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Safety evaluation of the food enzyme 1,4-α-glucan branching enzyme from the non-genetically modified *Geobacillus thermodenitrificans* strain TRBE14

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Abstract

The food enzyme 1,4-α-glucan branching enzyme ((1–4)-α-D-glucan:(1–4)-α-D-glucan 6-α-D-[(1–4)-α-D-glucano]-transferase; EC 2.4.1.18) is produced with the non-genetically modified *Geobacillus thermodenitrificans* strain TRBE14 by Nagase (Europa) GmbH. The production strain has been shown to qualify for the qualified presumption of safety (QPS) approach. The food enzyme is intended to be used in cereal-based processes, baking processes as well as meat and fish processing. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.29 mg TOS/kg body weight (bw) per day in European populations. Toxicological studies were not considered necessary given the QPS status of the production strain and the nature of the manufacturing process. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel noted that the food enzyme contains lysozyme, a known allergen. Therefore, allergenicity cannot be excluded. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

Keywords: food enzyme, 1,4-α-glucan branching enzyme, (1–4)-α-D-glucan:(1–4)-α-D-glucan 6-α-D-[(1–4)-α-D-glucano]-transferase, EC 2.4.1.18, *Geobacillus thermodenitrificans*

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1. Introduction

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

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

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

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

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

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

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

1.1.1. Background as provided by the European Commission

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

Five applications have been introduced by the companies 'Danisco US Inc.' for the authorisation of the food enzyme Cellulase from *Penicillium funiculosum* (strain DP-Lzc35), 'Advanced Enzyme Technologies Ltd.' for the authorisation of the food enzyme Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg (strain FL108SC), 'Avances Bioquimicos Alimentacion, S.L.' for the authorisation of the food enzyme Catalase from porcine livers and 'Nagase (Europa) GmbH' for authorisation of the food enzymes 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and Urase from Lactobacillus fermentum (strain 48/72).

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] 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 Cellulase from *Penicillium funiculosum* (strain DP-Lzc35), Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* agg (strain FL108SC), Catalase from porcine livers, 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and Urase from *Lactobacillus fermentum* (strain 48/72) 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 1,4-alpha-glucan branching enzyme from *G. stearothermophilus* (strain TRBE14).

Recent data identified the production microorganism as *Geobacillus thermodenitrificans* (Section 3.1). Therefore, this name will be used in this opinion instead of *Geobacillus stearothermophilus*.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme 1,4-alpha-glucan branching enzyme from *G. stearothermophilus* (strain TRBE14). The dossier was submitted on March 2015.

Additional information was requested from the applicant during the assessment process on 12 April 2022 and received on 20 October 2022 (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

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

3. Assessment

IUBMB nomenclature	1,4-α-Glucan branching enzyme
Systematic name	(1→4)-α-D-glucan:(1→4)-α-D-glucan 6-α-D-[(1→4)-α-D-glucano]-transferase
Synonyms	Amylo-(1,4-1,6)-transglycosylase; α-glucan-branching glycosyltransferase; amylose isomerase
IUBMB No	EC 2.4.1.18
CAS No	9001-97-2
EINECS No	849-290-8

1,4-α-Glucan branching enzymes catalyse the formation of α-1,6-glucosidic linkages in amylopectin by transferring linear α-1,4-glucan chains to the same or another molecule. The food enzyme under assessment is intended to be used in cereal-based processes, baking processes as well as meat and fish processing.

3.1. Source of the food enzyme

The 1,4-α-glucan branching enzyme is produced with the non-genetically modified bacterium *G. thermodenitrificans* strain TRBE14, which is deposited at the National Institute of Technology and Evaluation (NITE), Biological Resource Centre (Japan) with the deposit number [redacted].[4] The production strain was derived from the parental strain, [redacted].

[4] Additional data October 2022/Answer Q1.

The production strain was identified as *G. thermodenitrificans* by whole genome sequence (WGS), [redacted].[5]

The species *G. thermodenitrificans* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of acquired antimicrobial resistance (AMR) genes and toxigenic activity are verified for the specific strain used (EFSA, 2007; EFSA BIOHAZ Panel, 2020). The applicant demonstrated the absence of cytotoxicity in Vero cells using a lactate dehydrogenase assay. The production strain was analysed for the presence of antimicrobial resistance genes in the genome by WGS analysis. No genes of concern were identified. Therefore, the production strain is considered to qualify for the QPS approach to safety assessment.[6]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[7] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[8]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After completion of the fermentation and release of the intracellular enzyme by lysozyme, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is 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. Finally, the food enzyme is freeze-dried prior to analysis.[9] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[10]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The 1,4-α-glucan branching enzyme is a single polypeptide chain of [redacted] amino acids.[11] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches.[12] The food enzyme was tested for amylase, protease and lipase activities.[13] Only protease and amylase activities were detected. No other enzyme activities were reported.

The in-house determination of 1,4-α-glucan branching enzyme activity is based on the formation of 1,6-α-linkages in amylose, which is detected spectrophotometrically (reaction conditions: pH 7.5, 50°C, 10 min). The enzyme activity is expressed in Units/g (U/g). One U is defined as the amount of enzyme that causes a decrease in absorbance of the starch-iodine complex at 660 nm of 0.05% per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 45°C (pH 7.5) and a pH optimum around pH 7.2 (50°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 7.5). Enzyme activity decreased above 60°C, showing no residual activity above 70°C.[15]

[5] Additional data October 2022/Answer Q2_Report/Answer Q2_Appendix B.
[6] Additional data October 2022/Answer Q2_Report/Answer Q2_Appendix D.
[7] 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.
[8] Technical dossier/pg. 5; pg. 34/Annex 4/Additional data October 2022/Answer Q4a/Answer Q4b.
[9] Technical dossier/pg. 34–39.
[10] Technical dossier/pg. 36/Additional data October 2022/Answer Q5.
[11] Technical dossier/pg. 4, pg. 22/Annex 5.2/Additional data October 2022/Answer Q7.
[12] Additional data October 2022/Answer Q7.
[13] Technical dossier/pg. 4–5; pg. 27/Annex 2.3.
[14] Technical dossier/pg. 4, 25/Annex 2.4.
[15] Technical dossier/pg. 25–26.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation (Table 1).[16] The mean total organic solids (TOS) of the three food enzyme batches was 94.0% and the mean enzyme activity/TOS ratio was 613.3 U/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
1,4-α-Glucan branching enzyme activity	U/g[(a)]	675,000	604,000	449,000
Protein	%	80.1	70.0	81.1
Ash	%	0.4	0.4	0.4
Water	%	5.9	5.9	5.0
Total organic solids (TOS)[(b)]	%	93.7	93.7	94.6
Activity/ TOS	U/mg TOS	720	645	475

(a): U: Units (see Section 3.3.1).
(b): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three batches was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, arsenic was below the limit of detection (LoD) of the employed method.[17] For mercury and cadmium the average concentrations determined in the test batches were 0.02 and 0.06 mg/kg, respectively.[17,18] The Panel considered these concentrations as not of concern.

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).[17] No antimicrobial activity was detected in any of the tested batches.[17]

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

3.4. Toxicological data

No toxicological tests were provided by the applicant. As the production strain qualifies for the QPS approach of safety assessment and no issues of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel concluded that no toxicological studies other than assessment of allergenicity were necessary.

3.4.1. Allergenicity

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

The potential allergenicity of the 1,4-α-glucan branching enzyme produced with the non-genetically modified *G. thermodenitrificans* strain TRBE14 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, no match was found.[19]

No information was available on oral and respiratory sensitisation or elicitation reactions of this 1,4-α-glucan branching enzyme.

No allergic reactions upon dietary exposure to any 1,4-α-glucan branching enzyme have been reported in the literature.

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process it will be degraded and utilised by the microorganism for cell

[16] Technical dossier/pg. 24, pg. 39/Annex 2.1, Annex 2.2/Additional data October 2022/Answer Q8.
[17] Technical dossier/pg. 4, pg. 11, pg. 24, pg. 39/Annex 2.1.
[18] LODs: Pb = 0.05 mg/kg, As = 1 mg/kg; Hg and Cd = 0.01 mg/kg each.
[19] Technical dossier/pg. 6, pg. 48/Annex 5.2.

growth, cell maintenance and production of enzyme protein. In addition, the microbial 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 noted that egg white lysozyme, a known allergen, is used during the downstream processing of the food enzyme and is likely to be present in the final product.

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 due to the presence of lysozyme.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three 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[(e)]

Food manufacturing process[(a)]		Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]	
			Powder[(c)]	Liquid[(d)]
Cereal-based processes	Pasta	Wheat	0.18–**7.0**	0.14–5.4
	Cooked rice	Rice	1.8–176	1.4–136
Baking processes	Biscuits, cakes	Wheat	0.35–21.1	0.27–16.3
	Bread	Wheat	0.35–**21.1**	0.27–16.3
Meat and fish processing	Sausage	Pork	1.8–**17.6**	1.4–13.6
	Ham	Pork	1.8–17.6	1.4–13.6
	Fried chicken	Chicken	0.09–8.8	0.07–6.8
	Surimi	Fish paste	1.1–5.3	0.8–4.1

TOS, total organic solids.
(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 calculations.
(c): Based on 8.8% TOS.
(d): Based on 3.4% TOS.
(e): Additional information October 2022/Answer Q9.

In cereal-based processes, the food enzyme is added to flour during dough preparation[20] or to rice before cooking.[21] It results in a highly branched structure of starch, delaying retrogradation in pasta and cooked rice. The food enzyme–TOS remains in the final product.

In baking processes, the food enzyme is added to flour during dough preparation.[22] Branching of starch reduces staling, which improves the shelf-life of baked products. The food enzyme–TOS remains in the final product.

In meat and fish processing, the food enzyme is added to starch-containing meat or fish paste during preparation.[23] Branching of starch reduces the viscosity during mixing. The food enzyme–TOS remains in the final product.

Based on data provided on thermostability (see Section 3.3.1), the Panel considered that the 1,4-α-glucan branching enzyme is inactivated during all the above-mentioned food processes.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the

[20] Technical dossier/Figure 11.
[21] Technical dossier/Figure 14.
[22] Technical dossier/Figures 12 and 13.
[23] Technical dossier/Figures 15, 16, 17 and 18.

total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 0.29 mg TOS/kg bw per day in infants and toddlers at the 95th percentile.

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)	0.008–0.074 (12)	0.057–0.149 (15)	0.074–0.143 (19)	0.042–0.103 (21)	0.029–0.067 (22)	0.025–0.058 (23)
Min–max 95th percentile (number of surveys)	0.036–0.290 (11)	0.143–0.290 (14)	0.147–0.271 (19)	0.086–0.210 (20)	0.061–0.135 (22)	0.049–0.101 (22)

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)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
For cereal-based processes, the lower use level (for pasta) was chosen for calculation, instead of the higher use level (for cooked rice)	–
For meat and fish processing, the higher use level (for sausage and ham) was chosen for calculation, instead of the lower use levels (for fried chicken and surimi)	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular the 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

Since no toxicological assessment was considered necessary by the Panel, the margin of exposure was not calculated.

4. Conclusion

The Panel noted that the food enzyme contains lysozyme, a known allergen. Therefore, allergenicity cannot be excluded.

Based on the data provided, the outcome of the QPS assessment of the production strain and the absence of other concerns, the Panel concluded that the food enzyme 1,4-α-glucan branching enzyme produced with the non-genetically modified *G. thermodenitrificans* strain TRBE14 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

1,4-α-Glucan Branching Enzyme preparation from *Geobacillus stearothermophilus* TRBE14. March 2015. Submitted by Nagase (Europa) GmbH.

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Abbreviations

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
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
NITE	National Institute of Technology and Evaluation
QPS	qualified presumption of safety
TOS	total organic solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7834#support-information-section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

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

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