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Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Saccharomyces cerevisiae* strain LALL-LI

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is produced with the genetically modified *Saccharomyces cerevisiae* strain LALL-LI by Lallemand Inc. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism, but not from recombinant 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 0.42 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. Therefore, the Panel considered that toxicological tests are not needed for the assessment of this food enzyme. 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 by 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: food enzyme, triacylglycerol lipase, triacylglycerol acylhydrolase, lipase, *Saccharomyces cerevisiae*, 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 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.

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.

On 13 April 2022, a new application has been introduced by the applicant "Lallemand Inc." for the authorisation of the food enzyme Triacylglycerol lipase from a genetically modified strain of *Saccharomyces cerevisiae* (strain LALL-LI).

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: Triacylglycerol lipase from a genetically modified strain of *Saccharomyces cerevisiae* (strain LALL-LI), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.[2]

[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.

2. Data and Methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme triacylglycerol lipase from a genetically modified strain of *Saccharomyces cerevisiae* (strain LALL-LI).

Additional information was requested from the applicant during the assessment process on 17 February 2022 and consequently 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 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a) has been followed for the evaluation of the application.

3. Assessment

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase; triglyceride lipase
IUBMB No	EC 3.1.1.3
CAS No	9001-62-1
EINECS No	232-619-9

Triacylglycerol lipases catalyse, in the presence of water, the hydrolysis of the ester linkages in triacylglycerols, resulting in the generation of glycerol, free fatty acids, diacylglycerols and monoacylglycerols. The enzyme under assessment is intended to be used in baking processes.

3.1. Source of the food enzyme

The enzyme is produced with the genetically modified yeast *S. cerevisiae* LALL-LI, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with the deposit number DSM 34129.[3] The production strain was identified as *S. cerevisiae* by whole genome sequence (WGS) analysis.[4]

The species *S. cerevisiae* is included in the list of organisms for which the qualified presumption of safety (QPS) may be applied, provided that the absence of resistance to antimycotics used for medical treatment of yeast infections is verified in cases where viable cells are added to the food or feed chain (EFSA BIOHAZ Panel, 2022).

3.1.1. Characteristics of the parental microorganism

The parental strain is *S. cerevisiae* M10580, a yeast strain used in industrial bakery.[5]

3.1.2. Characteristics of the introduced sequences

The sequence encoding the triacylglycerol lipase ([redacted]) derives from *Fusarium oxysporum* and was codon-optimised for the expression in *S. cerevisiae*. The rest of the introduced sequences were all from *S. cerevisiae*.

[redacted]

[3] Technical dossier/Risk assessment/Source of the food enzyme/Annex 4.
[4] Technical dossier/Risk assessment/Source of the food enzyme/Annex 1.
[5] Technical dossier/Risk assessment/Source of the food enzyme.

[redacted]

An insertion cassette [redacted] was constructed [redacted][5]

Plasmid [redacted].[5]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to allow the production strain to synthesise triacylglycerol lipase from *F. oxysporum*.

For this purpose, the parental strain was co-transformed with the linearised [redacted] insertion cassette and the [redacted] plasmid by electroporation. The [redacted] cassette was integrated into the [redacted] by homologous recombination. Subsequently, the strain was cured from [redacted], resulting in the production strain *S. cerevisiae* LALL-LI.[5]

WGS analysis showed the integration of the full [redacted] cassette replacing the [redacted]. No other integration sites were detected.[4]

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 *S. cerevisiae* LALL-LI differs from the recipient strain in its capacity to produce triacylglycerol lipase from *F. oxysporum* and its ability to grow in the presence of [redacted] (due to the lack of [redacted]). The absence of [redacted] plasmid sequences, including the [redacted], was confirmed by WGS analysis.[4]

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[6], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[7]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[8] 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.[9]

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 triacylglycerol lipase is a single polypeptide chain of 331 amino acids.[10] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 35 kDa.[11] The food enzyme

[6] 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.
[7] Technical dossier/Manufacturing process of the food enzyme/p. 1.
[8] Technical dossier/Manufacturing process of the food enzyme/pp. 1–6.
[9] Technical dossier/Manufacturing process of the food enzyme/pp. 2–3.
[10] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 1 and Annex 28.
[11] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 5.

was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about 27 kDa, which, according to the applicant, differs from the calculated mass due to a Kex2-mediated truncation.[12] No other enzymatic activities were reported.

The in-house determination of the triacylglycerol lipase activity is based on the hydrolysis of tributyrin (reaction conditions: pH 7.0, 21°C, 10 min), measuring the release of butyric acid that is titrated with sodium hydroxide. The enzyme activity is expressed in Lallemand Baking Lipase Unit (LBLU). One LBLU is defined as the quantity of enzyme that produces 1 μM of butyric acid per minute under the assay conditions.[13]

The food enzyme has a temperature optimum around 34°C (pH 5.0) and a pH optimum around pH 5.0 (30°C). Thermostability was tested after a pre-incubation for 10 min at different temperatures. The activity decreased above 50°C, showing no residual activity above 70°C.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three large-scale pilot batches (Table 1).[15,16,17] The mean total organic solids (TOS) was 3.4% and the mean activity/TOS ratio 57.0 LBLU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches		
		1	2	3
Triacylglycerol lipase activity	LBLU/g[(a)]	2,348	2,237	1,127
Protein	%	1.8	1.3	1.3
Ash	%	2.0	1.8	1.9
Water	%	93.2	94.8	96.1
Total organic solids (TOS)[(b)]	%	4.8	3.4	2.0
Triacylglycerol lipase activity/TOS	LBLU/mg TOS	48.9	65.8	56.3

(a): LBLU: Lallemand Baking Lipase Unit (see Section 3.3.1).
(b): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three large-scale pilot batches was below 0.05 mg/kg,[18] 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, cadmium and mercury contents were below the limits of quantification (LoQ) of the employed methods.[19,20]

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).[21,22] No antimicrobial activity was detected in any of the tested batches.[23,24]

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

[12] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 1 and Annex 5.
[13] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/pp. 1–2 and Annex 29.
[14] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 2 and Annex 6.
[15] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme /pp. 2–3 and Annexes: 7, 8, 9, 10, 11, 12.
[16] Technical dossier/Risk assessment /Methods of Analysis/Annexes: 30, 31.
[17] Technical dossier/2022-12-19_Reply to ADR (1)/Chemical composition, properties and purity of the food enzyme/3. Chemical composition, properties and purity of the food enzyme v2.
[18] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3 and Annexes: 19, 20, 21.
[19] LoQs: Pb = 0.05 mg/kg; As = 0.1 mg/kg; Cd = 0.01 mg/kg; Hg = 0.005 mg/kg.
[20] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3 and Annexes: 19, 20.
[21] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3 and Annexes: 13, 14, 15, 16, 17.
[22] Technical dossier/Risk assessment /Methods of Analysis/Annex 24.
[23] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/p. 3 and Annex 18.
[24] Technical dossier/Risk assessment /Methods of Analysis/Annex 25.

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. One millilitre of product was centrifuged and most of the supernatant discarded. The pellet was then resuspended in the remaining liquid, spread on selective agar plates and incubated at 30°C for 24 h.[25] No colonies were produced. A positive control was included.[26]

The absence of recombinant DNA in the food enzyme was tested by polymerase chain reaction (PCR) analysis of three batches of the food enzyme tested in triplicate, targeting a 926-bp fragment that is specific for the triacylglycerol-lipase-encoding gene.[27] Amplification was observed in two replicates of one of the samples, with a limit of detection of 0.1 ng spiked DNA/g food enzyme.[28]

3.4. Toxicological data

No safety concerns arose from the genetic modification of the production strain (Section 3.1.4) and the absence of viable cells in the food enzyme was demonstrated (Section 3.3.4). Therefore, the production strain was considered to qualify for the QPS approach. In addition, no issue of concern arising from the production process of the food enzyme was identified (see Sections 3.1, 3.2 and 3.3). Consequently, the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary (EFSA CEP Panel, 2021a).

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 triacylglycerol lipase produced with the genetically modified *S. cerevisiae* strain LALL-LI 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 triacylglycerol lipase.

Occupational asthma caused by exposure to lipases has been reported (Brant et al., 2004). However, several studies have shown that adults with occupational asthma due to food enzymes may be able to ingest the corresponding allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any triacylglycerol lipase have been reported in the literature.

Yeast extract, 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 yeast biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues 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 1,000–2,000 LBLU/kg flour, corresponding to 17.5–35.1 mg TOS/kg flour.[30] It is added to flour during the

[25] Technical dossier/2022-12-19_Reply to ADR (1)bis/Methods of analysis/Annex 26.
[26] Technical dossier/2022-12-19_Reply to ADR (1)bis /Chemical composition, properties and purity of the food enzyme/Annex 22bis.
[27] Technical dossier/Risk assessment /Methods of Analysis/Annex 27.
[28] Technical dossier/Risk assessment/Chemical composition, properties and purity of the food enzyme/Annex 23.
[29] Technical dossier/Risk assessment/Allergenicity/pp. 1-2/Annex 28.
[30] Technical dossier/Risk assessment/Intended Use in Food and Use Levels, p.1.

preparation of the dough where it hydrolyses triglycerides present in the flour to facilitate handling of the dough, to improve its structure and behaviour, and to ensure a uniform volume of the bakery products.[30] The food enzyme–TOS remains in the baked foods.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the enzyme is inactivated by heat during baking 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 the 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 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 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 0.418 mg TOS/kg bw per day in infants at the 95th percentile.

Table 2: Summary of the 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)	0.007–0.098 (11)	0.075–0.210 (15)	0.084–0.203 (19)	0.046–0.124 (21)	0.035–0.076 (22)	0.034–0.077 (22)
Min–max 95th (number of surveys)	0.038–0.418 (9)	0.186–0.357 (13)	0.165–0.381 (19)	0.103–0.263 (20)	0.076–0.159 (22)	0.069–0.131 (21)

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 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	+

Sources of uncertainties	Direction of impact
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 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

As the production strain qualifies for the QPS approach to safety assessment and no issue of concern arising from the production process of the food enzyme was identified, toxicity tests were considered unnecessary by the Panel and the margin of exposure was not calculated.

4. Conclusions

Based on the data provided, the Panel concluded that the food enzyme triacylglycerol lipase produced with the genetically modified *S. cerevisiae* strain LALL-LI 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. Recombinant DNA was demonstrated to be present in one batch of the food enzyme.

5. Documentation as provided to EFSA

- Application for authorisation of the food enzyme triacylglycerol lipase from the genetically modified *Saccharomyces cerevisiae* strain LALL-LI. November 2022. Submitted by Lallemand Inc.
- Additional information. December 2022. Submitted by Lallemand Inc.

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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
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoQ	limit of quantification
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
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/full/10.2903/j.efsa.2023.8091#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
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).