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Safety evaluation of the food enzyme alpha-amylase from a genetically modified *Bacillus subtilis* (strain NBA)

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Abstract

The food enzyme alpha-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with a genetically modified strain of *Bacillus subtilis* strain NBA by DSM Food Specialities B.V. This α-amylase is intended to be used in baking processes. The genetic modifications do not give rise to safety concerns and the food enzyme is free from viable cells of the production organism and recombinant DNA. The parental strain meets the required qualifications to be considered as a Qualified Presumption of Safety (QPS) organism and is therefore presumed to be safe. Since the production strain is not cytotoxic and since the introduced genetic modifications do not raise safety concerns, the presumption of safety made for the parental strain is extended to the production strain. The conclusions on safety of the food enzyme are made following the QPS approach in relation to the production strain, with additional consideration of the conditions of manufacture. However, the Panel considers no toxicological studies other than assessment of allergenicity necessary. This is based on the QPS status of the production strain and the absence of any hazards from the product and downstream processing. Based on the maximum use level recommended for the baking processes and individual data from the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database, dietary exposure was estimated to be up to 0.093 mg TOS/kg body weight per day in European populations. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is considered low. Based on the data provided, the Panel concluded that this food enzyme does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, alpha-amylase, 4-α-D-glucan glucanohydrolase, EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase, *Bacillus subtilis*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 entered 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 European Union procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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.

Four applications have been submitted by Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and the companies 'DSM Food Specialities B.V.' and 'Amano Enzyme Inc.' for the food enzymes Bacillolysin from *Bacillus amyloliquefaciens* and/or *Bacillus subtilis*, Alpha-amylase from *Bacillus licheniformis*, Alpha-amylase from a genetically modified strain of *B. subtilis* (strain NBA) and Alpha-amylase from *Aspergillus oryzae* (strain AE-AA), respectively.

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 applications falls within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the EFSA to carry out the safety assessments on the food enzymes Bacillolysin from *B. amyloliquefaciens* and/or *B. subtilis*, Alpha-amylase from *B. licheniformis*, Alpha-amylase from a genetically modified strain of *B. subtilis* (strain NBA) and Alpha-amylase from

[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 1493/199, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 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, p. 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, p. 15–24.

A. oryzae (strain AE-AA) 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 alpha-amylase from a genetically modified (GM) strain of *B. subtilis* (strain NBA).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from a GM *B. subtilis* (strain NBA).

The additional information was requested from the applicant during the assessment process on 13 July 2017, on 23 February 2018 and on 28 November 2018 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 13 July 2017, the applicant requested a clarification teleconference, which was held on 10 August 2017.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009) and following the Scientific Opinion on Guidance on the risk assessment of GM microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011) and following the relevant existing guidances' of EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA CEF Panel, 2009) has been followed for the evaluation of this application with the exception of the exposure assessment, which was carried out in accordance with the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Alpha-amylase
Systematic name:	1,4-α-D-glucan glucanohydrolase
Synonyms:	4-α-D-glucan glucanohydrolase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6.

The enzyme α-amylase catalyses the hydrolysis of α-1,4-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides. It is intended to be used in baking processes.

3.1. Source of the food enzyme

The α-amylase is produced with a GM strain of *B. subtilis*.

The production strain *B. subtilis* NBA is deposited in the [redacted].[4] [redacted].[5]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted]

[4] Technical dossier/Additional information February 2019/Annex 2.
[5] Technical Dossier/1st submission/ Annex II-2

[redacted][7].

3.1.2. Characteristics of the introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

[redacted][8].

3.1.4. Safety aspects of the genetic modification

The production strain *B. subtilis* NBA differs from the recipient strain [redacted] in its capability to produce the α-amylase enzyme [redacted].

The absence of the antibiotic resistance genes used during the genetic modification was confirmed [redacted][9].

Phenotypic stability of the *B. subtilis* NBA strain was confirmed by its capacity to produce a constant level of the enzyme α-amylase measured in relation to the total organic solids (TOS) in three

[6] Technical dossier/Additional information April 2018/Q3.
[7] Technical dossier/Additional information January 2018/Annex 3.
[8] Technical Dossier/ 1st submission/Annex II-3.
[9] Technical Dossier/1st submission/ Annex II-11.

independent batches of the food enzyme. The genetic stability of the production strain *B. subtilis* NBA was confirmed [redacted] from three different commercial production batches.

The parental strain [redacted] meets the required qualifications to be considered as a Qualified Presumption of Safety (QPS) organism (identity established and the absence of acquired antibiotic resistance genes of concern and the absence of toxigenic activity demonstrated) and is therefore presumed to be safe. The production strain shares identity with the parental strain and also is not cytotoxic in tests made with [redacted]. Since the introduced genetic modifications do not raise safety concerns, the presumption of safety made for the parental strain can be extended to the production strain (EFSA BIOHAZ Panel, 2018).

3.2. Production of the food enzyme

The food enzyme is manufactured according to Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP) and in accordance with current Good Manufacturing Practice (GMP).[11]

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, cells are killed and the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. 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 weight material passes the filtration membrane and is discarded. The applicant provided information on the identity and analysis of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[12]

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

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The α-amylase is a single polypeptide chain of 719 amino acids (including a signal sequence of 33 amino acids).[13] The molecular mass, based on the amino acid sequence, with the signal sequence cleaved off, was calculated to be about 75 kDa.[13] The homogeneity of the food enzyme was investigated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis which showed an apparent molecular mass of about 66 kDa.[14] No enzymatic side activities were reported.

The in-house determination of α-amylase activity is based on the hydrolysis of a synthetic substrate *p*-nitrophenyl maltoheptaoside with a blocked non-reducing end (BPNPG7) in the presence of excess levels of α-glucosidase and amyloglucosidase. The oligosaccharide component of BPNPG7 is attacked by the α-amylase releasing *p*-nitrophenyl maltosaccharide fragments (reaction conditions: 37°C, pH 5.2) which are in turn hydrolysed by the other two enzymes to free glucose and *p*-nitrophenol which is determined spectrophotometrically at 405 nm. One New Baking Activity Unit (NBAU) is defined as the amount of enzyme required to release 1 micromole of *p*-nitrophenol per minute from BPNPG7 under the conditions described for the assay.[15]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 65°C (pH 5.0) and a pH optimum around pH 5.0 (37°C).[16] No information was provided on the thermostability of the enzyme. However, it is noted that the food enzyme mirrors that derived from a thermotolerant source and that the activity profile shows retention of approximately 80% of activity when assayed at 80°C.

[10] 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, 321 pp.
[11] Technical dossier/1st submission/p. 51–59.
[12] Technical dossier/1st submission/Annex I-7 and Additional information January 2018.
[13] Technical dossier/1st submission/p. 44.
[14] Technical dossier/1st submission/p. 42.
[15] Technical dossier/1st submission/Annex I-2.
[16] Technical dossier/1st submission/p. 46.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for four food enzyme batches, three batches used for commercialisation and one batch used for the toxicological tests (Table 1). The average TOS content of the three commercial enzyme batches was 7.0% (range 5.2–8.5%). The average enzyme activity/TOS ratio of the three batches for commercialisation is 13.4 Units/mg TOS.

Table 1: Compositional data provided for the food enzyme

Parameter	Unit	Batches			
		1	2	3	4[(a)]
α-amylase activity	Units/g batch[(b)]	866	876	945	814
Protein	%	4.1	3.5	3.0	3.9
Ash	%	1.6	1.8	1.1	1.2
Water	%	89.9	90.8	93.7	89.9
Total Organic Solids (TOS)[(c)]	%	8.5	7.4	5.2	8.9
α-amylase activity/mg TOS	Units/mg TOS	10.2	11.8	18.2	9.1

(a): Batch used for toxicological tests; Technical dossier/Additional information April 2018/ Annex 2-4.
(b): Units/g batch: α-amylase activity (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The food enzyme complies with the specification for lead (≤ 5 mg/kg)[17,18] as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and that the count of total coliforms should not exceed 30 CFU (Colony Forming Units) per gram.[19] No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).[19]

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the product was demonstrated [redacted] No growth of the *B. subtilis* production strain was detected.[20]

No recombinant DNA was found [redacted].[21]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats made with the food enzyme produced with *B. subtilis* NBA was provided. However, the Panel considers no toxicological studies other than assessment of allergenicity necessary. This is based on the QPS status of the production strain and the absence of any hazards from the product and downstream processing.

[17] Technical dossier/1st submission/p. 38, Annex 2.04 and Annex I-3.
[18] LOD: Pb = 1 mg/kg.
[19] Technical dossier/1st submission/Annex I-3.
[20] Technical dossier/Additional information January 2018.
[21] Technical dossier/1st submission/Annex II-14, Annex II-11; Technical dossier/Additional information January 2018.

3.4.1. Allergenicity

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

The potential allergenicity of this α-amylase produced with the GM *B. subtilis* strain NBA was assessed by comparing its amino acid sequence[22] with those of known allergens according to the EFSA Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on GM Organisms (EFSA GMO Panel, 2017). Using higher than 35% identity in a window of 80 amino acids as the criterion, two matches were found. The matching allergens were Asp o 21, an α-amylase from *A. oryzae* and Sch c 1 a glucoamylase produced by *Schizophyllum commune*.

No information is available on oral sensitisation or elicitation reactions of this α-amylase from *B. subtilis* strain NBA. Both glucoamylase from *S. commune* (Toyotome et al., 2014) and α-amylase from *A. oryzae* (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002) are known as occupational respiratory allergens associated with baker's asthma. However, several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucoamylase.

The Panel considers that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at the recommended use level up to 7.8 mg TOS/kg flour.[23]

In baking processes, the food enzyme is added to flour during the preparation of dough. The α-amylase hydrolyses starch from granules that have been damaged during milling and release fermentable sugars and dextrins. This reaction shortens the processing time and decreases dough viscosity. The latter facilitates the handling of the dough, resulting in more uniform products with better properties.[24]

The food enzyme remains in the dough. Considering the thermophilic nature of the microbial source and the activity of the food enzyme at 80°C (see Section 3.3.1), the α-amylase may not be fully inactivated during baking processes.

3.5.2. Dietary exposure estimation

For baking processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period 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

[22] Technical dossier/1st submission/p. 71.
[23] Technical dossier/1st submission/p. 64.
[24] Technical dossier/1st submission/Section: 3.2.1.4.A.

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 2 provides an overview of the derived exposure estimates across all surveys. Detailed average 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 2: 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 of means (number of surveys)	0.001–0.022 (10)	0.016–0.047 (14)	0.019–0.045 (19)	0.010–0.029 (18)	0.008–0.018 (19)	0.008–0.016 (18)
Min–max of 95th percentiles (number of surveys)	0.009–0.093 (8)	0.041–0.079 (12)	0.037–0.085 (19)	0.023–0.058 (17)	0.017–0.035 (19)	0.015–0.028 (18)

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 3.

Table 3: 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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment based on the description of the food process provided by the applicant	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

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

Based on the data provided, the Panel concludes that the food enzyme α-amylase produced with the GM *B. subtilis* strain NBA does not give rise to safety concerns under the intended conditions of use.

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

Documentation provided to EFSA

1) Dossier 'Alpha-amylase from *B. subtilis* (strain NBA)'. December 2014. Submitted by DSM Food Specialities.
2) Additional information, January 2018. Submitted by DSM Food Specialities B.V.
3) Additional information, April 2018. Submitted by DSM Food Specialities B.V.
4) Additional information, February 2019. Submitted by DSM Food Specialities B.V.
5) 'Summary report on GMM for α-amylase produced by *B. subtilis* strain NBA'. February 2016. Delivered by DTU (Denmark).
6) 'Summary report on technical data and dietary exposure related to α-amylase from a strain of *B. subtilis* (strain NBA [redacted])'. May 2016. Delivered by Hylobates Consulting and BiCT (Italy, Germany).
7) 'Summary report on genotoxicity and subchronic toxicity study related to alpha-amylase produced with a strain of *B. subtilis* (strain NBA) by DSM Food Specialities'. November 2016. Delivered by FoBiG (Germany).

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Abbreviations

CAS	Chemical Abstracts Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	Panel on Food Contact Materials, Enzymes, Processing Aids
CFU	Colony Forming Units
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation
GM	Genetically Modified
GMP	Good Manufacturing Practice
g	gram
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilo Dalton

NBAU	New Baking Activity Unit
QPS	Qualified Presumption of Safety
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TOS	Total Organic Solids
WHO	World Health Organisation

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.5681).

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: The contribution of FoodEx categories to the food enzyme–TOS dietary exposure

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, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(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).