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Safety evaluation of the food enzyme containing endo-polygalacturonase and cellulase from the non-genetically modified *Talaromyces cellulolyticus* strain NITE BP-03478

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, Jaime Aguilera, Magdalena Andryszkiewicz, Yi Liu, Giulio di Piazza, Sandra Rainieri, Rita Ferreira de Sousa and Andrew Chesson

Abstract

The food enzyme containing endo-polygalacturonase ((1–4)-α-D-galacturonan glycanohydrolase; EC 3.2.1.15) and cellulase (4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase; EC 3.2.1.4) activities is produced with the non-genetically modified *Talaromyces cellulolyticus* strain NITE BP-03478 by Meiji Seika Pharma Co., Ltd. It is intended to be used in eight food manufacturing processes: baking processes, brewing processes, fruit and vegetable processing for juice production, wine and wine vinegar production, fruit and vegetable processing for products other than juices, fruit and vegetable processing for refined olive oil production, coffee bean demucilation and grain treatment for starch production. Since residual amounts of total organic solids (TOS) are removed during three food processes (refined olive oil production, coffee bean demucilation and grain treatment for starch production), dietary exposure was not calculated for these food processes. For the remaining five food processes, dietary exposure was estimated to be up to 3.193 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 806 mg TOS/kg bw per day, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 252. A search for the similarity of the amino acid sequences of the food enzyme to known allergens was made and six matches with pollen allergens were found. The Panel considered that, under the intended conditions of use, the risk of allergic reactions by dietary exposure cannot be excluded, especially in individuals sensitised to pollen. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: food enzyme, endo-polygalacturonase, (1-4)-α-D-galacturonan glycanohydrolase, EC 3.2.1.15, and cellulase, 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4

Requestor: European Commission

Question numbers: EFSA-Q-2015-00370 and EFSA-Q-2016-00528

Correspondence: fip@efsa.europa.eu

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

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1. Introduction

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

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No. 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No. 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

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

1.1.1.1. First mandate (EFSA-Q-2015-00370 cellulase)

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

Five applications have been introduced by the companies "Meiji Seika Pharma Co., Ltd." for the authorisation of the food enzyme Cellulase from *Talaromyces cellulolyticus/Talaromyces pinophilus* (strain *Acremonium cellulolyticus*); "Danisco US Inc." for the authorisation of the food enzymes Aspergillopepsin I from a genetically modified strain of *Trichoderma reesei* (strain DP-Nzq40) and Triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33); "Neova Technologies Inc." for the authorisation of the food enzyme Trypsin and Chymotrypsin from porcine pancreatic glands, and "Novozymes A/S." for the authorisation of the food enzyme Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011 implementing Regulation (EC) No 1331/2008[3], 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 11 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, 11.03.2011, pp. 15–24.

1.1.1.2. Second mandate (EFSA-Q-2016-00528 polygalacturonase)

Only food enzymes included in the European 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 Bacillolysin from *Bacillus subtilis* and by the companies "Meiji Seika Pharma Co., Ltd" for the authorisation of the food enzyme Polygalacturonase from *Talaromyces cellulolyticus*/*Talaromyces pinophilus*, "Yakult Pharmaceutical Industry Co., Ltd." for the authorisation of the food enzyme Beta-galactosidase from *Sporobolomyces singularis* (YIT 10047), and "Bioresco Ltd." for the authorisation of the food enzymes Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM703) and Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM6420).

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

1.1.2. Terms of Reference

1.1.2.1. First mandate (EFSA-Q-2015-00370 cellulase)

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Cellulase from *Talaromyces cellulolyticus/Talaromyces pinophilus* (strain *Acremonium cellulolyticus*); Aspergillopepsin I from a genetically modified strain of *Trichoderma reesei* (strain DP- Nzq40), Triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33); Trypsin and Chymotrypsin from porcine pancreatic glands and Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.2.2. Second mandate (EFSA-Q-2016-00528 polygalacturonase)

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Bacillolysin from *Bacillus subtilis*, Polygalacturonase from *Talaromyces cellulolyticus/Talaromyces pinophilus*, Beta-galactosidase from *Sporobolomyces singularis* (YIT 10047), Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM703) and Cyclomaltodextrin glucanotransferase from a genetically modified strain of *E. coli* K12 (WCM105xpCM6420) 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 cellulase (EFSA-Q-2015-00370) and polygalacturonase (EFSA-Q-2016-00528) from *T. cellulolyticus/T. pinophilus* strain NITE BP-03478. The feasibility to combine the assessment of two EFSA question numbers in one single opinion is based on the following clarification from the applicant: (i) the production strain of the cellulase (EFSA-Q-2015-00370) and the polygalacturonase (EFSA-Q-2016-00528) is identical; (ii) the fermentation and purification process are identical for both enzymes; and (iii) toxicity and safety data are the same.[4]

2. Data and methodologies

2.1. Data

The applicant has submitted two dossiers in support of the application for authorisation of the food enzymes cellulase and polygalacturonase from *Talaromyces cellulolyticus* strain NITE BP-03478.

Additional information was requested from the applicant during the assessment process on 3 June 2020, 29 April 2021 and 19 October 2021, and was consequently provided (see 'Documentation provided to EFSA').

[4] EFSA-Q-2015-00370/Technical dossier/Additional data June 2020.

Following the request for additional data sent by EFSA on 3 June 2020, the applicant requested a clarification teleconference, which was held on 22 July 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 guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

The food enzyme contains two declared activities:

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

Endo-polygalacturonases catalyse the random hydrolysis of α–(1–4) glycosidic bonds between galacturonic acid residues in polygalacturonans, resulting in their progressive depolymerisation.

IUBMB nomenclature: Cellulase
Systematic name: 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
Synonyms: carboxymethyl cellulase; β-1-4-glucanase
IUBMB No.: EC 3.2.1.4
CAS No.: 9012-54-8
EINECS No.: 232-734-4

Cellulases catalyse the random hydrolysis of 1–4-β-glycosidic linkages in cellulose and other β-glucans, resulting in the generation of shorter β-D-glucan chains.

The food enzyme under assessment is intended to be used in eight food manufacturing processes: baking processes, brewing processes, fruit and vegetable processing for juice production, wine and wine vinegar production, fruit and vegetable processing for products other than juices, fruit and vegetable processing for refined olive oil production, coffee bean demucilation and grain treatment for starch production.

3.1. Source of the food enzyme

The food enzyme is produced with the non-genetically modified filamentous fungus *Talaromyces cellulolyticus* ACC8105, which is deposited at the National Institute for Technology and Evaluation (NITE) Patent Microorganism Depository (Japan) with the deposit number NITE BP-03478.[5]

T. cellulolyticus ACC8105 is a derivative of the wild-type strain *T. cellulolyticus* Y-94 (CBS 136886, formerly known as *Acremonium cellulolyticus*) obtained by conventional mutagenesis and selection for cellulolytic activity. *T. cellulolyticus* Y-94 was isolated from soil and identified by sequence analysis of the ITS1, ITS2, 5.8 rRNA, RPB1 and β-tubulin genes (Fujii et al., 2014). Its whole genome sequence is available.[6]

3.2. Production of the food enzyme

The production plant is licensed by the Ministry of Health, Labour and Welfare Government of Japan in accordance with provision of Paragraph 1, article 13 of the Pharmaceutical Affairs Law of

[5] EFSA-Q-2015-00370/Technical dossier/Additional data September 2021/Annex AI1.
[6] https://www.ncbi.nlm.nih.gov/bioproject/PRJDB3250.

Japan, which requires implementation of a Hazard Analysis and Critical Control Point plan and is considered equivalent to European standards and legislation.[7]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded. [redacted] is then added to the food enzyme concentrate. Finally, the food enzyme preparation is filtered and spray-dried prior to analysis.[8] 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.[9]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The endo-polygalacturonase is a single polypeptide chain of [redacted] amino acids with a calculated molecular mass of [redacted] kDa.[10] Cellulolytic activity results from the action of an enzyme complex of [redacted] different enzymes with single peptide chains ranging from [redacted] to [redacted] amino acids and molecular masses ranging from [redacted] to [redacted] kDa.[11] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The observed complexity of the protein profiles reflects the fact that the food enzyme is derived from a wild-type fungal strain without protein fractionation.[12] The food enzyme was tested for β-glucosidase, endo-1,4-β-xylanase, α-L-arabinofuranosidase, endo-β-1,4-galactanase and cellulose 1,4-β-cellobiosidase activities, and all were detected. No other enzyme activities were reported.

The in-house determination of endo-polygalacturonase activity is based on the hydrolysis of polygalacturonic acid with a consequent increase in reducing groups (reaction conditions: pH 4.5, 37°C, 30 min). The reaction is stopped and an iodine solution is added. The enzymatic activity is determined by titration with sodium thiosulfate using starch as reagent indicator. The enzyme activity is expressed in Polygalacturonase Units (PU)/g. One PU is defined as the amount of enzyme that generates 1 μmol of galacturonic acid equivalents in 1 h under the assay conditions.[13]

To account for the different activity against soluble and insoluble cellulose, the in-house determination of cellulase activity is performed following two methods:

To measure activity against soluble glucans, the activity is determined based on the random hydrolysis of carboxymethylcellulose, resulting in the generation of free reducing groups (reaction conditions: pH 4.0, 40°C, 30 min). The enzymatic activity is determined spectrophotometrically by measuring the release of reducing carbohydrates, which react with arsenic molybdate producing a colour and quantified relative to a glucose standard. The enzyme activity is expressed in carboxymethyl cellulose units (CMCU)/g. One CMCU corresponds to the amount of enzyme required to generate 1 μmol of glucose equivalents per minute under the conditions of the assay.[14]

For the hydrolysis of the insoluble cellulose, the activity is determined based on the hydrolysis of Avicel, a crystalline cellulose (reaction conditions: 50°C, 30 min). The enzymatic activity is determined spectrophotometrically by measuring the release of reducing carbohydrates, which react with 3,5 dinitrosalicylic acid producing a colour. The activity is expressed in Avicelase units (AU)/g. One AU corresponds to the amount of enzyme required to generate 1 μmol of glucose equivalents per minute under the conditions of the assay.[15]

The endo-polygalacturonase activity has a temperature optimum around 50°C (pH 4.5) and a pH optimum around pH 4.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme

[7] EFSA-Q-2016-00528/Technical dossier/ Annex A23 and EFSA-Q-2015-00370/Technical dossier/Annex A22.
[8] EFSA-Q-2016-00528/Technical dossier/Annex A22 and EFSA-Q-2015-00370/Technical dossier/Annex A21.
[9] EFSA-Q-2015-00370/Technical dossier/ Additional data January 2021/2016-0528/Annex AI2.
[10] EFSA-Q-2016-00528/Technical dossier/Annex A1.
[11] EFSA-Q-2015-00370/Technical dossier/Annex A1.
[12] EFSA-Q-2016-00528/Technical dossier/Annex A3 and EFSA-Q-2015-00370/Technical dossier/Annex A2.
[13] EFSA-Q-2016-00528/Technical dossier/Annex A12.
[14] EFSA-Q-2015-00370/Technical dossier/Annex A10.
[15] EFSA-Q-2015-00370/Technical dossier/Annex A11.

for 10 min at different temperatures at pH 4.5. Endo-polygalacturonase activity decreased above 50°C showing approximately 30% residual activity at 65°C.[16] Cellulase activity showed a temperature optimum around 70°C (pH 4.0) and a pH optimum around pH 4.5 (40°C).[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation[18] (1–3) and two batches (4 and 5) produced for the toxicological tests[19,20] (Table 1). The mean total organic solids (TOS) of the three food enzyme preparation batches is 80.9% and the mean enzyme activity/TOS ratios are 1,004.9 PU/mg TOS, 9.3 CMCU/mg TOS and 1.6 AU/mg TOS.

Table 1: Compositional data of the food enzyme preparation

Parameter	Unit	Batches				
		1	2	3	4[(a)]	5[(b)]
Endo-polygalacturonase activity	PU/g[(c)]	780,000	870,000	790,000	NA[(d)]	NA
Cellulase activity (soluble fraction)	CMCU/g[(e)]	7,210	7,800	7,520	9,470	7,400
Cellulase activity (insoluble fraction)	AU/g[(f)]	1,260	1,320	1,300	1,320	1,210
Protein	%	53.0	54.9	54.5	NA	NA
Ash	%	0.5	0.5	0.6	0.6	0.6
Water	%	1.8	1.9	2.1	2.1	2.1
Diluent ([redacted])	%	16.6	16.6	16.6	16.6	16.7
Total organic solids (TOS)[(g)]	%	81.1	81.0	80.7	80.7	80.6
Endo-polygalacturonase activity/TOS	PU/mg TOS	961.8	1,074.1	978.9	–	–
Cellulase activity (soluble substrate)/TOS	CMCU/mg TOS	8.9	9.6	9.3	11.7	9.2
Cellulase activity (insoluble substrate)/TOS	AU/mg TOS	1.6	1.6	1.6	1.6	1.5

(a): Batch used for the bacterial reverse mutation test.
(b): Batch used for the *in vitro* mammalian chromosomal aberration test and repeated dose 90-day oral toxicity study in rodents.
(c): PU: Polygalacturonase Unit (see Section 3.3.1).
(d): NA: not analysed.
(e): CMCU: Carboxymethyl cellulose Unit (see Section 3.3.1).
(f): AU: Avicelase Unit (see Section 3.3.1).
(g): TOS calculated as 100% − % water − % ash − % diluent.

3.3.3. Purity

The lead content in six commercial batches was below 0.05 mg/kg, 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, arsenic and cadmium were below the limits of quantification (LoQs) of the employed methods.[21,22]

The food enzyme preparation complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[23] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Talaromyces*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of aflatoxin B1, nivalenol, deoxynivalenol, ochratoxin A, sterigmatocystin and zearalenone was examined in three batches of the food enzyme preparation and all were below the limits of detection (LoDs) of the applied analytical methods.[24,25] Adverse effects

[16] EFSA-Q-2016-00528/Technical dossier/Annex 13.
[17] EFSA-Q-2015-00370/Technical dossier/Annex A12.
[18] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annexes AI4.
[19] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI10.
[20] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI7.
[21] EFSA-Q-2016-00528/Technical dossier/Additional data January 2021/Annex AI4-AI5.
[22] LoQ: Pb = 0.05 mg/kg; As = 0.1 mg/kg; Cd = 0.01 mg/kg; Hg = 0.01 mg/kg.
[23] EFSA-Q-2016-00528/Technical dossier/Additional data January 2021/Annex AI5.
[24] EFSA-Q-2016-00528/Technical dossier/Annex A27 and EFSA-Q-2015-00370/Technical dossier/Annex A27.
[25] LoDs: Aflatoxin B1 = 0.001 mg/kg; nivalenol = 0.05 mg/kg; deoxynivalenol = 0.05 mg/kg; ochratoxin A = 0.05 mg/kg; sterigmatocystin = 0.05 mg/kg; zearalenone = 0.05 mg/kg.

caused by the possible presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4. Viable cells of the production strain

The absence of the production strain in the food enzyme was demonstrated [redacted].[26]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided. The batches 4 and 5 (Table 1) used in these studies have similar composition and activity/TOS values as the batches used for commercialisation, and thus are considered suitable as test items.

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 Guidelines 471 and 472 (OECD, 1983a,b) and following Good Laboratory Practice (GLP).[27]

Five strains of *Salmonella* Typhimurium (TA98, TA100, TA1535, TA1537 and TA1538) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. A dose-range finding experiment was performed using six concentrations ranging from 4.88 to 5,000 μg food enzyme/plate, corresponding to 3.94 to 4,035 μg TOS/plate. Two separate main experiments were carried out using five concentrations of the food enzyme (313–5,000 μg food enzyme/plate, corresponding to 252.6, 504.4, 1,009, 2,017.5 and 4,035 μg TOS/mL).

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

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

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out Chinese hamster lung cells according to OECD Test Guideline 473 (OECD, 1997) and following GLP. [28]

A dose-finding study was performed at concentrations ranging from 5 to 5,000 μg/mL, corresponding to 4.03–4,030 μg TOS/mL, and no inhibition of cell growth by 50% or more was observed except for the highest concentration used in the continuous treatment. Based on these results, duplicate cell cultures were exposed to the food enzyme at 1,250, 2,500 and 5,000 μg/mL (corresponding to 1,007.5, 2,015 and 4,030 μg TOS/mL), in a short-term treatment (6 h followed by 18 h recovery period) with and without metabolic activation (S9-mix) and at 650, 1,250, 2,500 and 5,000 μg/mL (corresponding to 503.7, 1,007.5, 2,015 and 4,030 μg TOS/mL) in a continuous treatment (24 h) in the absence of S9-mix.

The relative cell growth rate at 5,000 μg/mL was 84, 89.1 and 57.4% of negative control values with the short-term treatment without S9-mix, with S9-mix and with continuous treatment, respectively. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and were within the range of the laboratory historical solvent control data.

The Panel concluded that food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

[26] EFSA-Q-2016-00528/Technical dossier/Additional data September 2021/Annex AI3.
[27] EFSA-Q-2016-00528/Technical dossier/Annex S1 and EFSA-Q-2015-00370/Technical dossier/Annex S1.
[28] EFSA-Q-2016-00528/Technical dossier/Annex S3 and EFSA-Q-2015-00370/Technical dossier/Annex S3.

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.[29] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses corresponding to 80.6, 241.8 and 806 mg TOS/kg bw per day. Controls received the vehicle (injection water). A recovery control and high-dose groups, each comprising 5 males and 5 females, terminated 4 weeks after the end of treatment, were included in the study.

No mortality was observed.

The feed consumption was statistically significantly decreased in low-dose males at week 2 (−9%) and 3 (−10%). The Panel considered these changes as not toxicologically relevant as they were only recorded sporadically, they were only observed in one sex, there was no dose–response relationship and there were no statistically significant changes in the body weight during the dosing period and in the final body weight.

Statistically significant changes in organ weights included a decrease in epididymis absolute weight in mid-dose males (−10%), an increase in the relative kidney weight in mid- and high-dose females (+8% at both dose levels) and in the relative adrenal weight of low-dose females (+18%). The Panel considered these changes as not toxicologically relevant as they were only observed in one sex (kidneys, adrenals), the changes were small (kidneys), there was no dose–response relationship (epididymis, kidneys, adrenals) and the changes were not accompanied by histopathological changes.

No other statistically significant or biologically relevant differences were reported.

The Panel identified a no observed adverse effect level (NOAEL) of 806 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the endo-polygalacturonase and cellulase produced with the non-genetically modified *T. cellulolyticus* 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, six matches were found. The matching allergens were all polygalacturonases: Jun a 2, produced by *Juniper ashei* (mountain cedar), Pla a 2 produced by *Platanus ascerifolia* (London plane tree), Phl p 13 produced by *Phleum pretenese* (timothy grass), Cha o 2 produced by *Chamaecyparis obtuse* (Japanase cypress), Cry j 2 produced by *Crytomeria japonica* (Japanese cedar) and Pla or 2 produced by *Platanus orientalis* (oriental plane), all known as pollen allergens.[30]

All matches found were respiratory allergens. Respiratory allergy following occupational inhalation of cellulase have been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma caused by an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Armentia et al., 2009). This can be, however, different in the case of pollen. Due to cross-reactivity of certain vegetable food allergens with pollen, pollen-sensitised individuals may develop allergic reactions after ingestion of these cross-reactive vegetable foods. Individuals suffering from this so-called Pollen Food Allergic Syndrome experience mild effects in and around their moth after ingestion of the food. The allergens that match with the food enzyme that is subject of this evaluation were all polygalacturonases, and these allergens are found to be linked to the occurrence of this syndrome (Mastrorilli et al., 2019). In addition, polygalacturonase from papaya has been identified as a food allergen (Sarkar et al., 2018), but the prevalence of papaya food allergy is low and there was no sequence homology with allergens identified in papaya.

No information was available on oral sensitisation or elicitation reactions of this food enzyme. Information on adverse reactions upon ingestion of other cellulases in individuals sensitised through the respiratory route has not been reported.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, especially in individuals sensitised to pollen.

[29] EFSA-Q-2015-00370/Technical dossier/Annex S6_RJ13500.
[30] EFSA-Q-2015-00370/Technical dossier/Annexes F32-F46.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in eight food processes. Intended uses and the recommended use levels are summarised in Table 2.

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

Food manufacturing process[(a)]		Raw material (RM)	Recommended dosage of the food enzyme (mg TOS/kg RM)[(b)]
Baking process		Flour	161.6–**258.56**
Brewing process		Cereals	0.0808–**6.06**
Fruit and vegetable processing for juice production		Fruits and vegetables	0.1616–**16.16**
Wine and wine vinegar production		Grapes	0.1616–**16.16**
Fruit and vegetable processing for products other than juices	To produce puree	Fruits and vegetables	0.1616–**16.16**
	To peel	Oranges with peel	161.6–4,040
	To peel	Peaches with peel and stone	161.6–2020
Fruit and vegetable processing for refined olive oil production		Olive paste after milling	32.32–80.8
Coffee bean demucilation		Coffee cherries	80.8
Grain treatment for starch production (corn only)		Corn kernel	80.8

TOS: total organic solids.
(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold are used for calculations.
(c): EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI35.

In all the intended food manufacturing processes, the food enzyme hydrolyses cell wall components (glucans and polygalacturonans) in plant raw materials. The disruption of the structure reduces the viscosity, thus improving the pressing ability, releasing cellular components of sensory properties.

In baking processes, the food enzyme is added to flour during dough preparation.[31] The food enzyme–TOS remains in the dough.

In brewing processes, the food enzyme is added to malt during saccharification.[32] The food enzyme–TOS remains in the beer.

In fruit and vegetable juice production, the food enzyme is added to the mash during mash treatment and again added to the raw juice during depectinisation.[33] The food enzyme–TOS remains in the juice.

For wine and wine vinegar making, the food enzyme is added to grape during mashing.[34] The food enzyme–TOS remains in the wine and wine vinegar.

For the production of other fruit and vegetable products such as puree, the food enzyme is added to the mash during mash treatment. The food enzyme–TOS remains in the puree. Whole fruits (e.g. peach) or peeled fruit (e.g. orange) can be soaked in the food enzyme solution to facilitate the removal of soft skins.[35] Followed by washing with water, those fruits can be made into many different types of products (e.g. canned or dried).[36] Although most of the food enzyme remains in the washing water, the applicant did not quantify the extent of TOS removal in the final fruits products. Therefore,

[31] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI36.
[32] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI38.
[33] EFSA-Q-2015-00370/Technical dossier/Annex A24.
[34] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI39.
[35] EFSA-Q-2015-00370/Technical dossier/Additional data January 2021/2016-0528/Annex AI40.
[36] EFSA-Q-2015-00370/Technical dossier/EFSA-Q-2016-00528/Annex 40.

all food groups covered under the 'fruit and vegetable processing for products other than juices' are included in the exposure calculation, using the use level given for puree production.

In olive oil production, the food enzyme is added to the olive fruits during crushing and milling.[37] Chiefly the polygalacturonase activity is used to hydrolyse pectin present in the cell walls, facilitating the release of oil retained in the cells, and thus increasing extraction yield.

The term 'olive oil' is defined in the Regulation (EU) No 1308/2013[38] as 'composed of refined olive oils and virgin olive oils'. The term 'virgin olive oils' means 'oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action, or by re-esterification process and any mixture with oils of other kinds'.

In accordance with the law, the use of enzymes is not permitted in the production of virgin olive oils in the European Union. Therefore, this assessment is limited to the use of this food enzyme in the production of refined olive oil only. The food enzyme–TOS is removed from the refined olive oil by repeated washing during the refinement process (EFSA CEP Panel, 2021b).

In coffee processing, the food enzyme is added to green coffee berry during pulping and fermentation to degrade the mucilage.[39] The food enzyme–TOS is removed from the coffee bean by repeated washing (EFSA CEP Panel, 2021b).

To produce corn starch, maize kernels are treated with the food enzyme during steeping.[40] The food enzyme–TOS is removed from the corn starch by repeated washing (EFSA CEP Panel, 2021b).

Based on data provided on thermostability (see Section 3.3.1), the cellulase and endo-polygalacturonase would be inactivated by heat in most of the food processes, but may remain active in wine, wine vinegar, and juices, depending on the pasteurisation conditions.

3.5.2. Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021a), a dietary exposure was calculated only for the remaining food manufacturing processes where the food enzyme–TOS remains in the final foods: baking process, brewing process, fruit and vegetable processing for juice production, wine and wine vinegar production and fruit and vegetable processing for products other than juices.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 43 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 3.193 mg TOS/kg bw per day in infants at the 95th percentile.

[37] EFSA-Q-2015-00370/Technical dossier/Additional data March 2022/Annex AI6.
[38] Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007.
[39] Technical dossier EFSA-Q-2016-00528/Annex A42.
[40] Technical dossier EFSA-Q-2016-00528/Annex A44.

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	Toddlers	Children	Adolescents	Adults	The elderly
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.086–0.897 (12)	0.629–1.897 (15)	0.731–1.687 (19)	0.392–0.971 (21)	0.288–0.633 (22)	0.288–0.594 (23)
Min–max 95th percentile (number of surveys)	0.395–3.193 (11)	1.509–2.887 (14)	1.439–2.981 (19)	0.861–1.954 (20)	0.629–1.270 (22)	0.569–1.004 (22)

TOS: total organic solid.

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	
Exposure to food enzyme–TOS was calculated based on the recommended maximum use level	+
A lower use level was used to calculate dietary exposure in fruit and vegetable products other than juices	–
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of three processes from the exposure assessment: – Olive oil production, – Coffee bean demucilation, – Grain treatment for starch production	–

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

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

The exclusion of three food manufacturing processes from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (806 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.086–1.897 mg TOS/kg bw per day at the mean and from 0.395 to 3.193 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 252.

4. Conclusions

Based on the data provided, the removal of TOS during refined olive oil production, coffee bean demucilation and grain treatment for starch production, and the derived margin of exposure for the remaining food manufacturing processes, the Panel concluded that the food enzyme containing endo-polygalacturonase and cellulase produced with the non-genetically modified *Talaromyces cellulolyticus* strain NITE BP-03478 does not give rise to safety concerns under the intended conditions of use.

5. Remark

This food enzyme contains cellulase activity. One of the intended uses declared by the applicant is the manufacturing of fruit juice. The Panel notes that cellulase is not among the enzymes authorised in fruit juices according to the EU legislation.[41]

In accordance with Regulation (EU) No 1308/2013, the use of this food enzyme in producing virgin olive oils is excluded in this evaluation.

Documentation provided to EFSA

1) Application for the Authorisation of the food enzyme Cellulase derived from *Acremonium cellulolyticus*. May 2015. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
2) Application for the Authorisation of the food enzyme Pectinase derived from *Acremonium cellulolyticus*. April 2016. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
3) Additional data. June 2020. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
4) Clarification data. September 2020. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
5) Additional data. January 2021. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
6) Additional data. September 2021. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.
7) Additional data. March 2022. Submitted by RDA Scientific Consultants GmbH on behalf of Meiji Seika Pharma Co., Ltd.

References

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.

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

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? Allergy, 52, 346–349.

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), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

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,

[41] 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.

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

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and, Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmentl Medicine 60, 802–804.

FAO/WHO (Food and Agriculture Organization of the United Nations/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, 63–67. Available online: http://www.fao.org/3/a-a0675e.pdf

Fujii T, Hoshino T, Inoue H and Yano S, 2014. Taxonomic revision of the cellulose-degrading fungus *Acremonium cellulolyticus* nomen nudum to *Talaromyces* based on phylogenetic analysis. FEMS Microbiology Letters, 351, 32–41.

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.en-75

Mastrorilli C, Cardinale F, Giannetti A and Caffarelli C, 2019. Pollen-food allergy syndrome: a not so rare disease in childhood. Medicina, 55(10), 641.

OECD (Organisation for Economic Co-Operation and Development), 1983a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Genetic Toxicology: Salmonella typhimurium, Reverse Mutation Assay. Available online: https://www.oecd.org/env/ehs/testing/45125466.pdf

OECD (Organisation for Economic Co-Operation and Development), 1983b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 472: Genetic Toxicology: Escherichia coli, Reverse Mutation Assay. Available online: https://www.oecd.org/env/ehs/testing/45125467.pdf

OECD (Organisation for Economic Co-Operation and Development), 1997. 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

Sarkar MB, Sircar G, Ghosh N, Das AK, Jana K, Dasgupta A and Bhattacharya SG, 2018. Cari p 1, a novel polygalacturonase allergen from papaya acting as respiratory and food sensitizer. Frontiers in Plant Science, 9, 823.

Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
LoQ	limit of quantification
OECD	Organisation for Economic Cooperation and Development
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.2023.7840#support-information-section).

The file contains two sheets, corresponding to two tables.

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

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).