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Safety evaluation of the food enzyme maltogenic α-amylase from the genetically modified *Saccharomyces cerevisiae* strain LALL-MA

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Abstract

The food enzyme maltogenic α-amylase (4-α-D-glucan α-maltohydrolase; EC 3.2.1.133) is produced with the genetically modified *Saccharomyces cerevisiae* strain LALL-MA by Lallemand Baking Solutions. 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. The food enzyme is intended to be used in baking processes. Based on the maximum use level recommended for the baking processes and individual data from the EFSA Comprehensive European Food Consumption Database, dietary exposure was estimated to be up to 0.059 mg total organic solids (TOS)/kg body weight per day in European populations. As the production strain of *S. cerevisiae* meets the requirements for a Qualified Presumption of Safety (QPS) approach, no toxicological data are required. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and six matches were found. The Panel considered that under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be 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, glucan 1,4-α-maltohydrolase, 4-α-D-glucan α-maltohydrolase, EC 3.2.1.133, maltogenic α-amylase, *Saccharomyces cerevisiae*, genetically modified microorganism

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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 "Lallemand Baking Solutions" for the authorisation of the food enzyme maltogenic alpha-amylase produced by a genetically modified strain of *Saccharomyces cerevisiae* (strain [redacted]).

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

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the following food enzyme: maltogenic alpha-amylase produced by a genetically

[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, 11.3.2011, pp. 15–24.

modified strain *of Saccharomyces cerevisiae* (strain [redacted]) in accordance with Article 29 of Regulation (EC) No 178/2002, and Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme maltogenic α-amylase from a genetically modified *S. cerevisiae* (strain [redacted]).

Additional information was requested from the applicant during the assessment process on 5th October 2020 and was 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) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

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

3. Assessment

IUBMB nomenclature	glucan 1,4-α-maltohydrolase
Systematic name	4-α-D-glucan α-maltohydrolase
Synonyms	maltogenic α-amylase; 1,4-α-D-glucan α-maltohydrolase
IUBMB No.	EC 3.2.1.133
CAS No.	160611-47-2
EINECS No.	Not available

The maltogenic amylase catalyses the hydrolysis of (1,4)-α-D-glucosidic linkages in starch polysaccharides (amylose and amylopectin), to successively remove maltose from the non-reducing chain ends.[4] The enzyme is intended to be used in baking processes.

3.1. Source of the food enzyme

The maltogenic α-amylase is produced with the genetically modified yeast *Saccharomyces cerevisiae* strain LALL-MA ([redacted]), which is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany) with deposit number [redacted].[5] The production strain was taxonomically identified as *S. cerevisiae* by whole genome sequence (WGS) analysis.[6]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain, which is also the recipient strain, is *S. cerevisiae* [redacted] is deposited at the American Type Culture Collection (ATCC, USA), with deposit number [redacted].[7]

3.1.2. Characteristics of introduced sequences

The sequence encoding the maltogenic α-amylase [redacted]

[4] Technical dossier/1st submission/p. 13.
[5] Technical dossier/Additional data December 2020/Appendix 4.
[6] Technical dossier/1st submission/Appendix 5.
[7] Technical dossier/1st submission/Appendix 4.

[redacted]

3.1.3. Description of the genetic modification process

[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 *S. cerevisiae* LALL-MA differs from the recipient strain [redacted]

The species *S. cerevisiae* is included in the list of organisms for which the Qualified Presumption of Safety (QPS) may be applied (EFSA, 2007; EFSA BIOHAZ Panel, 2020), with the qualification of absence of resistance to antimycotics used for medical treatment of yeast infections in cases where viable cells are added to the food or feed chain. However, as no viable cells of the production strain remain in the product (Section 3.3.4) this qualification does not apply. The production strain was unequivocally identified as *S. cerevisiae*. Therefore, in the absence of concerns arising from the genetic modifications, the production strain *S. cerevisiae* LALL-MA is considered to qualify for the QPS approach to safety assessment.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[10]

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 [redacted]. The enzyme is released from the wet biomass [redacted] of the yeast cells, and then further purified and concentrated, [redacted] in which enzyme protein is retained while most of the

[8] Technical dossier/1st submission/p.24 and Additional data December 2020/pp. 9–10 and Appendix 5.
[9] 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.
[10] Technical dossier/1st submission/p. 75.

low molecular weight material [redacted] is discarded. Finally, the food enzyme was [redacted]-dried prior to analysis.[11] 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.[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 maltogenic α-amylase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted]. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, and 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], consistent with the expected mass of the enzyme.[13] The food enzyme was tested for lipase and glucoamylase activities and none were detected. Minor protease side activities have been reported by the applicant.[14]

The in-house determination of maltogenic α-amylase activity is based on hydrolysis of an insoluble starch linked to a blue dye (reaction conditions: pH 5.5, 60°C, 15 min). The enzymatic activity is determined by measuring the release of the dye spectrophotometrically at 620 nm and comparing against an internal standard.[15] The enzyme activity is expressed in Lallemand Baking JUN Units (LBJU)/g. One LBJU is defined as the amount of enzyme required to hydrolyze 0.175 μmol of glucosidic linkages per minute in a starch substrate under the conditions of the assay.

The food enzyme has a temperature optimum around 80°C (pH 5.0) and a pH optimum around pH 5.5 (60°C). Thermostability was tested after a pre-incubation of the food enzyme for 10 min at different temperatures (pH 5.0). Maltogenic α-amylase activity decreased above 90°C showing no residual activity above 95°C after 10 min.[16]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four batches used for commercialisation[17] (Table 1). The average total organic solids (TOS) of the four food enzyme batches for commercialisation was 15.1%. The average enzyme activity/TOS ratio of the four food enzyme batches for commercialisation is 399.3 LBJU/mg TOS.

Table 1: Compositional data of the food enzyme preparation

Parameters	Unit	Batches			
		1	2	3	4
Maltogenic amylase activity	LBJU/g batch(a)	62,550	82,700	64,240	32,300
Ash	%	2.4	4.9	2.0	3.0
Water	%	4.0	6.0	3.3	7.1
Excipient(b)	%	80	72	80	75
Total organic solids (TOS)(c)	%	13.6	17.1	14.7	14.9
Activity/mg TOS	LBJU/mg TOS	459.9	483.6	437.0	216.8

(a): LBJU: Lallemand Baking JUN Units (see Section 3.3.1).
(b): [redacted].
(c): TOS calculated as 100% – % water – % ash – % excipient.

[11] Technical dossier/1st submission/pp. 32–37 and Additional data December 2020/pp. 4–7.
[12] Technical dossier/1st submission/pp. 34, 83–87 and Additional data December 2020/pp. 7–8 and 30–35.
[13] Technical dossier/1st submission/pp. 9–10.
[14] Technical dossier/1st submission/pp. 11 and 17–18.
[15] Technical dossier/1st submission/pp. 62–64.
[16] Technical dossier/1st submission/pp. 15–16.
[17] Technical dossier/1st submission/pp. 12, 62–64 and Additional data December 2020/pp. 1–2 and 18–21.

3.3.3. Purity

The lead content in the four commercial batches was below 0.05 mg/kg which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[18,19]

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[20] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[21]

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 four independent batches analysed in triplicate. [redacted] Some colonies were produced, but not derived from the production strain, as demonstrated by polymerase chain reaction (PCR) analysis.[22]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of four batches in triplicate. [redacted].[23]

3.4. Toxicological data

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

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 the maltogenic α-amylase produced with the genetically modified *S. cerevisiae* strain LALL-MA 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, six matches were found. The matching allergens are: Asp o 21 and Asp o 21.0101 (TAKA amylase A and α-amylase A type-1/2 from *Aspergillus oryzae*), Sch c 1.0101 (glycoside hydrolase family 15 from *Schizophyllum commune* or Split Gill fungus), Aed a 4.0101 (probable maltase from *Aedes aegypti* or yellow fever mosquito maltase produced), Asp f 13.0101 (uncleaved alkaline protease from *Aspergillus fumigatus*) and Asp f 13 (partial alkaline protease from *Aspergillus fumigatus*).[24]

No information on oral and respiratory sensitisation or elicitation reactions of this maltogenic α-amylase is available.

α-Amylase from *Aspergillus oryzae* (Brisman and Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998; Brisman, 2002), serine protease from *Aspergillus fumigatus* (Kurup et al., 2002) and glucoamylase from *Schizophyllum commune* (Toyotome et al., 2014) are known as occupational respiratory allergens associated with 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). Taking into account the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in literature that focused on allergic reactions upon

[18] Technical dossier/Additional data December 2020/pp. 1–2, 9 and 18–21.
[19] LoDs: Pb = 0.017 mg/kg and 0.05 mg/kg.
[20] Technical dossier/Additional data December 2020/pp. 1–2, 18–21 and 22–29.
[21] Technical dossier/1st submission/p. 65 and Additional data December 2020/pp. 1–2 and 18–21.
[22] Technical dossier/Additional data December 2020/pp. 10–11.
[23] Technical dossier/1st submission/p. 39 and Additional data December 2020/pp. 11–16.
[24] Technical dossier/1st submission/pp. 48–51 and Additional data December 2020/Appendix 6.

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 and serine protease. Serine protease produced by *S. commune* is associated with allergic reactions to insect bites, but allergic reactions after oral exposure have not been reported. In addition, no allergic reactions upon dietary exposure to any maltogenic α-amylase have been reported in the literature. For these reasons, it can be concluded that an allergic reaction upon oral ingestion of maltogenic amylase, produced by the genetically modified *S. cerevisiae* strain LALL-MA, in individuals respiratory sensitised to α-amylase, serine protease produced by *A. fumigatus* or glucoamylase produced by *S. commune* cannot be excluded, but the likelihood is considered to be low.

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 occurring 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 maximum use level of 2,000 LBJU/kg flour,[25] corresponding to 5 mg TOS/kg flour.[26]

In baking processes, the food enzyme is added to flour during the preparation of dough. The maltogenic α-amylase hydrolyses starch and releases maltose. 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 (increased firmness, reduced oil absorption and less stockiness).

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is expected that the maltogenic α-amylase may not be fully inactivated during some baking processes.

3.5.2. Dietary exposure estimation

Chronic dietary exposure was estimated by using the FEIM-baking calculator.[27] The calculation combines the maximum recommended use level (see Section 3.5.1) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data.[28] Exposure from individual 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 average 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 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 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 European countries (Appendix B).

[25] Technical dossier/1st submission/p. 41 & Additional data December 2020/p. 3.
[26] Technical dossier/Additional data December 2020/p. 3.
[27] https://zenodo.org/record/4382037#.X-rMgthKjD4
[28] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

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 mean (number of surveys)	0.001–0.014 (12)	0.011–0.030 (16)	0.012–0.029 (19)	0.007–0.018 (20)	0.005–0.011 (22)	0.005–0.010 (21)
Min–max 95th percentile (number of surveys)	0.005–0.059 (10)	0.027–0.051 (14)	0.024–0.054 (19)	0.015–0.037 (19)	0.011–0.021 (22)	0.010–0.018 (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	+
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	+/–

+: 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 concluded that the food enzyme maltogenic α-amylase produced with the genetically modified *S. cerevisiae* strain LALL-MA 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 as provided to EFSA

1) Application for authorisation of a maltogenic α-amylase enzyme from a modified strain of *Saccharomyces cerevisiae* in accordance with Regulation (EC) No 1331/2008 and Regulation (EC) No 1332/2008. April 2020. Submitted by Lallemand Baking Solutions.
2) Additional information. December 2020. Submitted by Lallemand Baking Solutions.

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Abbreviations

ATCC	American Type Culture Collection
bw	body weight
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
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
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
LoD	limit of detection
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
qPCR	Quantitative 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/10.2903/j.efsa.2021.6434#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 one 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, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, United Kingdom
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, 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, Slovenia, Spain, Sweden, United Kingdom
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, United Kingdom
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, 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).