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Safety evaluation of the food enzyme β-galactosidase from the genetically modified *Kluyveromyces lactis* strain KLA

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Abstract

The food enzyme β-galactosidase (β-D-galactoside galactohydrolase; EC 3.2.1.23) is produced with the genetically modified *Kluyveromyces lactis* strain KLA by DSM Food Specialties B.V. The genetic modifications did not give rise to safety concerns. The food enzyme was considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used for the lactose hydrolysis in milk processing, production of fermented milk products and whey processing. It is also intended for lactose hydrolysis in milk products at home. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 11.876 mg TOS/kg body weight per day in European populations. The production strain of the food enzyme fulfils the requirements for the Qualified Presumption of Safety (QPS) approach to safety assessment. As no concerns arising from its genetic modification or from the manufacturing process have been identified, the Panel considered that toxicological tests are not needed for the assessment of this food enzyme. A search for 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 by dietary exposure cannot be excluded, but the likelihood for this to occur is low. The Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: β-Galactosidase, β-D-galactoside galactohydrolase; EC 3.2.1.23, lactase, *Kluyveromyces lactis*, genetically modified microorganism

Requestor: European Commission

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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 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.

An application has been introduced by the applicant "DSM Food Specialties B.V." for the authorisation of the food enzyme β-galactosidase from a genetically modified strain of *Kluyveromyces lactis* (strain KLA).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls 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 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 assessment on the following food enzyme: β-galactosidase from a genetically modified strain of *Kluyveromyces lactis* (strain KLA), in

[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.

accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β-galactosidase from a genetically modified strain of *K. lactis* strain KLA.

Additional information was requested from the applicant during the assessment process on 12 January 2022 and subsequently provided (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 '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	β-galactosidase
Systematic name	β-D-galactoside galactohydrolase
Synonyms	Lactase; β-D-lactosidase
IUBMB No	3.2.1.23
CAS No	9031-11-2
EINECS No	232-864-1

β-Galactosidases catalyse the hydrolysis of lactose to its monosaccharide units, D-galactose and D-glucose. The food enzyme is intended to be used for the lactose hydrolysis in milk processing, production of fermented milk products and whey processing. The food enzyme is also intended for lactose hydrolysis in infant formula and follow-on formula at home.

3.1. Source of the food enzyme

The β-galactosidase is produced with the genetically modified yeast *K. lactis* strain KLA [redacted], which is deposited at the Westerdijk Fungal Biodiversity Institute culture collection (CBS, The Netherlands), with deposit number [redacted].[4] The production strain was identified as *K. lactis* by whole genome sequence (WGS) analysis [redacted].[5]

The species *K. lactis* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied (EFSA BIOHAZ Panel, 2020).[6]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted]

[4] Technical dossier/Annex II-12.
[5] Technical dossier/Annex II-2.
[6] https://zenodo.org/record/4917383#.YYqMLBrMKUk

3.1.2. Characteristics of introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

The objective of the genetic modification was to allow the production strain to overproduce β-galactosidase. [redacted]

[redacted]

[redacted]

[7] Technical dossier/Annexes II-6 to II-10.
[8] Technical dossier/Annex II-3.
[9] Technical dossier/Annex II-4.
[10] Technical dossier/Annex II-5.
[11] Technical dossier/Annex II-11.
[12] Technical dossier/Additional information April 2022/Q-2021-00311 Additional information to EFSA on KLA part II.

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.

[redacted].[5]

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, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, cells are killed [redacted] to aid the release of the intracellular enzyme. The solid biomass is then removed from the fermentation broth by filtration. The filtrate containing the enzyme is further purified and concentrated, including an ultrafiltration step in which the 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 β-galactosidase is a single polypeptide chain of 1,025 amino acids.[17] The molecular mass of the mature protein, calculated from the amino acid sequence, is 117.6 kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about 116 kDa, consistent with the expected mass of the enzyme.[18] No other enzymatic activities were reported.[19]

The in-house determination of β-galactosidase activity is based on the hydrolysis of lactose (reaction conditions: pH 6.5, 37°C, 30 min). The enzymatic activity is determined by measuring the release of glucose spectrophotometrically at 340 nm using a commercial glucose hexokinase test. The enzyme activity is expressed in Lactose Hydrolysis Units (LHU)/g. One LHU is defined as the amount of enzyme that liberates 0.81 μmol glucose per minute under the conditions of the assay.[20]

The food enzyme has a temperature optimum around 39°C (pH 6.5) and a pH optimum between pH 6.5 and pH 7.0 (37°C). Thermostability was tested after an incubation of the food enzyme for 2 min at different temperatures in phosphate buffer (pH 6.5) and in lactose-free milk. No enzyme activity was detected following pretreatment at 55°C in phosphate buffer and at 60°C in lactose-free milk.[21]

[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/p. 49/Annex I-6.
[15] Technical dossier/p. 49-57/Annex I-7.
[16] Technical dossier/Annex I-8.
[17] Technical dossier/p. 41/Annex I-5.
[18] Technical dossier/p. 39.
[19] Technical dossier/p. 42–43.
[20] Technical dossier/p. 42/Annex I-2.
[21] Technical dossier/p. 43–44.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[22] The mean total organic solids (TOS) of the three food enzyme batches is 16.6% and the mean enzyme activity/TOS ratio is 178.9 LHU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
β-galactosidase activity	LHU/g batch[(a)]	25,800	31,800	31,500
Protein	%	10.4	12.3	12.6
Ash	%	0.5	0.4	0.5
Water	%	85.1	81.9	81.8
Total organic solids (TOS)[(b)]	%	14.4	17.7	17.7
Activity/mg TOS	LHU/mg TOS	179.2	179.7	178.0

(a): LHU: Lactose Hydrolysis 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 0.1 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).[23,24]

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).[23] No antimicrobial activity was detected in any of the tested batches.[23]

The residual amounts of [redacted] were measured in three batches of the food enzyme and the results were below the limit of quantification (LoQ) of the method used, raising no concern.[25,26]

The Panel considered that the information provided on the purity of the food enzyme is 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 [redacted][27]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted][28]

3.4. Toxicological data

As the production strain qualifies for the QPS approach for safety assessment and as 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 assessment of allergenicity were necessary.

[22] Technical dossier/p. 38/Annexes: I-1, I-2, I-3.
[23] Technical dossier/p. 41/Annexes: I-3, I-4.
[24] LoD: Pb = 0.01 mg/kg.
[25] Technical dossier/ Additional information April 2022/Q-2021-00311 Additional information to EFSA on KLA part I.
[26] LoQ for [redacted] = 10 mg/kg.
[27] Technical dossier/Annex II-13.
[28] Technical dossier/Annex II-14.

3.5. Allergenicity

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

The potential allergenicity of the β-galactosidase produced with the genetically modified *K. lactis* strain KLA 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.[29]

No information was available on oral and respiratory sensitisation or elicitation reactions of this β-galactosidase. Occupational exposure to β-galactosidases have been reported to lead to respiratory and skin sensitisation (Mapp, 2001). Several studies have shown that occupationally sensitised adults may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Only one case of an anaphylactic reaction due to lactase, where the lactase was ingested as a tablet, has been reported (Voisin and Borici-Mazi, 2016).

[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 fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

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 of such reactions to occur is low.

3.6. Dietary exposure

3.6.1. Intended use of the food enzyme

The food enzyme is intended to be used in three industrial food manufacturing processes and for home use 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[(d)]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b),(c)]
Lactose hydrolysis in milk processing	Milk	2–**130**
Production of fermented milk products	Milk	2–**130**
Whey processing	Liquid whey	2–**130**
Lactose hydrolysis in milk products at home	Infant formula, follow-on formula, and other milk products	18–**21**

(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): Based on 178.96 LHU/mg TOS.
(c): The numbers in bold were used for calculation.
(d): Technical dossier/p.64 & Additional information April 2022.

Two different dairy materials can be treated with this food enzyme: milk or whey. β-Galactosidase hydrolyses lactose to release glucose and galactose. The treatment makes milk more suitable for lactose-intolerant individuals and sweeter.[30] Adding β-galactosidase together with microbial cultures during fermentation results in lactose-reduced yoghurt.[31] Treatment of the cheese whey or whey

[29] Technical dossier/p. 70-71/Annex I-11.
[30] Technical dossier/p. 85.
[31] Technical dossier/p. 86.

permeate results in lactose-reduced and sweeter whey syrups.[32] No separation step is applied to remove the food enzyme TOS from the treated milk, fermented milk products or whey syrup.

The enzymatically treated milk or whey can be consumed directly, but also can be used as an ingredient in a large variety of foods. This includes infant formula, follow-on formula and foods for special medical purposes.[33] The enzymatic treatment also prevents the sandiness caused by lactose crystallisation in frozen desserts such as ice cream.

The applicant provided a lower use level for treating milk at home.[34] Consumers may choose to add β-galactosidase into milk in order to produce lactose-reduced milk or yoghurt on their own. The lower use level compared to the one applied in industrial applications was explained by longer processing time when compared to regular dairy processing.[34]

Based on data provided on thermostability (see Section 3.3.1), it is expected that the β-galactosidase is inactivated during the pasteurisation step.

3.6.2. 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 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 41 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 about 11.876 mg TOS/kg body weight (bw) per day in infants 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)	0.258–3.223 (11)	0.363–4.804 (15)	0.971–4.133 (19)	0.186–1.530 (21)	0.194–0.670 (22)	0.075–0.604 (22)
Min–max 95th percentile (number of surveys)	1.111–11.876 (9)	5.053–11.121 (13)	2.227–6.847 (19)	0.652–3.210 (20)	0.588–1.936 (22)	0.598–1.389 (21)

3.6.3. 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.

[32] Technical dossier/p. 87.
[33] Technical dossier/p. 89 and Additional information April 2022.
[34] Technical dossier/Additional information April 2022/Part I.

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	+/–
Consumption survey for infants below 3 months of age are not included, due to limited availability	+/–
Model assumptions and factors	
The use of an assumption that the 50% of dairy protein in regular infant formula and follow-on formula are from milk and 50% from whey	+/–
Exposure from whey processing considered both cheese whey and acid whey as raw material, although this food enzyme targets only cheese whey	+
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	+/–

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 of 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.

3.7. 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, the Panel concluded that the food enzyme β-galactosidase produced with the genetically modified *K. lactis* strain KLA does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Application for authorization of β-galactosidase from a genetically modified strain of *Kluyveromyces lactis* (strain KLA). September 2021. Submitted by DSM Food Specialties B.V.

Additional information. April 2022. Submitted by DSM Food Specialties B.V.

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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
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
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
LoD	limit of detection
LoQ	limit of quantification
PCR	polymerase chain reaction
QPS	Qualified Presumption of Safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization
WGS	whole genome sequence

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.2022.7575#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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, 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).