of juices	Fruits and vegetables	0.2–**2.0**
• Production of wine and wine vinegar	Grapes	0.1–**11.0**
Processing of yeast and yeast products	Yeast cells, yeast extracts or yeast cell walls	10–**50.0**

[a]The name has been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The numbers in bold represent the maximum recommended use levels, which were used for calculation.

In the production of brewed products, the food enzyme is added to cereals during mashing[45] to degrade cell wall glucans, promoting the release of starch and protein and increasing the brewing yield. The food enzyme can also be added during fermentation to reduce beer turbidity.[46] The food enzyme–TOS remain in the beer.

In the production of juices, the food enzyme is added to crushed fruits and vegetables during mash treatment.[47] The hydrolysis of β-glucans reduces viscosity and improves the extraction of fruit or vegetable components, such as colour, from plant tissues.[48] The food enzyme–TOS remain in the juice.

In the production of wine and wine vinegar, the food enzyme can be added during several steps: crushing and maceration, pressing, fermentation, clarification and stabilisation.[49] The enzymatic treatment reduces viscosity and improves the extraction of sensory active components from the grape. The food enzyme–TOS remain in the wine.

[42]Technical dossier/Additional data, 2 May 2024/Attachment 9.

[43]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.

[44]Technical dossier/p. 67; Technical dossier/Additional data May 2024/Response to EFSA Question 11.

[45]Technical dossier/p. 61.

[46]Technical dossier/p. 61.

[47]Technical dossier/p. 62.

[48]Technical dossier/p. 93.

[49]Technical dossier/p. 63.

In yeast processing, the food enzyme is added to yeast cells, yeast extracts or yeast cell walls.[50] The endo-1,3(4)-β-glucanase degrades cell walls glucans, improving the extraction process of cellular components from yeast. The food enzyme–TOS remain in the final yeast products, which are ingredients of a variety of final foods (e.g. wine, soups, savoury snacks, bouillons).[51]

Based on data provided on thermostability (see Section 3.3.1) it is expected that the food enzyme is inactivated in brewed products and in yeast products. However, it may remain in its active form in juices, wines and wine vinegars, depending on the processing 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, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.110 mg TOS/kg bw per day in elderly people at the 95th percentile.

TABLE 3 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**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)	0.001–0.013 (12)	0.005–0.050 (15)	0.001–0.028 (19)	0.001–0.017 (21)	0.005–0.024 (22)	0.003–0.034 (23)
Min–max 95th percentile (number of surveys)	0.003–0.051 (11)	0.024–0.083 (14)	0.003–0.086 (19)	0.003–0.054 (20)	0.023–0.082 (22)	0.015–0.110 (22)

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.

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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; – uncertainty with potential to cause underestimation of exposure.

[50]Technical dossier/p. 64.

[51]Technical dossier/p. 97; Additional data May 2024/Response to EFSA Question 11.

The conservative approach applied to estimate the exposure to the 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 (2229 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.050 mg TOS/kg bw per day at the mean and from 0.003 to 0.110 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 20,264.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme endo-1,3(4)-β-glucanase produced with the non-genetically modified *T. versatilis* strain PF8 does not give rise to safety concerns under the intended conditions of use.

5 | REMARK

The use of this endo-1,3(4)-β-glucanase from the non-genetically modified *T. versatilis* strain PF8 is not considered to raise a safety concern when used in the production of fruit and vegetable juices. However, the Panel noted that according to the Directive 2012/12/EU,[52] the use of endo-1,3(4)-β-glucanase is not permitted in the treatment of fruits for juice production.

6 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier "Application for authorisation of beta-glucanase from *Talaromyces versatilis* in accordance with Regulation (EC) No. 1331/2008". 27th February 2015. Submitted by Erbslöh Geisenheim AG.

Additional information. 2nd May 2024. Submitted by Erbslöh Geisenheim AG.

ABBREVIATIONS

A/G ratio	albumin/globulin ratio
β-glucanase-U	Unit of β-glucanase
bw	body weight
CAS	Chemical Abstracts Service
CHL/IU	Chinese hamster lung fibroblasts cell line
[redacted]	[redacted]
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 FEZ Panel	EFSA Panel on Food Enzymes
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
IgE	Immunoglobulin E
IUBMB	International Union of Biochemistry and Molecular Biology
i.v.	intravenously
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MNBN	bi-nucleated cells with micronuclei
MNPCE	micronucleated polychromatic erythrocytes
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OECD	Organisation for Economic Co-operation and Development

[52]Directive 2012/12/EU of the European Parliament and of the Council of 19 April 2012 amending Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption. OJ L 115, 27.4.2012, p. 1–11. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:115:0001:0011:EN:PDF.

RM	raw material
SPF	specific pathogen free
TOS	total organic solids
WHO	World Health Organization

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CONFLICT 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.

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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.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting 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, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Republic of North Macedonia*, Serbia*, 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, the Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, 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, Montenegro*, the Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion, however, they are included in Appendix B for testing purpose.

[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).