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SCIENTIFIC OPINION

Safety evaluation of the food enzyme glucan 1,4-α-maltohydrolase from the genetically modified *Bacillus subtilis* strain BABSC

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Abstract

The food enzyme glucan 1,4-α-maltohydrolase (4-α-D-glucan α-maltohydrolase, EC 3.2.1.133) is produced with the genetically modified *Bacillus subtilis* strain BABSC by Advanced Enzyme Technologies Ltd. The requirements for the qualified presumption of safety (QPS) approach have not been met. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in baking processes and starch processing for the production of glucose syrups and other starch hydrolysates. Since residual amounts of total organic solids (TOS) are removed, dietary exposure was not calculated for starch processing for the production of glucose syrups and other starch hydrolysates. For baking processes, the dietary exposure was estimated to be up to 0.101 mg TOS/kg body weight per day in European populations. No toxicological studies were provided by the applicant. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and one match with a respiratory allergen was found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood is low. In the absence of appropriate data to fully characterise the production strain, the Panel was unable to conclude on the safety of the food enzyme under the intended conditions of use.

KEYWORDS

4-α-D-glucan α-maltohydrolase, *Bacillus subtilis*, EC 3.2.1.133, food enzyme, genetically modified microorganism, glucan 1,4-α-maltohydrolase, maltogenic amylase

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 on food enzymes.

An application has been introduced by the applicant Advanced Enzyme Technologies Ltd. for the authorisation of the food enzyme: maltogenic amylase from a genetically modified strain of *Bacillus subtilis* (strain BABSC).

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 amylase from a genetically modified strain of *Bacillus subtilis* (strain BABSC) 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 glucan 1,4-α-maltohydrolase from a genetically modified *Bacillus subtilis* strain BABSC.

[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.

Additional information was requested from the applicant during the assessment process on 20 January 2023 and received on 24 August 2023 (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, 2009) 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 CEF Panel, 2009) 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. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Glucan 1,4-α-maltohydrolase
Systematic name	4-α-D-glucan α-maltohydrolase
Synonyms	Maltogenic α-amylase
IUBMB No	EC 3.2.1.133
CAS No	160611-47-2
EINECS No	630-523-5

Glucan 1,4-α-maltohydrolases catalyse the hydrolysis of (1 → 4)-α-D-glucosidic linkages in starch polysaccharides and release maltose units from the non-reducing chain ends. The enzyme under assessment is intended to be used in baking processes and starch processing for the production of glucose syrups and other starch hydrolysates.

3.1 | Source of the food enzyme

The glucan 1,4-α-maltohydrolase is produced with the genetically modified bacterium *B. subtilis* strain BABSC, which is deposited at the American Type Culture Collection (USA) with the deposit number [redacted][4] The applicant made a [redacted] which was considered not sufficiently reliable for the identity of *Bacillus* species.[5]

The species *B. subtilis* 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 absence of cytotoxicity of the production strain *B. subtilis* BABSC to VERO cells could not be demonstrated.[6] The whole genome sequencing (WGS) of the production strain was interrogated for the presence of antimicrobial resistance genes using one database with thresholds of > 80% similarity and > 70% coverage. No genes of concern were detected.[7]

In the absence of appropriate data provided by the applicant, despite being requested,[8] an unequivocal taxonomic identity of the production strain and the absence of cytotoxic activity were not demonstrated. As a consequence, the production strain could not be considered to qualify for the QPS approach.

3.1.1 | Characteristics of the parental and recipient microorganisms

The parental microorganism is *B. subtilis* strain 168, for which the complete genome sequence is publicly available (NC_000964.3; Kunst et al., 1997; Barbe et al., 2009).

[redacted]

[4]Technical dossier/Annex I p. 18.

[5]Technical dossier/Annex I and Additional data August 2023.

[6]Technical dossier/Annex J; ADD DATA_AUGUST 2023/2. Additional Info_Maltogenic amylase_ Jan 23.pdf.

[7]Technical dossier/Additional data August 2023.

[8]Request for additional information/January 2023.

3.1.2 | Characteristics of introduced sequences

The sequence encoding the glucan 1,4-α-maltohydrolase is [redacted]

3.1.3 | Description of the genetic modification process

The aim of the genetic modification is to enable the production strain to produce the glucan 1,4-α-maltohydrolase [redacted]

3.1.4 | Safety aspects of the genetic modification

The production strain *B. subtilis* strain BABSC differs from the recipient strain in its capacity to produce the glucan 1,4-α-maltohydrolase from [redacted]

The absence of the antimicrobial resistance genes used during the genetic modification was confirmed by WGS analysis.[11]

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

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 enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded. Finally, the food enzyme was spray-dried prior to analysis.[14] 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.[15]

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 glucan 1,4-α-maltohydrolase is a single polypeptide chain of 686 amino acids.[16] The molecular mass of the mature protein, calculated from the amino acid sequence, is 75.15 kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a single

[9]Technical dossier/Annex M.

[10]Technical dossier/Annex M p. 23 and Additional data August 2023.

[11]Technical dossier/ Additional data August 2023.

[12]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.

[13]Technical dossier/Risk assessment data/p. 26/Annex F and Additional data August 2023.

[14]Technical dossier/Risk assessment data/pp. 26–32/Annex G.

[15]Technical dossier/Risk assessment data/Annex G and Additional data August 2023.

[16]Technical dossier/Risk assessment data/p. 5.

major protein band corresponding to an apparent molecular mass of about 66 kDa, consistent with the expected mass of the enzyme.[17] No other enzymatic activities were reported.[18]

The in-house determination of the enzyme activity is based on the hydrolysis of maltotriose to maltose and glucose (reaction conditions: pH 5.0, 37°C, incubation time 30 min). The released glucose is quantified with a commercial test based on the use of glucose dehydrogenase. The enzyme activity is expressed in maltogenic amylase unit (MAN U)/g. One MAN U is defined as the amount of enzyme that hydrolyses 1 µmol of maltotriose per minute under the conditions of the assay.[19]

The food enzyme has a temperature optimum between 60 and 70°C (pH 5.0) and a pH optimum around pH 5.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 120 min at different temperatures (pH 5.0). The enzyme activity was retained at temperatures up to 75°C; thereafter, it decreased with a residual activity of 35% at 80°C.[20]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three food enzyme batches (Table 1).[21] The mean total organic solids (TOS) of the three batches was 85.2% and the mean enzyme activity/TOS ratio was 78.1 MAN U/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
Glucan 1,4-α-maltohydrolase	MAN U/g[a]	65,427	64,573	69,433
Protein	%	62.1	62.0	64.1
Ash	%	7.6	8.1	7.3
Water	%	7.2	7.3	7.0
Total organic solids (TOS)[b]	%	85.2	84.6	85.7
Glucan 1,4-α-maltohydrolase activity/TOS ratio	MAN U/mg TOS	76.8	76.4	81.0

[a]MAN U: maltogenic amylase unit (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,[22] 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, the concentrations of arsenic, cadmium and mercury were below the limits of quantification (LoQ) of the employed methods.[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).[25] No antimicrobial activity was detected in any of the tested batches.[26]

The Panel considered that the information provided on the purity of the food enzyme was 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 in three independent batches analysed in triplicate. [redacted] No colonies were produced. A positive control was included.[27]

[17]Technical dossier/Additional data August 2023.
[18]Technical dossier/Risk assessment data/p. 10.
[19]Technical dossier/Risk assessment data/pp. 9–10/Annex C.
[20]Technical dossier/Risk assessment data/pp. 10–12/Annex C.
[21]Technical dossier/Risk assessment data/p. 6/Annex A3.
[22]Technical dossier/Risk assessment data/p. 6/Annex A3.
[23]Technical dossier/Risk assessment data/Annex D.
[24]LoQs: Pb, As, Cd = 0.1 mg/kg each; Hg = 0.025 mg/kg.
[25]Technical dossier/Risk assessment data/p. 6, p. 9/Annex A3.
[26]Technical dossier/Risk assessment data/p. 6, p. 9/Annex A3.
[27]Technical dossier/Annex N and Additional data August 2023.

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.[28]

3.4 | Toxicological data

Claiming the QPS approach for the production strain, the applicant did not provide toxicological data. The Panel did not request toxicological studies, as further shortcomings concerning the molecular characterisation of the production strain were not adequately addressed by the applicant (see Section 3.1).

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 glucan 1,4-*α*-maltohydrolase with the genetically modified *B. subtilis* strain BABSC 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, one match was found (using the FARRP (AllergenOnline) database).[29] The matching allergen is Asp o 21, an *α*-amylase produced by *Aspergillus oryzae*, known as a respiratory occupational allergen.

No information was available on oral and respiratory sensitisation or elicitation reactions of this glucan 1,4-*α*-maltohydrolase.

α-Amylase from *A. oryzae* (Brisman, 2002; Brisman & Belin, 1991; Quirce et al., 1992, 2002; Sander et al., 1998) is known as occupational respiratory allergen 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 (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004). Taking into account the wide use of *α*-amylase as a food enzyme, only a low number of case reports has been described in the literature that focused on allergic reactions upon oral exposure to *α*-amylase in individuals respiratorily sensitised to *α*-amylase (Baur & Czuppon, 1995; Kanny & Moneret-Vautrin, 1995; Losada et al., 1992; Moreno-Ancillo et al., 2004; Quirce et al., 1992). In addition, no allergic reactions upon dietary exposure to any glucan 1,4-*α*-maltohydrolase have been reported in the literature.

The Panel considered that 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 two food manufacturing processes 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.[30]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Baking processes	Flour	0.85–**8.52**
Starch processing for production of glucose syrups and other starch hydrolysates	Starch	12.77–34.06

[a]The name has been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The number in bold were used for calculation.

In baking processes, the food enzyme is added to flour during the preparation of the dough.[31] The maltogenic *α*-amylase hydrolyses amylose and amylopectin and releases maltose. The conversion of starch lowers the rate of retrogradation,

[28]Technical dossier/Additional data August 2023/Annexes 4 and 5.

[29]Technical dossier/Annex L.

[30]Technical dossier/3.2 Risk assessment data, p. 37.

[31]Technical dossier/3.2 Risk assessment data, Figure 3.2.1.4–1.

thereby reducing staling, improving crumb structure and increasing the shelf life of bakery products.[32] The food enzyme–TOS remains in the final baked foods.

In starch processing for production of glucose syrups and other starch hydrolysates, the food enzyme is added during the saccharification step.[33] The hydrolysis of starch results in higher yields of maltose.[34] The food enzyme–TOS is removed in the final processed foods by treatment with activated charcoal or similar, and with ion-exchange resins (EFSA CEP Panel, 2021).

Based on data provided on thermostability (see Section 3.3.1), the maltogenic *α*-amylase may remain active in baked products depending on the specific food manufacturing conditions.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), a dietary exposure was not calculated for starch processing for production of glucose syrups and other starch hydrolysates. A dietary exposure was calculated only for baking processes, where the food enzyme–TOS remains in the final foods.

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 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 0.101 mg TOS/kg body weight (bw) per day in infants at the 95th percentile.

TABLE 3 Summary of the estimated dietary exposure to 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)	0.002–0.024 (11)	0.018–0.051 (15)	0.020–0.049 (19)	0.011–0.030 (21)	0.008–0.019 (22)	0.008–0.019 (22)
Min–max 95th percentile (number of surveys)	0.009–0.101 (9)	0.045–0.087 (13)	0.040–0.092 (19)	0.025–0.064 (20)	0.018–0.038 (22)	0.017–0.032 (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 4.

[32]Technical dossier/3.3 Risk management data, p. 6.

[33]Technical dossier/3.2 Risk assessment data, Figure 3.2.1.4–2.

[34]Technical dossier/3.3 Risk management data, p. 8.

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	+/–
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	+/–
Exclusion of one process from the exposure assessment: Starch processing for glucose syrups production and other starch hydrolysates	–

+: 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 an overestimation of the exposure.

The exclusion of one food manufacturing process (starch processing for production of glucose syrups and other starch hydrolysates) from the exposure assessment was based on > 99% of TOS removal. This is not expected to have an impact on the overall estimate derived.

3.6 | Margin of exposure

In the absence of toxicological studies provided by the applicant, the margin of exposure could not be calculated.

4 | CONCLUSIONS

In the absence of appropriate data to fully characterise the production strain, the Panel was unable to conclude on the safety of the food enzyme glucan 1,4-α-maltohydrolase produced with the genetically modified *B. subtilis* strain BABSC 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 authorisation of Maltogenic amylase from genetically modified *Bacillus subtilis* (strain BABSC) in accordance with Regulation (EC) No 1331/2008. May 2017. Submitted by Advanced Enzyme Technologies Ltd.

Additional information. August 2023. Submitted by Advanced Enzyme Technologies Ltd.

ABBREVIATIONS

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
GLP	good laboratory practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
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

QUESTION NUMBER

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NOTE

The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the 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).