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Safety evaluation of a food enzyme containing endo-polygalacturonase and pectin lyase activities from the non-genetically modified *Aspergillus tubingensis* strain NZYM-PE

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, Yrjö Roos, Magdalena Andryszkiewicz, Ana Criado, Yi Liu, Simone Lunardi, Francesco Pesce and Andrew Chesson

Abstract

The food enzyme with the declared activities endo-polygalacturonase ((1–4)-α-D-galacturonan glycanohydrolase; EC 3.2.1.15) and pectin lyase ((1–4)-6-O-methyl-α-D-galacturonan lyase; EC 4.2.2.10) is produced with the non-genetically modified *Aspergillus tubingensis* strain NZYM-PE by Novozymes A/S. It is intended to be used in four food manufacturing processes: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices, refined olive oil production and wine and wine vinegar production. Since residual amounts of total organic solids (TOS) are removed during production, dietary exposure was not calculated for refined olive oil production. For the remaining three food processes, it was estimated to be up to 0.132 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 (NOAEL) of 1,430 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure above 10,833. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and 13 matches were found, including one food allergen (papaya). The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, in particular for individuals sensitised to papaya, but that the risk will not exceed that of consumption of papaya. In addition, oral allergy reactions cannot be excluded in pollen-sensitised individuals. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

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Correspondence: fip@efsa.europa.eu

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

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Table of contents

1. Introduction

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

'Food enzyme' means a product obtained from plants, animals or 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 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, 2009a) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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 on food enzymes.

Five applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP), and by the companies "DSM Food Specialties B.V" and "Novozymes A/S" for the authorisation of the food enzymes Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A_2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-B-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO), respectively.

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

[1] 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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger*, Phospholipase A_2 from a genetically modified strain of *Aspergillus niger* (strain PLA), Pectinesterase from a genetically modified strain of *Aspergillus niger* (strain PME), Endo-1,4-B-xylanase from a genetically modified strain of *Aspergillus niger* (strain XEA) and Maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (strain NZYM-SO) 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 the food enzyme Pectinase, Poly-galacturonase, Pectin esterase, Pectin lyase and Arabanase from *Aspergillus niger* submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2015-00038-42. During a meeting between EFSA, the European Commission and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP),[5] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2015-00038-42. This data package, identified as EFSA-Q-2022-00543, concerns a food enzyme containing polygalacturonase and pectin lyase activities that is produced with *Aspergillus tubingensis* strain NZYM-PE and submitted by Novozymes A/S.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme containing endo-polygalacturonase and pectin lyase activities from the non-genetically modified *Aspergillus tubingensis* strain NZYM-PE.

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 current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021a) has been followed for the evaluation of the application.

3. Assessment

The food enzyme under application 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.

[4] Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevanceOJ L 168, 28.6.2012, pp. 21–23.

[5] The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes

IUBMB nomenclature	Pectin lyase
Systematic name	(1–4)-6-*O*-methyl-α-D-galacturonan lyase
Synonyms	Pectin trans-eliminase, polymethylgalacturonic transeliminase, pectin methyltranseliminase
IUBMB No	EC 4.2.2.10
CAS No	9033-35-6
EINECS No	232-894-5

Pectin lyases catalyse a β-eliminative cleavage of 1,4-α-D-galactosiduronic linkages in galacturonans to produce oligosaccharides with 4-deoxy-6-*O*-methyl-α-D-galact-4-enuronosyl groups at their non-reducing ends.

The enzyme under assessment is intended to be used in four food manufacturing processes: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices, refined olive oil production and wine and wine vinegar production.

3.1. Source of the food enzyme

The food enzyme containing endo-polygalacturonase and pectin lyase activities is produced with the non-genetically modified filamentous fungus *Aspergillus tubingensis* strain NZYM-PE (Ap18), which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with the deposit number [redacted][6] The production strain was identified as *A. tubingensis* by [redacted][7]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [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.[10] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[11]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The food enzyme contains [redacted] endo-polygalacturonases of [redacted] amino acids and a pectin lyase of [redacted] amino acids.[12] The molecular masses of the mature proteins, calculated from the amino acid sequences, are [redacted] kDa, respectively.[12] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[13] A consistent protein pattern was observed across all batches. The gel showed protein bands consistent with the expected masses of the enzymes, together with other bands of lesser staining intensity. The food enzyme was tested for

[6] Technical dossier/Annex 7.
[7] Technical dossier/Annex 6.
[8] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[9] Technical dossier/p. 47/Annex 8.
[10] Technical dossier/pp. 47–50.
[11] Technical dossier/p. 50, p. 52/Annex 9.
[12] Technical dossier/pp. 32-33/Annex 1.
[13] Technical dossier/p. 35.

α-amylase, lipase and protease activities.[14] Only α-amylase activity was detected. No other enzymatic activities were reported.

The in-house determination of endo-polygalacturonase activity is based on the hydrolysis of polygalacturonic acid with a consequent release of reducing groups determined using a colorimetric assay (reaction conditions: pH 4.5, 50°C, 5 min). Enzyme activity is expressed as polygalacturonase units (PGNU)/g. One PGNU is defined as the amount of enzyme producing reducing groups equivalent to 1 mg of galacturonic acid under the conditions of the assay.[15]

The in-house determination of pectin lyase activity is based on the cleavage of pectin (reaction conditions: pH 5.5, 45°C, 10 min). The enzymatic activity is determined by measuring the release of unsaturated Δ-4,5 polygalacturonides, which are determined spectrophotometrically at 235 nm. Enzyme activity is expressed in PECtin Transeliminase Units (PN)/g (PECTU(PN)/g). One PECTU(PN) is defined as the quantity of the enzyme releasing 1 μmol of unsaturated Δ-4,5 polygalacturonides per minute under the conditions of the assay.[16]

The optimum temperature is around 60°C (pH 4.0) for the endo-polygalacturonase and around 40°C (pH 4.0) for pectin lyase activities. The optimum pH is around pH 4.0 (37°C) for endo-polygalacturonase and around pH 4.0 (37°C) for pectin lyase activities. Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.0). Both activities decreased at temperatures greater than 70°C, with no residual activity detected at 80°C.[17]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation, among which batch 1 was used for the genotoxicity tests (Table 1). In addition, batch 4 was produced for the 90-day toxicity study.[18] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 14% and the mean enzyme activity/TOS ratio was 261.6 PGNU(PE)/mg TOS for the endo-polygalacturonase and 94.7 PECTU(PN)/mg TOS for the pectin lyase, respectively.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1[(a)]	2	3	4[(b)]
Endo-polygalacturonase activity	PGNU(PE)/g[(c)]	33,800	32,800	43,400	11,880
Pectin lyase activity	PECTU(PN)/g[(d)]	15,000	14,000	10,200	NA
Protein	%	11.2	11.1	10.6	6.1
Ash	%	0.9	0.6	0.8	8.4
Water	%	84.7	87.0	84.0	77.3
Total organic solids (TOS)[(e)]	%	14.4	12.4	15.2	14.3
Endo-polygalacturonase activity/TOS	PGNU(PE)/mg TOS	234.7	264.5	285.5	83.1
Pectin lyase activity/TOS	PECTU(PN)/mg TOS	104.2	112.9	67.1	NA[(f)]

(a): Batch used for Ames and *in vitro* micronucleus tests.
(b): Batch used for repeated oral toxicity study in rats.
(c): PGNU(PE)/g: see Section 3.3.1.
(d): PECTU(PN)/g: see Section 3.3.1.
(e): TOS calculated as 100% – % water – % ash.
(f): NA: not analysed.

3.3.3. Purity

The lead content in all batches was below 0.5 mg/kg,[19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

[14] Technical dossier/pp. 42-43/Annex 3.03, Annex 3.04, Annex 3.05.
[15] Technical dossier/pp. 37-39/Annex 3.01.
[16] Technical dossier/pp. 37-39/Annex 3.02.
[17] Technical dossier/pp. 41-42/Annex 5.01, Annex 5.02.
[18] Technical dossier/p. 33/Annexes 2.01–2.03, Annex 3.01, Annex 3.02, Annex 4.
[19] Technical dossier/p. 35/Annex 2.04, Annex 4.

In addition, arsenic, mercury and cadmium contents were below the limits of detection (LoD) of the employed methods.[19,20]

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).[21] No antimicrobial activity was detected in any of the tested batches.[22]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The applicant did not provide information on the potential secondary metabolites produced under the conditions of fermentation which might contribute to the food enzyme-TOS. This issue was 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

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.[23]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian micronucleus test and a repeated dose 90-day oral toxicity study in rats, were provided.

The batch 1 (Table 1) used in genotoxicity studies was one of the batches intended for commercialisation and was considered suitable as a test item. In addition, batch 4 (Table 1), used for the repeated dose 90-day study, had a lower activity/TOS value compared to the batches used for commercialisation and was also considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. 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, 2020) and following good laboratory practice (GLP).[24]

Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2*uvr*A(pKM101) were used with or without metabolic activation (S9-mix), applying the treat and wash assay. A dose-range finding study and two experiments were carried out in triplicate.

The first experiment was performed with TA1535 and WP2*uvr*A strains using seven concentrations of the food enzyme ranging from 5.4 to 5,000 μg TOS/plate, and in TA1537, TA98 and TA100 strains using five concentrations from 52 to 5,000 μg TOS/plate. The second experiment was carried out in all five tester strains, using five concentrations ranging from 492 to 5,000 μg TOS/plate. No cytotoxicity was observed at any concentration. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values, in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme containing polygalacturonase and pectin lyase activities did not induce gene mutations under the test conditions applied in this study.

3.4.1.2. *In vitro* mammalian cell micronucleus test

The *in vitro* mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.[25]

[20] LoDs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Hg = 0.03 mg/kg; Cd = 0.05 mg/kg.
[21] Technical dossier/p. 36/Annexes 2.06–2.09, Annex 4.
[22] Technical dossier/p. 35/Annex 2.05, Annex 4.
[23] Technical dossier/Annex 2.10.
[24] Technical dossier/Annex 10.01.
[25] Technical report/Annex 10.02.

A dose-range finding study and two separate experiments were 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 the first experiment, cells were exposed to the food enzyme at nine concentrations ranging from 200 to 5,000 μg TOS/mL in a short-term treatment (3 h exposure and 27 h recovery period) either with or without S9-mix and scored for the frequency of binucleated cells with micronuclei (MNBN) at 200, 2,500 and 5,000 μg TOS/mL. In the second experiment, cells were exposed to the food enzyme at nine concentrations ranging from 1 to 150 μg TOS/mL in a long-term treatment (24 h exposure and 24 h recovery period) without S9-mix, and scored for MNBN at concentrations of 1, 50 and 100 μg TOS/mL.

No cytotoxicity was seen either in the short-term 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 any concentrations tested.

The Panel concluded that the food enzyme containing polygalacturonase and pectin lyase activities did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study was performed following GLP[26] and in accordance with OECD Test Guideline 408 (OECD, 1981) with the following deviations: serum ornithine decarboxylase (ODC) was not measured and microscopic examination of lungs at low and intermediate doses was not performed. The Panel considered that these deviations are minor and do not impact the evaluation of the study.

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 14.3, 143 or 1,430 mg TOS/kg bw per day. Controls received the vehicle (purified water obtained by reverse osmosis).

One high-dose male died following blood sampling procedures on day 87 (week 13). The Panel considered the death as incidental based on its isolated occurrence and lack of test article-related microscopic findings in this, or any other rat, in the study.

Salivation after dosing increased from day 1 throughout the study duration in high-dose males and females, reaching 8/10 males and 5/10 females on day 78. The Panel considered this change test item-related, possibly associated with the organoleptic properties of the food enzyme and/or with the gavage procedure. However, it was not regarded as adverse based on the transient presentation and the lack of effect on the overall health status.

Haematological investigations revealed a statistically significant decrease in neutrophils (NEU) in high-dose males (−39%), in monocytes (MONO) in low- and mid-dose males (−33% in both cases) and in large unstained cells (LUC) in low-dose males (−50%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all), there was no dose–response relationship (MONO and LUC), there were no changes in other relevant parameters (other white blood cell parameters) and there were no histopathological changes in lymphohematopoietic tissues or organs.

Clinical chemistry investigations revealed statistically significant changes in alanine aminotransferase (ALT), with increase in low-dose males (+16%) and decrease in high-dose males and females (−11% and − 35%, respectively), decrease in aspartate aminotransferase (AST) in mid- and high-dose females (−19% and − 26%, respectively), decrease in total proteins (TP) in high-dose males (−6%), decrease in albumin (ALB) in mid-dose females (−8%), decrease in α-2-globulin in low-dose females (−20%), decrease in γ-globulin in mid-dose males (−33%), decrease in albumin/globulin ratio in low-dose males (−11%), changes in sodium (Na), with decrease in high-dose males and mid-dose females (−0.7% and − 1%, respectively) and increase in low-dose females (+0.7%), decrease in potassium (K) in low-dose females (−9%), increase in chloride (Cl) in mid-dose males (+2%) and decrease in mid-dose females (−1%), decrease in calcium (Ca) in mid- and high-dose males (−4% and −3%, respectively) and in females at all doses (−4%, −5% and − 3%, respectively) and decrease in phosphorus (Phos) in high-dose males (−20%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (AST, TP, ALB, alpha-2-globulin, gamma-globulin, albumin/globulin, K), there was no consistency between the changes in males and females (ALT), there was no dose–response relationship (ALT, ALB, alpha-2-globulin, gamma-globulin, albumin/

[26] Technical dossier/Annex 10.03.

globulin, all electrolytes) and there were no histopathological changes in the liver (ALT, AST and proteins).

Statistically significant changes in organ weights included increase in absolute kidney weight in high-dose males and females (+9% and +13%, respectively), and relative testes weight in mid- and high-dose males (+19% and +33%, respectively). The Panel considered increase in kidney weight as not toxicologically relevant, as the changes were small, they were within the historical control values and did not correlate with histopathological findings (males).

The microscopic examination revealed increased mineralisation in the kidneys from females at mid- and high dose compared with controls; the finding was dose-related in terms of incidence, only reaching statistical significance at the high dose and it was localised in the corticomedullary junction (1/10 rats affected in control and low-dose groups, 4/10 in mid-dose and 10/10 in high-dose) and the medulla (1/10 in controls, 2/10 in mid-dose and 9/10 in high-dose). The Panel noted that renal mineralisation is frequently found along the corticomedullary junction in rats, particularly in females, as a background finding and is regarded as of no clinical consequence (Ritskes-Hoitinga and Beynen, 1992; Frazier et al., 2012). In this study, considering the dose relationship observed, an effect of the test item could not be ruled out and the Panel considered the increased incidence of renal mineralisation observed in females a test item exacerbation of a background change. However, the Panel did not regard microscopic renal changes as adverse taking into consideration that: (i) there was no evidence of associated degenerative changes in the kidneys, (ii) there was no concurrent alteration in serum biomarkers of renal function (i.e. urea and creatinine), (iii) there was no correlated increase in serum Ca, while the minimal decrease in serum Ca observed was regarded as incidental and of no toxicological relevance.

In addition, in the testes from the control group, an unusual high incidence of degeneration of the germinal epithelium was observed in the seminiferous tubules not seen in the high-dose group. Degenerative changes in the control group were variably associated with interstitial cell hyperplasia (observed in 2/10 rats), reduced sperm content in the epididymides (in 3/10) and accounted for the flaccidity observed macroscopically in one male, and for the lower mean weight of testes in control group when compared to historical control data.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1,430 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the endo-polygalacturonases and pectin lyase produced from the non-genetically modified *A. tubingensis* strain NZYM-PE was assessed by comparing its amino acid sequences 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, no match was found for the pectin lyase, but 13 matches were found for the two endo-polygalacturonases. Twelve of them were with pollen allergens: Sor h 13.0101 from *Sorghum halepense* (Johnson grass), Pla or 2.0101 from *Platanus orientalis* (oriental plane tree), Cry j 2.0101 from *Cryptomeria japonica* (Japanese cedar), Pla a 2.0101 from *Platanus acerifolia* (London plane tree), Cha o 2.0101 from *Chamaecyparis obtusa* (Japanese cypress), Jun a 2.0101 from *Juniperus ashei* (mountain cedar), Phl p 13.0101 from *Phleum pratense* (thimothy), Zea m 13 from *Zea mays* (maize), gi|338930674 from *Paspalum notatum* (bahiagrass), Sal k 6.01, partial (gi|589912883) from *Salsola kali* (prickly saltwort), gi|73913442 from *Lilium longiflorum* (trumpet lily) and Ole e 14.0101 from *Olea europaea* (olive tree). The remaining match was with the food allergen Cari p 1.0101 from *Carica papaya* (papaya).

No information is available on oral and respiratory sensitisation or elicitation reactions of these endo-polygalacturonase and pectin lyase enzymes.

Endo-polygalacturonases are allergens often present in grass and tree pollen. The oral allergy syndrome, i.e. allergic reactions mainly in the mouth, is associated with sensitisation to pollen such as from cedar trees (Midoro-Horiuti et al., 2003) and grasses. Such reactions are seldomly leading to severe systemic anaphylaxis.

Cari p 1 (Cari p 1.0101) is the main allergen present in *C. papaya*, described as both a food and a respiratory allergen (Sarkar et al., 2018). Several studies reported occupational rhinitis and asthma in workers of industries where papain is handled (Baur and Fruhmann, 1979; Baur et al., 1982; Niinimaki et al., 1993; Soto-Mera et al., 2000; Van Kampen et al., 2005). In other studies, allergy to papaya-derived products unrelated to occupational exposure has also been described. Garcia-Ortega et al. (1991) showed that administration of chymopapain for chemonucleolysis resulted in sensitisation in some patients. Mansfield and Bowers (1983) reported severe systemic allergic reactions mediated by papain-specific IgE in some individuals that ingested papain-containing meat tenderiser. Sensitisation to papaya does not typically occur from eating papaya fruit. However, once sensitised, individuals may suffer allergic reactions following any type of exposure to papaya or papaya-derived products (Morton, 1987).

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[27]), is used as raw material. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from this source are present in the food enzyme.

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

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in four food processes at the recommended use levels summarised in Table 2.[28]

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

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Fruit and vegetable processing for juice production	Fruit and vegetable	0.2–**3**
Fruit and vegetable processing for products other than juices	Fruit and vegetable	0.3–**5**
Refined olive oil production	Olives	2–8
Wine and wine vinegar production	Grapes	0.2–**2**

(a): The name has been harmonised 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 were used for calculation.

In fruit and vegetable processing for juice production, the food enzyme is added to fruits or vegetables during mash treatment and to the pomace during the second mash treatment. It can also be added to the raw juice during depectinisation.[29] The endo-polygalacturonase degrades galacturonans in the cell wall, improving the processability, resulting in increased yield and avoidance of haziness.[30] The food enzyme-TOS remains in the juices.

In fruit and vegetable processing for products other than juices, the food enzyme is added to fruits or vegetables during the maceration step, where it catalyses the breakdown of pectins,[31] improving processability. The food enzyme-TOS remains in the final products.

[27] 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.
[28] Technical dossier/p. 56.
[29] Technical dossier/p. 78.
[30] Technical dossier/p. 55.
[31] Technical dossier/p. 79.

In refined olive oil production, the food enzyme is added to the olive paste during the malaxation step. The food enzyme catalyses the breakdown of pectins in the cell wall, facilitating the release of oil from vacuoles and thus increasing the yield.[32]

The term 'olive oil' is defined in the Regulation (EU) No 1308/2013[33] 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).

The production processes of olive oil and palm oil are very similar. The applicant provided a theoretical calculation, showing that > 99.96% of the food enzyme-TOS could be removed in olive oil.[34] The residual amounts of enzyme in crude palm oil were below the LoD of the method.[35,36] Although equivalent analytical data were not available for olive oil, the Panel considered that only negligible amounts of enzyme TOS (< 1%) would remain in refined olive oils.

In wine and wine vinegar production, the food enzyme is added during the crushing and maceration, the clarification, and before the ageing and filtration steps.[37] It is used to improve processability, to increase yield and to improve clarification.[28] The food enzyme-TOS remains in the final products.

Based on data provided on thermostability (see Section 3.3.1), the food enzyme is expected to be inactivated by heat in fruit and vegetable processing for products other than juices, but may remain active in wine and wine vinegar, and in 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 food manufacturing processes where the food enzyme-TOS remains in the final foods: fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices and wine and wine vinegar production.

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

Table 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. The highest dietary exposure was estimated to be about 0.132 mg TOS/kg bw per day in toddlers and children at the 95th percentile.

[32] Technical dossier/pp. 80–81.
[33] 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. OJ L 347, 20.12.2013, p. 671–854.
[34] Technical dossier/p.82.
[35] Technical dossier/Annexes 12.01 and 12.02.
[36] LoD = 10 ng/mL.
[37] Technical dossier/p. 84.

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)	0.005–0.068 (12)	0.016–0.092 (15)	0.008–0.055 (19)	0.003–0.030 (21)	0.004–0.020 (22)	0.002–0.018 (23)
Min–max 95th (number of surveys)	0.016–0.131 (11)	0.059–0.132 (14)	0.028–0.132 (19)	0.012–0.088 (20)	0.014–0.065 (22)	0.011–0.045 (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	
Exposure to food enzyme-TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of one process from the exposure assessment: - Refined olive oil production	–

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

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 overestimation of the exposure.

The exclusion of one food manufacturing process from the exposure assessment was based on > 99% of TOS removal. This is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (1,430 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.002–0.092 mg TOS/kg bw per day at the mean and from 0.011 to 0.132 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MOE) of at least 10,833.

4. Conclusions

Based on the data provided, the removal of TOS during refined olive oil production and the derived margin of exposure for the remaining food manufacturing processes, the Panel concluded that the food enzyme containing endo-polygalacturonase and pectin lyase activities produced with the non-genetically modified *Aspergillus tubingensis* strain NZYM-PE does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Dossier "Polygalacturonase and pectin lyase produced by *Aspergillus tubingensis* (strain NZYM-PE)". August 2022. Submitted by Novozymes A/S.

References

Baur X and Fruhmann G, 1979. Papain-induced asthma: diagnosis by skin test, RAST, and bronchial provocation test. Clinical Allergy, 9, 75–81.

Baur X, Konig G, Bencze K and Fruhmann G, 1982. Clinical symptoms and results of skin test, RAST, and bronchialprovocation test in thirty-three papain workers: evidence for strong immunogenic potency and clinically relevant "Proteolytic Effects of Airborne Papain". Clinical Allergy, 12, 9–17.

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

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

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

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

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

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

FAO/WHO (Food and Agriculture Organization of the United 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

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawas A, Durchfeld-Meyer B and Bube A, 2012. Proliferative and non proliferative lesions of the rat and mouse urinary system. Toxicologic Pathology, 40, 14S–86S.

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

Garcia-Ortega P, Ramirez Ferreiras W, Sancho A, Urias S and Cistero A, 1991. Sensitisation to chymopapain in patients treated with chemonucleosysis. Medicina Clínica, 96, 410–412.

Mansfield LE and Bowers CH, 1983. Systemic reaction to papain in a non-occupational setting. The Journal of Allergy and Clinical Immunology, 71, 371–374.

Midoro-Horiuti T, Mathura V, Schein CH, Braun W, Yu S, Watanabe M, Lee JC, Brooks EG and Goldblum RM, 2003. Major linear IgE epitopes of mountain cedar pollen allergen Jun a 1 map to the pectate lyase catalytic site. Molecular Immunology, 40, 555–562. https://doi.org/10.1016/s0161-5890(03)00168-8

Morton JF, 1987. In: F Julia (ed). Papaya. Morton (publisher) Fruits of Warm Climates, Miami, Florida. pp. 336–346. Available on the website of the New Crop Resource Online Programme, Purdue University, http://www.hort.purdue.edu/newcrop/morton/papaya_ars.html

Niinimaki A, Reijula K, Pirila T and Koistinen AM, 1993. Papain-induced allergic rhinoconjunctivitis in acosmetologist. The Journal of Allergy and Clinical Immunology, 92, 492–449.

OECD (Organisation for Economic Co-Operation and Development), 1981. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 12 May 1981. 10 pp. Available online: https://www.oecd.org/env/ehs/testing/45123549.pdf

OECD (Organisation for Economic Co-Operation and Development), 2016. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 487: In vitro mammalian cell micronucleus test. 29 July 2016. 29 pp. Available online: https://www.oecd-ilibrary.org/docserver/9789264264861-en.pdf?expires=1660741037&id=id&accname=guest&checksum=AF31053300A9B34FFB06238A4AE35598

OECD (Organisation for Economic Co-Operation and Development), 2020. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 29 June 2020. 11 pp. Available online: https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1660741244&id=id&accname=guest&checksum=913CB0A044880B3F24CBF2A547CF413C

Ritskes-Hoitinga J and Beynen AC, 1992. Nephrocalcinosis in the rat: a literature review. Progress in Food & Nutrition Science, 16, 85–124.

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. https://doi.org/10.3389/fpls.2018.00823

Soto-Mera MT, Lopez-Rico MR, Filgueira JF, Vilamil E and Cidras R, 2000. Occupational allergy to papain. Allergy, 55, 983–984.

Van Kampen V, Merget R and Bruning T, 2005. Occupational allergies to papain. Pneumologie, 59, 405–410.

Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
GLP	good laboratory practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LOD	limit of detection
MOE	margin of exposure
NOAEL	no observed adverse effect level
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

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 one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, 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, 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, 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*, 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*, 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*, 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).