Adopted: 19 September 2024

DOI: 10.2903/j.efsa.2024.9038

SCIENTIFIC OPINION

efsa JOURNAL

Safety evaluation of the food enzyme carboxypeptidase C from the genetically modified *Aspergillus niger* strain PEG

EFSA Panel on Food Enzymes (FEZ) | Holger Zorn | José Manuel Barat Baviera | Claudia Bolognesi | Francesco Catania | Gabriele Gadermaier | Ralf Greiner | Baltasar Mayo | Alicja Mortensen | Yrjö Henrik Roos | Marize L. M. Solano | Monika Sramkova | Henk Van Loveren | Laurence Vernis | Simone Lunardi | Magdalena Andryszkiewicz | Ana Criado | Yi Liu

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme carboxypeptidase C (EC 3.4.16.5) is produced with the genetically modified *Aspergillus niger* strain PEG by DSM Food Specialties B.V. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in nine food manufacturing processes. Dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 2.053 mg TOS/kg body weight (bw) per day in European populations. The toxicity studies were carried out with a xylanase obtained from *A. niger* strain XEA. The Panel considered this food enzyme as a suitable substitute for the carboxypeptidase to be used in the toxicological studies, because both strains were derived from the same recipient strain, the location of the inserts was comparable, no partial inserts were present and the production methods were essentially the same. 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 1850 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 901. A homology search for the amino acid sequence of the food enzyme to known allergens was made and one match with a wheat allergen was found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, especially in wheat-allergic individuals, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

Aspergillus niger, carboxypeptidase C, EC 3.4.16.5, EFSA-Q-2021-00315, EFSA-Q-2015-00445, food enzyme, genetically modified microorganism, serine carboxypeptidase I

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 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.

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

The European Commission mandated EFSA to evaluate the safety of this food enzyme in 2015 and in 2021.

1.1.1 | Background as provided by the European Commission in 2015

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 companies "DSM Food Specialties B.V." for the authorisation of the food enzyme Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG); "Advanced Enzyme Technologies Ltd." for the authorisation of the food enzymes Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC) and Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC); "Danisco US Inc." for the authorisation of the food enzyme Glucan 1,4-a-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24), and "Amano Enzyme Inc." for the authorisation of the food enzyme Catalase from *Aspergillus niger* (strain AE-CN).

Following the requirements of Article 12.1 of Commission Regulation (EU) 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 contains all the elements required under Chapter II of that Regulation.

1.1.2 | Terms of Reference in 2015

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG), Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC), Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC), Glucan 1,4-a-maltotetraohydrolase from a genetically modified strain of *Bacillus*

[1]Regulation (EC) No. 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No. 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, pp. 15–24.

licheniformis (strain DP-Dzf24) and Catalase from *Aspergillus niger* (strain AE-CN) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.3 | Background as provided by the European Commission in 2021

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.

Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG) is a food enzyme included in the Register of food enzymes to be considered for inclusion in the European Union (EU) Community list and thus subject to a risk assessment by the European Food Safety Authority (EFSA). In the initial dossier with reference EFSA-Q-2015-00445, the applicant requests for the authorisation of the above food enzyme in dairy processing, meat/protein processing and the production of flavourings in accordance with Regulation (EC) No 1331/2008.

On 8 April 2021, a new application has been introduced by the applicant "DSM Food Specialties B.V." for an extension of the conditions of use authorisation of the above food enzyme in yeast processing and plant-based products (dairy alternatives).

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

Taking into account that the above food enzyme is subject to a risk assessment by the European Food Safety Authority (EFSA), in accordance with Regulation (EC) No 1331/2008, it is appropriate to address the safety of the proposed extension of the condition of use within the scientific opinion evaluating the safety of that food enzyme.

1.1.4 | Terms of Reference in 2021

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to carry out the safety assessments on an extension of the condition of use for the following food enzyme: Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzyme and food flavourings.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's requests to carry out the safety assessment of food enzyme Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* strain PEG (submitted in 2015) and under extended conditions of uses (submitted in 2021).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Carboxypeptidase C from a genetically modified strain of *A. niger* (strain PEG).

Additional information was requested from the applicant during the assessment process on 18 October 2021 and 15 December 2022, and received on 23 September 2022 and 6 September 2023, respectively (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 18 October 2021, the applicant requested a clarification tele-conference on 30 November 2021, after which the applicant provided additional data on 23 September 2022.

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, 2009a) 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, 2009b) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Carboxypeptidase C
Systematic name	–
Synonyms	Carboxypeptidase Y; serine carboxypeptidase I; cathepsin A; lysosomal protective protein; deamidase; lysosomal carboxypeptidase A; phaseolin
IUBMB No	3.4.16.5
CAS No	9046-67-7
EINECS No	232-934-1

Carboxypeptidase C catalyses the hydrolysis of the peptide bond at the C-terminal amino acid residue of proteins and peptides with broad specificity, releasing amino acids and peptides of shorter length. The food enzyme is intended to be used in nine food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): processing of dairy products for the production of (1) cheese, (2) fermented dairy products, (3) flavouring preparations and (4) modified milk proteins; processing of meat and fish products for the production of (5) modified meat and fish products and (6) protein hydrolysates; processing of plant- and fungal-derived products for the production of (7) plant-based analogues of milk and milk products and (8) protein hydrolysates and (9) processing of yeast and yeast products.

3.1 | Source of the food enzyme

The carboxypeptidase C is produced with the genetically modified filamentous fungus *A. niger* strain PEG, which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with the deposit number [redacted][4] The production strain was identified as *A. niger* by [redacted]

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental strain is *A. niger* [redacted]

3.1.2 | Characteristics of introduced sequences

The sequence encoding the carboxypeptidase C [redacted]

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise carboxypeptidase C [redacted]

[4]Technical dossier/Additional data September 2022/Annex 1.
[5]Technical dossier/Additional data September 2022/Annex 2.
[6]Technical dossier/1st submission/Annex II-3.
[7]Technical dossier/1st submission/Annexes II-7 and II-8.

[redacted]

3.1.4 | Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* PEG differs from the recipient strain [redacted]

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

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[11] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. 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.[13] 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.[14]

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 carboxypeptidase C is a single polypeptide chain of 467 amino acids.[15] The molecular mass, calculated from the amino acid sequence, is around 52 kDa.[16] The food enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about 70 kDa.[17] No other enzymatic activities were reported.[18]

The in-house determination of carboxypeptidase C activity is based on the hydrolysis of the dipeptide furylacryloyl-Phe-Ala (reaction conditions: pH 4.5, 37°C) and determined by measuring the decrease of absorbance at 340 nm. The enzyme activity is expressed in CPGU (Carboxy Peptidase G Unit)/g. One CPGU is the amount of enzyme needed to decrease the absorbance at 340 nm by one absorbance unit per minute under the conditions of the assay.[19]

[8]Technical dossier/1st submission/Annex II-3.
[9]Technical dossier/1st submission/Annex II.
[10]Technical dossier/Additional data September 2022/Annex 2.
[11]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.
[12]Technical dossier/1st submission/pp. 61/Annex I-5.
[13]Technical dossier/1st submission/pp. 61–69/Annex I-6.
[14]Technical dossier/1st submission/Annex I-7.
[15]Technical dossier/1st submission/pp. 50.
[16]Technical dossier/1st submission/pp. 50.
[17]Technical dossier/1st submission/pp. 48.
[18]Technical dossier/1st submission/pp. 51–52.
[19]Technical dossier/1st submission/pp. 51/Annex I-2.

The food enzyme has a temperature optimum between 40°C and 45°C (pH 4.5) and a pH optimum around pH 4.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme at different temperatures and times (2–60 min, pH 4.5). Carboxypeptidase C activity decreased above 45°C, showing no residual activity after 5 min pre-incubation at 60°C.[20]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1).[21] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 10.2% and the mean enzyme activity/TOS ratio was 44.1 U/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Carboxypeptidase C activity	CPGU/g[a]	3970	4441	4630
Protein	%	4.6	4.7	4.5
Ash	%	0.5	0.4	0.5
Water	%	91.7	90.0	86.3
Total organic solids (TOS)[b]	%	7.8	9.6	13.2
Activity/TOS ratio	U/mg TOS	50.9	46.3	35.1

[a]CPGU: Carboxy Peptidase G Unit (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg which complies with the specification for lead as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[22,23]

The food enzyme complies with the microbiological criteria for total coliforms, *E. coli* and *Salmonella* as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[24] No antimicrobial activity was detected in any of the tested batches.

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of fumonisins, ochratoxin A, aflatoxins, trichothecenes and zearalenone were examined in the three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[25,26] Adverse effects due to the potential 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 was sufficient.

3.3.4 | Viable cells and DNA 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.[27]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. [redacted] with a limit of detection of 10 ng spiked DNA/g food enzyme.[28]

[20]Technical dossier/1st submission/pp. 52–54.

[21]Technical dossier/1st submission/pp. 47/Annex I-3.

[22]Technical dossier/1st submission/pp. 49–50/Annexes: I-3 and I-4.

[23]LOD: Pb = 0.006 mg/L sample solution.

[24]Technical dossier/1st submission/pp. 49–50/Annexes: I-3 and I-4.

[25]Technical dossier/1st submission/pp. 49–50/Annexes: I-3 and I-4 and Additional data September 2022.

[26]LoQs: ochratoxin A = 0.1 μg/kg; fumonisins = 10 μg/kg; aflatoxins = 0.1 μg/kg, trichothecenes = 10 μg/kg; zearalenone = 3 μg/kg.

[27]Technical dossier/Additional data September 2022/Annex 3.

[28]Technical dossier/Additional data September 2023/Annex 1.

3.4 | Toxicological data

3.4.1 | Choice of test item

No toxicological studies were provided for the carboxypeptidase produced with *A. niger* PEG. Instead, the applicant argued that the assessment of the carboxypeptidase could be based on toxicological data from another food enzyme – a xylanase produced with *A. niger* XEA, previously submitted to EFSA (Question No EFSA-Q-2015-00045) following the EFSA guidance (EFSA CEF Panel, 2009b).

The production strain of the xylanase was developed from the same recipient strain [redacted] as that of the carboxypeptidase under assessment, using a similar genetic modification system, with a specific gene of interest in each case. The genetic modification in *A. niger* PEG only differs from that of *A. niger* XEA in the gene of interest and in the number of expression cassettes inserted in the genome. The expression cassettes of both production strains were inserted [redacted]. No rounds of mutagenesis have been applied in the development of the production strains from the recipient and all the genetic modifications have been described throughout and raise no concerns. Therefore, the genetic differences between *A. niger* PEG and *A. niger* XEA are not expected to result in a different toxigenic potential.

The batch of xylanase food enzyme from *A. niger* XEA, used for toxicological studies, was produced according to a standard procedure similar to the one described in Section 3.2 of this opinion. The data provided by applicant, the raw materials used and the steps involved in the manufacturing of the xylanase and carboxypeptidase food enzymes are essentially the same. In both manufacturing processes, the temperature and pH conditions used during fermentation are similar. Therefore, the compositions of TOS apart from the enzyme protein itself is comparable.

Taking the molecular and technical data into account, the Panel considered the xylanase produced with *A. niger* XEA as a suitable substitute for the carboxypeptidase produced by *A. niger* PEG in the toxicological studies.

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, all made with the substitute food enzyme.

3.4.2 | Genotoxicity

3.4.2.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation assay (Ames test) was performed according to the OECD Test Guideline 471 (OECD, 1997a), and following Good Laboratory Practice (GLP) in four strains of Salmonella Typhimurium (TA98, TA100, TA1535, TA1537) and *E. coli* WP2uvrApKM 101, in the presence and absence of metabolic activation, applying the plate incorporation assay.

The effect of xylanase activity on S9-mix was tested and it was observed that the test item did not inhibit the activity of S9-mix. Two independent experiments were carried out in triplicate using five concentrations of the food enzyme ranging from 50 to 5000 µg dry matter/plate of the food enzyme (corresponding to 49 to 4930 µg TOS/plate). No precipitation or significant cytotoxicity were observed in any strain at any dose level tested. Upon treatment with the food enzyme, there was no significant increase in the number of revertant colonies in any tester strain, both in the presence and absence of metabolic activation.

Therefore, the Panel concluded that the food enzyme did not induce gene mutations in the bacterial reverse mutation assay under the test conditions employed for this study.

3.4.2.2 | *In vitro mammalian chromosomal aberration test*

The in vitro mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in Chinese hamster ovary cells (CHO).

Based on the results obtained in a dose-range finding test, the cells were treated with 1250, 2500 and 5000 µg dry matter/mL (corresponding to 1233, 2466 and 4932 µg TOS/mL) applying a short-term treatment (3 + 17 h of recovery) in the presence and absence of S9-mix, and with 750, 3000 and 5000 µg dry matter/mL(corresponding to 740, 2959 and 4932 µg TOS/mL) applying a continuous treatment (20 + 0 h) in the absence of S9-mix. No precipitation or significant changes in pH were detected. Cytotoxicity, measured as mitotic inhibition, did not exceed 23% of concurrent negative control values at any concentration of the food enzyme.

No statistically significant increase in the frequency of chromosomal aberrations was observed in the short term treated cultures compared to the negative controls both in the presence and absence of metabolic activation. After continuous treatment in the absence of S9-mix, a statistically significant increase in the frequency of aberrant cells was observed only at 5000 µg dry matter/mL (0 vs. 2.5% aberrant cells at 0 and 5000 µg dry matter/mL, respectively). However, the increase was slightly above the historical negative control range (0–2) that was not considered robust because it was based only on six experiments. Therefore, the increase was not considered biological relevant and the Panel concluded that the food enzyme did not induce chromosomal aberrations under the experimental conditions employed for this study.

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

A repeated dose 90-day oral toxicity study in rodents was performed according to OECD test guideline 408 (OECD, 1998) and following GLP.

Groups of 10 male and 10 female Wistar rats received daily via gavage for at least 90 days dose levels of 0 (double distilled water as vehicle), 400, 1600 and 6400 mg food enzyme/kg bw per day in a volume of 10 mL/kg bw per day, corresponding to 0, 116, 463 and 1852 mg TOS/kg bw per day (referred to as control, low-, mid- and high-dose groups).

No treatment-related deaths or effects on clinical signs, body weight and body weight gains, food consumption, ophthalmoscopic examinations, organ weights and organ weight ratios, and macroscopic or microscopic pathology were observed.

In the functional observation battery tests a lower grip strength value was observed in forelimbs of males in the low-dose group and a higher grip strength value in hindlimbs of the mid-dose group. In females a significantly higher grip strength value was observed in forelimbs of the mid- and high-dose groups and in hindlimbs of the high-dose group. All these changes were considered to be incidental findings since they lacked dose relationship. Significantly higher values of landing foot splay were observed in mid and high-dose males and females and were also considered to be incidental, as there was no change in gait observed in these animals.

In haematology evaluation significant incidental increases were observed in mid-dose males for mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) (also in high-dose males), platelets, prothrombin time, neutrophils and decreased values for lymphocytes. The higher levels of MCHC in mid- and high-dose males were considered incidental, as the corresponding changes were not observed in red blood cell counts and haemoglobin. In females, a significantly increased mean corpuscular volume (MCV) was seen in low- and mid-dose animals, a higher level of haematocrit in mid-dose females and an increased neutrophil percentage with lower lymphocyte percentage in high-dose females. These changes were minor and were considered as incidental and attributed to normal biological variation.

In clinical chemistry evaluation some parameters were only affected in males. Minor increased sodium and chloride levels were observed in the high-dose group which were considered to be attributed to normal biological variation. The dose-related increased creatinine levels at mid- and high-doses groups were considered as incidental, as there were no corresponding histopathological changes in the kidneys.

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

The Panel identified a no observed adverse effect level (NOAEL) based on the high-dose level of this repeated dose 90-day oral toxicity study of 1852 mg TOS/kg bw per day.

3.4.4 | Allergenicity

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

The potential allergenicity of the carboxypeptidase C produced with the *A. niger* strain PEG 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, one match was found. The matching allergen was a carboxypeptidase, a food allergen from wheat (*Triticum aestivum*).

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

Carboxypeptidase from wheat is not a major allergen and IgE binding has been reported (Weichel et al., 2006). *Aspergillus* species are known to cause respiratory allergy (Shen & Han, 1998). Oral allergic reactions to *Aspergillus* do occur (Xing et al., 2022) but are rare.

[redacted] a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will mostly be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. The Panel, however, considered that residual amounts of potentially allergenic proteins could still be present in the food enzyme.

Overall, the Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme is low but cannot be excluded, especially in wheat-allergic patients. However, the likelihood of such reactions will not exceed the likelihood of allergic reactions to wheat.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

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

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[29,30]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of dairy products		
• Production of cheese	Milk	0.38–**5.65**
• Production of fermented dairy products	Milk	0.38–**5.65**
• Production of flavouring preparations from dairy products	Dairy products (e.g. cheese, cream, butter)[31]	56.5–**188.3**
• Production of modified milk proteins[32]	Whey proteins	226.0–**753.1**
Processing of meat and fish products		
• Production of modified meat and fish products	Meat[33]	1.67–**8.37**
• Production of protein hydrolysates from meat and fish proteins[34]	Protein concentrates or isolates from meat and fish	226.0–**753.1**
Processing of plant- and fungal-derived products		
• Production of plant-based analogues of milk and milk products	Cereals, legumes, oilseeds, nuts, etc.	Up to **0.61**
• Production of protein hydrolysates from plants and fungi[35]	Protein concentrates or isolates from plants	226.0–**753.1**
Processing of yeast and yeast products	Yeast culture, yeast cell walls, yeast extracts	Up to **204**

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

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

In the production of cheese, the carboxypeptidase C is added to milk during the coagulation step[36] to reduce the ripening time and the bitterness of cheese.[37] The food enzyme–TOS remain in cheeses.

In the production of fermented dairy products, the food enzyme is added during the coagulation and fermentation step.[38] The action of carboxypeptidase C improves the sensory properties of the final foods.[39] The food enzyme–TOS remain in these fermented products like yoghurt or sour cream.

In the production of flavouring preparations from dairy products, carboxypeptidase C is added to dairy products (e.g. curd, cheese) during the hydrolysis step.[40] It is used to reduce the bitterness of the final products and to accelerate the processing time.[41] The food enzyme–TOS remain in these enzyme-modified dairy ingredients (EMDI), which are subsequently used as an ingredient in the formulation of a variety of foods, such as processed cheese, cheese sauce, cheese powder, salad dressing and snack foods.

In the production of modified milk proteins, the food enzyme is added to whey proteins to achieve the desired degree of hydrolysis.[42] Addition of carboxypeptidase C can also enhance the flavour of the resulting milk protein products (e.g. whey protein hydrolysates, whey concentrates and whey protein isolates), which are subsequently used as ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes. The food enzyme–TOS remain in the final foods.

In the production of protein hydrolysates, the food enzyme is added to a variety of partially purified proteins from plant (e.g. soy, pea) and animal (e.g. meat and fish trimmings) sources.[43,44] The carboxypeptidase C is used to achieve the desired degree of hydrolysis, to increase the yield and to reduce the bitterness.[45] The food enzyme–TOS remain in the final hydrolysates, which are added to a variety of final foods (e.g. soups, bouillons, snacks, dressings) to enhance the flavour.

[29]Technical dossier of EFSA-Q-2021-00315/p. 12.
[30]Additional information September 2022 of EFSA-Q-2015-00445/Part I/p. 6.
[31]Additional information September 2022 of EFSA-Q-2015-00445/Part I/Answer 8.
[32]Additional information September 2022 of EFSA-Q-2015-00445/Part I/Answer 10.
[33]Additional information September 2022 of EFSA-Q-2015-00445/Part I/Answer 9.
[34]Additional information September 2022 of EFSA-Q-2015-00445/Part I/Answer 10.
[35]Additional information September 2022 of EFSA-Q-2015-00445/Part I/Answer 10.
[36]Technical dossier 2015-00445/p. 72.
[37]Technical dossier 2015-00445/p. 104.
[38]Technical dossier 2015-00445/p. 73.
[39]Technical dossier 2015-00445/p. 106.
[40]Technical dossier 2015-00445/p. 74.
[41]Technical dossier 2015-00445/p. 107.
[42]Additional information September 2022/Answer to question 10.
[43]Additional information September 2022/Answer to question 10.
[44]Technical dossier 2015-00445/p. 76.
[45]Technical dossier 2015-00445/p. 111.

In the production of modified meat and fish products, the carboxypeptidase C is added to meat during fermentation to shorten the ripening time.[46,47] The food enzyme–TOS remain in these fermented meat products such as sausages.

In the production of plant-based analogues of milk and milk products, the food enzyme is added to a slurry of milled plant materials to increase yield and to enhance flavour.[48] The food enzyme–TOS remain in the final foods.

In the processing of yeast and yeast products, the food enzyme is added to different yeast components (yeast cells, yeast extracts and yeast cell walls) in combination with other enzymes to improve sensory properties of the treated yeast products.[49] The food enzyme–TOS remain in the final foods that are made with or from yeast products.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied, it is expected that the food enzyme is inactivated in the food manufacturing processes listed in Table 2, except for cheeses, fermented dairy products and fermented meat products, in which the enzyme remains in its active form, depending on the processing conditions.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSEFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 2.053 mg TOS/kg bw per day in infants at the 95th percentile.

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

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.158–0.985 (12)	0.177–0.525 (15)	0.110–0.216 (19)	0.030–0.125 (21)	0.024–0.104 (22)	0.015–0.078 (23)
Min–max 95th percentile (number of surveys)	0.401–2.053 (11)	0.420–1.142 (14)	0.252–0.546 (19)	0.075–0.394 (20)	0.053–0.317 (22)	0.038–0.243 (22)

Abbreviation: TOS, total organic solids.

3.5.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

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)	+

(Continues)

[46]Technical dossier 2015-00445/p. 75.
[47]Technical dossier 2015-00445/p. 108.
[48]Technical dossier 2021-00315/p. 9.
[49]Technical dossier 2021-00315/p. 10.

TABLE 4 (Continued)

Sources of uncertainties	Direction of impact
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
For the production of meat and fish products, the calculation is made with the default list of FoodEx categories that is broader than meat	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

3.6 | Margin of exposure

A comparison of the NOAEL (1850 mg TOS/kg bw per day) identified from the 90-day rat study with the derived exposure estimates of 0.015–0.985 mg TOS/kg bw per day at the mean and from 0.038 to 2.053 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 901.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme carboxypeptidase C produced with the genetically modified *A. niger* strain PEG does not give rise to safety concerns under the intended conditions of use.

The Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of carboxypeptidase C from a genetically modified strain of *Aspergillus niger* in accordance with Regulation (EC) No 1331/2008. February 2015. Submitted by DSM Food Specialties.

Application for extension of use of carboxypeptidase C from a genetically modified strain of *Aspergillus niger* in accordance with Regulation (EC) No 1331/2008. March 2021. Submitted by DSM Food Specialties.

Additional information. September 2022 and September 2023. Submitted by DSM Food Specialties.

ABBREVIATIONS

ANI	average nucleotide identity
bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
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
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MoE	margin of exposure

OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization

ACKNOWLEDGEMENTS
The Panel wishes to thank the following for the support provided to this scientific output: Andrew Chesson, Lieve Herman.

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

REQUESTOR
European Commission

QUESTION NUMBERS
EFSA-Q-2015-00445, EFSA-Q-2021-00315

PANEL MEMBERS
José Manuel Barat Baviera, Claudia Bolognesi, Francesco Catania, Gabriele Gadermaier, Ralf Greiner, Baltasar Mayo, Alicja Mortensen, Yrjö Henrik Roos, Marize LM Solano, Monika Sramkova, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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

REFERENCES
Andersen, M. R., Salazar, M. P., Schaap, P. J., van de Vondervoort, P. J., Culley, D., Thykaer, J., Frisvad, J. C., Nielsen, K. F., Albang, R., Albermann, K., Berka, R. M., Braus, G. H., Braus-Stromeyer, S. A., Corrochano, L. M., Dai, Z., van Dijck, P. W., Hofmann, G., Lasure, L. L., Magnuson, J. K., … Baker, S. E. (2011). Comparative genomics of citric-acid-producing Aspergillus niger ATCC 1015 versus enzyme-producing CBS 513.88. *Genome Research*, *21*(6), 885–897.

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

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

EFSA (European Food Safety Authority). (2009b). 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*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2011.1305

EFSA (European Food Safety Authority). (2011). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. 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*, *17*(6), 5741. 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, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., 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., … Chesson, A. (2021). Scientific Guidance for the submission of dossiers on food enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Apergi, K., … Chesson, A. (2023). Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, *21*(7), 8094. https://doi.org/10.2903/j.efsa.2023.8094

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010a). Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. 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. https://www.fao.org/3/a-a0675e.pdf

Frisvad, J. C., Møller, L. L. H., Larsen TO, Kumar, R., & Arnau, J. (2018). Safety of the fungal workhorses of industrial biotechnology: Update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, *102*, 9481–9515. https://doi.org/10.1007/s00253-018-9354-1

OECD (Organisation for Economic Co-Operation and Development). (1997a). OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-Operation and Development). (1997b). OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: *In vitro* mammalian chromosomal aberration test. 21 July 1997. 10 pp. 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. https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Pel, H. J., de Winde, J. H., Archer, D. B., Dyer, P. S., Hofmann, G., Schaap, P. J., Turner, G., de Vries, R. P., Albang, R., Albermann, K., Andersen, M. R., Bendtsen, J. D., Benen, J. A., van den Berg, M., Breestraat, S., Caddick, M. X., Contreras, R., Cornell, M., Coutinho, P. M., … Stam, H. (2007). Genome sequencing and analysis of the versatile cell factory Aspergillus niger CBS 513.88. *Nature Biotechnology*, *25*(2), 221–231.

Sgorbati, B., Scardovi, V., & Leblanc, D. (1982). Plasmids in the Genus Bifidobacterium. *Journal of General Microbiology*, *128*. https://doi.org/10.1099/00221287-128-9-2121

Shen, H. D., & Han, S. H. (1998). Characterization of allergens of Penicillium and Aspergillus species. *Journal of Microbiology, Immunology, and Infection*, *31*, 141–145.

van Dijck, P. W., Selten, G. C., & Hempenius, R. A. (2003). On the safety of a new generation of DSM *Aspergillus niger* enzyme production strains. *Regulatory Toxicology and Pharmacology*, *38*(1), 27–35.

Weichel, M., Vergoossen, N. J., Bonomi, S., Scibilia, J., Ortolani, C., Ballmer-Weber, B. K., Pastorello, E. A., & Crameri, R. (2006). Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. *Allergy*, *61*(1), 128–135. https://doi.org/10.1111/j.1398-9995.2006.00999.x

Xing, H., Wang, J., Sun, Y., & Wang, H. (2022). Recent advances in the allergic cross-reactivity between fungi and foods. *Journal of Immunology Research*, 7583400. https://doi.org/10.1155/2022/7583400

SUPPORTING INFORMATION

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

How to cite this article: EFSA FEZ Panel (EFSA Panel on Food Enzymes), Zorn, H., Barat Baviera, J. M., Bolognesi, C., Catania, F., Gadermaier, G., Greiner, R., Mayo, B., Mortensen, A., Roos, Y. H., Solano, M. L. M., Sramkova, M., Van Loveren, H., Vernis, L., Lunardi, S., Andryszkiewicz, M., Criado, A., & Liu, Y. (2024). Safety evaluation of the food enzyme carboxypeptidase C from the genetically modified *Aspergillus niger* strain PEG. *EFSA Journal*, *22*(10), e9038. https://doi.org/10.2903/j.efsa.2024.9038

APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey
Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey

APPENDIX B

Population groups considered for the exposure assessment

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

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

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