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SCIENTIFIC OPINION

Safety evaluation of the food enzyme endo-1,4-β-xylanase from the genetically modified *Bacillus velezensis* strain AR-112

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Abstract

The food enzyme endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase, EC 3.2.1.8) is produced with the genetically modified *Bacillus velezensis* strain AR-112 by AB Enzymes GmbH. 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 baking processes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.024 mg TOS/kg body weight (bw) per day in European populations. As the production strain *B. velezensis* strain AR-112 meets the requirements for the qualified presumption of safety (QPS) approach to safety assessment and no issue of concern arose from the production process, no toxicological data are required. 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 considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure cannot be excluded, 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

4-β-D-xylan xylanohydrolase, *Bacillus velezensis*, EC 3.2.1.8, endo-1,4-β-xylanase, food enzyme, genetically modified microorganism

CONTENTS

1 | INTRODUCTION

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

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

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

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

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

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

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

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008[3] on food enzymes.

On 03 February 2022, a new application has been introduced by the applicant "AB ENZYMES GmbH" for the authorisation of the food enzyme endo-1,4-β-xylanase from a genetically modified strain of *Bacillus velezensis* (AR-112).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: endo-1,4-β-xylanase from a genetically modified strain of *Bacillus velezensis* (AR-112), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[4]

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-β-xylanase from a genetically modified *Bacillus velezensis* strain AR-112.

Additional information was requested from the applicant during the assessment process on 21 September 2022 and received on 22 March 2023 (see 'Documentation provided to EFSA').

[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]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.

[4]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.

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 relevant guidance documents of the EFSA Scientific Committee.

The 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) have been followed for the evaluation of the application.

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[5] and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 31 October to 21 November 2023, for which no comments were received.

3 | ASSESSMENT

IUBMB nomenclature	Endo-1,4-β-xylanase
Systematic name	4-β-D-xylan xylanohydrolase
Synonyms	Xylanase; endo-1,4-D-β-xylanase
IUBMB No	EC 3.2.1.8
CAS No	9025-57-4
EINECS No	232-800-2

Endo-1,4-β-xylanases catalyse the random hydrolysis of 1,4-ß-D-xylosidic linkages in xylans (including arabinoxylans) resulting in the generation of (1- > 4)-ß-D-xylan oligosaccharides. The enzyme under this assessment is intended to be used in baking processes.

3.1 | Source of the food enzyme

The endo-1,4-β-xylanase is produced with the genetically modified bacterium *B. velezensis* strain AR-112 [redacted] which is deposited [redacted][6] The production strain was identified as *B. velezensis* by whole genome sequencing (WGS) analysis, with an average nucleotide identity value [redacted][7]

The species *B. velezensis* 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, 2022).

The production strain *B. velezensis* AR-112 was found not to be cytotoxic to VERO cells.[8] The WGS of the production strain was interrogated for the presence of antimicrobial resistance genes using two databases [redacted][9]

Therefore, the production strain is considered to qualify for the QPS approach.

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental strain is *B. velezensis* [redacted] the genes [redacted] were deleted.[10]

[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[6]Technical dossier/ Source of the food enzyme/Annex 1.

[7]Technical dossier/Additional data March 2023 Annex 2.

[8]Technical dossier/ Source of the food enzyme/Annex 4.

[9]Technical dossier/Additional data March 2023 Annex 3.

[10]Technical dossier/ Source of the food enzyme/Annex 5.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the endo-1,4-β-xylanase [redacted]

3.1.3 | Description of the genetic modification process

The aim of the genetic modification was to enable the production strain [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 *B. velezensis* strain AR-112 differs from the recipient strain in its capacity to [redacted]. The absence of the antimicrobial resistance genes used during the genetic modification was confirmed by WGS.

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,[13] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[14]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] fermentation system with conventional process controls in place. After completion of the fermentation the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[15] 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.[16]

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

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The endo-1,4-β-xylanase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is around [redacted] kDa.[17] The food enzyme was analysed by sodium dodecyl sulfate-

[11]Technical dossier/ Source of the food enzyme/Annexes 7,8.

[12]Technical dossier/ Source of the food enzyme/Annex 6.

[13]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.

[14]Technical dossier/ Manufacturing process of the food enzyme/dossier p.29.

[15]Technical dossier/ Manufacturing process of the food enzyme/Annex 11.

[16]Technical dossier/ Manufacturing process of the food enzyme/Annex 10.

[17]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 14.

polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected mass of the enzyme. The protein profile also included bands of lesser staining intensity.[18] No other enzyme activities were reported.

The in-house determination of endo-1,4-β-xylanase activity is based on [redacted] The enzyme activity is expressed in XylH/g. [redacted]

The food enzyme has a temperature optimum around [redacted] and a pH optimum around pH [redacted] Thermostability was tested after a pre-incubation of the food enzyme at 85°C at different times [redacted] Enzyme activity decreased by [redacted] of pre-incubation, showing no residual activity after [redacted] of pre-incubation at 85°C.[22]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 12.4% and the mean enzyme activity/TOS ratio was 27.0 XylH/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
Endo-1,4-β-xylanase activity	XylH/g[a]	5497	2981	2033
Protein	%	8	5.6	5.65
Ash	%	0.5	0.5	0.5
Water	%	84.1	87.8	89.3
Total organic solids (TOS)[b]	%	15.4	11.7	10.2
Activity/TOS	XylH/mg TOS	35.7	25.5	19.9

[a]XylH: Xylanase 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 0.05 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, arsenic, mercury and cadmium contents were below the limits of quantification (LoQ) of the employed methods.[24,25]

The food enzyme preparation complies with the criteria for total coliforms, *Escherichia coli* and *Salmonella* as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[26]

The presence of aflatoxin B1, B2, G1, G2, fumonisin B1, B2, B3, ochratoxin A, deoxynivalenol, sterigmatocystin, zearalenone, T2-toxin and HT2-toxin was examined in the three food enzyme preparation batches and were below the LoQ of the applied analytical methods.[27,28] For aflatoxin B1 and aflatoxin G1, the average concentration determined in one of the commercial batches was 0.3 μg/kg and 0.1 μg/kg, respectively. The Panel considered these concentrations as not of concern.

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

[18]Technical dossier/Additional data March 2023 Annex 13.

[19]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 16.

[20]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 17/ Appendix 3.

[21]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 17/ Appendix 2.

[22]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 17/ Appendix 1.

[23]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 15/Appendix A, Certificate of Analysis & dossier p. 34.

[24]LoQs: Pb, Cd, Hg = 0.05 mg/kg each; As = 0.5 mg/kg.

[25]Technical dossier/Chemical composition, properties and purity of the food enzyme/Annex 15/ Appendix C & Certificates of analysis 2021 – Annex 20/Method 4 & Methods and LoDs.

[26]Technical dossier/Chemical composition, properties and purity of the food enzyme/Annex 15/Appendix C & Certificates of analysis 2021 – Annex 20/Method 2–7 & Methods and LoDs.

[27]LoQ: aflatoxins B1, B2, G1 and G2 = 0.1 μg/kg each; fumonisins B1, B2, B3 = 20 μg/kg each; ochratoxin A = 2 μg/kg; sterigmatocystin = 10 μg/kg; deoxynivalenol = 20 μg/kg; zearalenone = 10 μg/kg; T2-toxin = 10 μg/kg; HT2-toxin = 10 μg/kg.

[28]Technical dossier/Chemical composition, properties and purity of the food enzyme/Annex 15/Appendix A & Certificates of analysis 2021.

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.[29]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify [redacted] with a limit of detection of 10 ng spiked DNA/g food enzyme.[30]

3.4 | Toxicological data

As the production strain qualifies for the QPS approach of safety assessment and no issue of concern arising from the production process of the food enzyme were identified (see Sections 3.1, 3.2 and 3.3), the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary (EFSA CEP Panel, 2021).

3.4.1 | Allergenicity

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

The potential allergenicity of the endo-1,4-β-xylanase produced with the genetically modified *B. velezensis* strain AR-112 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.[31]

No information is available on oral and respiratory sensitisation or elicitation reactions of this endo-1,4-β-xylanase.

Respiratory allergy, e.g. baker's asthma, following occupational exposure to xylanase has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al., 1998; Merget et al., 2001). However, several studies have shown that adults with occupational asthma caused by an enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Poulsen, 2004).

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the 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 considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is 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 a recommended use level of 0.3–2 mg TOS/kg flour.[32]

In baking processes, the food enzyme is added to flour during the preparation of the dough.[33] The endo-1,4-β-xylanase hydrolyses (arabino)xylans, which interacts with gluten and binds water, thus reducing the dough viscosity and shortening the processing time. The decrease in viscosity facilitates the handling of the dough and results in more uniform products. The food enzyme–TOS remains in the final foods.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that the enzyme is inactivated during baking processes.[34]

[29]Technical dossier/Additional data March 2023/Annex 1.

[30]Technical dossier/ Chemical composition, properties and purity of the food enzyme/Annex 19.

[31]Technical dossier/Allergenicity/pp. 43–44 and Annex 21.

[32]Technical dossier/Risk assessment/Intended uses.

[33]Technical dossier/Risk assessment/Intended uses.

[34]Technical dossier/Risk assessment/Reaction and fate in food.

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, 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 2 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 was estimated to be 0.024 mg TOS/kg body weight (bw) per day in infants at the 95th percentile.

TABLE 2 Summary of the estimated dietary exposure to the 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)	0–0.006 (11)	0.004–0.012 (15)	0.005–0.012 (19)	0.003–0.007 (21)	0.002–0.004 (22)	0.002–0.004 (22)
Min–max 95th percentile (number of surveys)	0.002–0.024 (9)	0.011–0.020 (13)	0.009–0.022 (19)	0.006–0.015 (20)	0.004–0.009 (22)	0.004–0.007 (21)

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	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

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

3.6 | Margin of exposure

Given the QPS status of the production strain and the lack of hazards resulting from the food enzyme manufacturing process, toxicity tests were considered unnecessary by the Panel and the margin of exposure was not calculated.

4 | CONCLUSIONS

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process of the food enzyme, the Panel concluded that the food enzyme endo-1,4-β-xylanase produced with the genetically modified *B. velezensis* strain AR-112 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 an endo-1,4-β-xylanase from a genetically modified strain of *Bacillus velezensis*. January 2022. Submitted by AB Enzymes GmbH.

Additional information. March 2023. Submitted by AB Enzymes GmbH.

ABBREVIATIONS

AMR	antimicrobial resistance
bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural 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
LoQ	limit of quantification
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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

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SUPPORTING INFORMATION

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

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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
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, 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, 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, the 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, the 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, the 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).