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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme α-amylase from the non-genetically modified *Bacillus licheniformis* strain AE-TA

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) | Claude Lambré | José Manuel Barat Baviera | Claudia Bolognesi | Pier Sandro Cocconcelli | Riccardo Crebelli | David Michael Gott | Konrad Grob | Evgenia Lampi | Marcel Mengelers | Alicja Mortensen | Gilles Rivière | Inger-Lise Steffensen | Christina Tlustos | Henk Van Loveren | Laurence Vernis | Holger Zorn | Yrjö Roos | Jaime Aguilera | Magdalena Andryszkiewicz | Kyriaki Apergi Daniele Cavanna | Cristina Fernàndez-Fraguas | Natalia Kovalkovicova | Silvia Peluso | Giulio di Piazza | Yi Liu | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme α-amylase (4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the non-genetically modified microorganism *Bacillus licheniformis* strain AE-TA by Amano Enzyme Inc. The food enzyme is intended to be used in eight food manufacturing processes. Since residual amounts of food enzyme-total organic solids (TOS) are removed in two food manufacturing processes, dietary exposure was calculated only for the remaining six processes. It was estimated to be up to 0.056 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 approach to safety assessment. Consequently, in the absence of other concerns, the Panel considered that toxicological studies were not needed for the safety 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 two matches with respiratory allergens were found. The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded (except for the production of distilled alcohol), but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

4-α-D-glucan glucanohydrolase, amylase, *Bacillus licheniformis*, EC 3.2.1.1, food enzyme, glycogenase, α-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.

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 1332/20082 on food enzymes.

Four applications have been submitted by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and by the companies "DSM Food Specialties B. V." and "Amano Enzyme Inc." for the food enzymes Bacillolysin from *Bacillus amyloliquefaciens and/or Bacillus subtilis*, α-amylase from *Bacillus licheniformis*, Alpha-amylase from a genetically modified strain of *Bacillus subtilis* (strain NBA) and Alpha-amylase from *Aspergillus oryzae* (strain AE-AA) respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/20083, the Commission has verified that the four applications fall 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 safety assessments on the food enzymes Bacillolysin from *Bacillus amyloliquefaciens and/or Bacillus subtilis*, Alpha-amylase from *Bacillus licheniformis*, Alpha-amylase from a genetically modified strain of *Bacillus subtilis* (strain NBA) and Alpha-amylase from *Aspergillus oryzae* (strain AE-AA) in accordance with Article 17.3 of Regulation (EC) No 1332/20082 on food enzymes.

[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.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme α-amylase from *Bacillus licheniformis* submitted by AMFEP.

The application was submitted initially as a joint dossier[4] and identified as the EFSA-Q-2014-00911. During an ad hoc meeting between EFSA, the European Commission and AMFEP,[5] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the joint dossier EFSA-Q-2014-00911. This data package, identified as EFSA-Q-2022-00549, concerns the food enzyme α-amylase that is produced with *Bacillus licheniformis* strain AE-TA and submitted by Amano Enzyme Inc.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from a non-genetically modified *Bacillus licheniformis* strain (AE-TA).

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, 2009b) 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	α-Amylase
Systematic name	4-α-D-glucan glucanohydrolase
Synonyms	1,4-α-D-glucan glucanohydrolase
IUBMB no	EC 3.2.1.1
CAS no	9000-90-2
EINECS no	232–565-6

α-Amylases catalyse the hydrolysis of 1,4-α-glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins. The food enzyme under this assessment is intended to be used in eight food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): processing of cereals and other grains for the production of (1) baked products, (2) brewed products, (3) non-wine vinegar, (4) cereal-based products other than baked, (5) glucose syrups and other starch hydrolysates and (6) distilled alcohol, and processing of plant- and fungal-derived products for the production of (7) refined and unrefined sugar and (8) plant-based analogues of milk and milk products.

3.1 | Source of the food enzyme

The α-amylase is produced with the non-genetically modified bacterium *Bacillus licheniformis* strain AE-TA, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan) with the deposit number [redacted].[6] The production strain was identified as *B. licheniformis* by whole genome sequence (WGS) analysis,

[4]Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes Text with EEA relevance OJ L 168, 28.6.2012, p. 21–23.

[5]The full detail is available at the https://www.efsa.europa.eu/en/events/event/ad-hoc-meeting-industry-association-amfep-joint-dossiers-food-enzymes

[6]Technical Dossier/Annex and Reference/Annex/Annex 5.

[redacted].[7] *B. licheniformis* AE-TA was derived from the parental strain [redacted].

The species *Bacillus licheniformis* 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 BIOHAZ Panel, 2020, 2022). A cytotoxicity test made with culture supernatants indicated that the production strain *B. licheniformis* AE-TA did not induce cell damage to Vero cells using the lactate dehydrogenase assay.[8] The WGS of the production strain was interrogated for the presence of antimicrobial resistance genes using two regularly maintained databases. No genes of concern were identified with thresholds above 80% of identity and 70% of coverage. Therefore, the production strain qualifies for the QPS approach to safety assessment.[9]

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[10] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[11]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or 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.[12] 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.[13]

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 α-amylase is a single polypeptide chain of [redacted] amino acids.[14] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by size exclusion chromatography. The chromatograms of the three batches for commercialisation showed similar patterns.[15] No other enzymatic activities were reported.[16]

The in-house determination of α-amylase activity is based on the hydrolysis of starch (reaction conditions: pH 5.5, 37°C, 30 min) and determined by measuring the release of reducing sugars using a titrimetric method. The enzyme activity is expressed in Unit (U)/g. One unit is the amount of enzyme which catalyses the increase of reducing activity equivalent to 1.0 mg of glucose per minute under the conditions of the assay.[17]

The food enzyme has a temperature optimum around 90°C (pH 6.0) and a pH optimum around pH 6.5 (80°C). Thermostability was tested after a pre-incubation of the food enzyme for 60 min at different temperatures (pH 5.0). The enzyme activity decreased above 90°C, showing no residual activity above 95°C.[18,19]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[20] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 7.2% and the mean enzyme activity/TOS ratio was 69.1 U/mg TOS.

[7]Technical Dossier/Annex and Reference/Annex/Annex 4.
[8]Technical Dossier/Annex and Reference/Annex/Annex 7.
[9]Technical Dossier/Annex and Reference/Annex/Annex 4.
[10]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.
[11]Technical dossier/pp. 36–37/Annex 8.
[12]Technical dossier/pp. 36–43/Annex 9.
[13]Technical dossier/Annex 10.
[14]Technical dossier/p. 28.
[15]Technical dossier/p. 26.
[16]Technical dossier/p. 29.
[17]Technical dossier/pp. 28–29/Annex 2.
[18]Technical dossier/pp. 29–30.
[19]Technical dossier/Additional data Dec 2023/Additional Information about alpha-amylase from B. licheniformis AE-TA.
[20]Technical dossier/p. 26/Annexes: 1, 2, 3.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches		
		1	2	3
α-Amylase activity	U/g[a]	4790	5090	4980
Protein	%	5.23	5.56	5.38
Ash	%	13.9	14.3	14.0
Water	%	79.0	78.3	79.0
Total organic solids (TOS)[b]	%	7.1	7.4	7.0
α-Amylase activity/TOS	U/mg TOS	67.5	68.8	71.1

[a]UNIT: see Section 3.3.1.
[b]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity

The lead content of the three batches was below 0.05 mg/kg,[21] 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 cadmium and mercury concentrations were below the limits of quantification (LoQ) of the employed methods. For arsenic, the average concentration determined in the commercial batches was 0.03 mg/kg.[22,23] The Panel considered this concentration as not of concern.

The food enzyme preparation 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).[24] No antimicrobial activity was detected in any of the tested batches.[25]

The presence of aflatoxins (B1, B2, G1, G2), deoxynivalenol, HT-2 toxin, T-2 toxin, zearalenone, ochratoxin A and sterigmatocystin was examined in the three food enzyme batches. All were below the LoQs of the applied analytical methods.[26,27]

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.[28]

The absence of DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. [redacted].[29]

3.4 | Toxicological data

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

3.4.1 | Allergenicity

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

[21]Technical dossier/p. 27/Annexes: 1, 3.
[22]Technical dossier/p. 27/Annexes: 1, 3.
[23]LoQs: Pb = 0.005 mg/kg; As = 0.02 mg/kg; Cd, Hg = 0.001 mg/kg each.
[24]Technical dossier/p. 27/Annexes: 1, 3.
[25]Technical dossier/p. 27/Annexes: 1, 3.
[26]Technical dossier/Annex 3.
[27]LoQs: aflatoxins B1, B2, G1, G2 = 0.2μg/kg; deoxynivalenol = 20 μg/kg; HT-2 toxin = 10 μg/kg; T-2 toxin = 10 μg/kg; Zearalenone = 10 μg/kg; ochratoxin A = 0.5 μg/kg; sterigmatocystin: < 10 μg/kg.
[28]Technical dossier/Additional data Dec 2023/Annex 1.
[29]Technical dossier/Additional data Dec 2023/Annex 2.

The potential allergenicity of the α-amylase produced with the *Bacillus licheniformis* strain AE-TA 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, two matches were found. The matching allergens were α-amylases produced by *A. oryzae,* both known as occupational respiratory allergens.[30]

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

Several studies have shown that adults with occupational asthma to a food enzyme (as described for α-amylase from *A. oryzae*) may be able to ingest the corresponding allergen 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 food enzyme, only a low number of case reports of allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase have been described in literature (Baur & Czuppon, 1995; Kanny & Moneret-Vautrin, 1995; Losada et al., 1992; Moreno-Ancillo et al., 2004; Quirce et al., 1992).

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used as raw material. In addition, [redacted], known sources of allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from these sources are present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded (except for production of distilled alcohol), 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 eight 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.[32,33]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[b]	
Processing of cereals and other grains			
• Production of baked products	Flour	**2.1**	
• Production of brewed products	Cereals	**0.4**	
• Production of non-wine vinegar	Cereals	**850.7**	
• Production of cereal-based products other than baked	Cereals (e.g. wheat, rice, corn, oat)[34]	**4.3**	Infant cereals
		2.1	Other products
• Production of glucose syrups and other starch hydrolysates	Starch	4.3	
• Production of distilled alcohol	Cereals	177.4	
Processing of plant- and fungal-derived products			
• Production of refined and unrefined sugar	Sugar beet and sugar cane	**0.2**	
• Production of plant-based analogues of milk and milk products	Oat flour, almond flour, rice flour, buckwheat, pulses, legumes, oil seeds, nuts, etc.	**4.3**	

[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 numbers in bold represent the maximum recommended use levels, which were used for calculation.

[30]Technical dossier/pp. 52–53/Annex 11.

[31]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[32]Technical dossier/p. 47.

[33]Additional data December 2023.

[34]Additional data December 2023/Answer to question 7.

In the production of baked products, the food enzyme is added to flour during the dough preparation.[35] The hydrolysis of α-amylase reduces the viscosity of the dough and increases the volume of the final product.[36] The food enzyme-TOS remains in the final baked foods.

In production of brewed products, for beer production, the food enzyme is added to cereals during the mashing step[37] and, for the production of products like sake, it could be also added during the liquefaction, the pre-saccharification or the fermentations steps.[38] Together with other saccharifying enzymes, the α-amylase degrades starch from the raw material into dextrins and fermentable sugars. The food enzyme-TOS remains in the final brewed products.

In the production of non-wine vinegar, the food enzyme is added to milled grains or cereals during slurry mixing.[39] The α-amylase acts on the starch present in different grains and cereals. The food enzyme-TOS remains in the final processed foods.

In the production of cereal-based products other than baked, such as breakfast cereals, the food enzyme is added to the cereal slurry.[40] The hydrolysis by *α*-amylase reduces the viscosity of the slurry, facilitating the downstream processing steps, such as extrusion.[41] The food enzyme-TOS remains in the final foods.

In the production of glucose syrups and other starch hydrolysates, the food enzyme is added to starch during liquefaction.[42] It degrades starch into dextrins and reduces the viscosity of the gelatinised starch. The food enzyme-TOS is removed from the final glucose syrups by treatment with activated charcoal or similar and ion-exchange resins. The same was concluded for other starch hydrolysates (EFSA CEP Panel, 2023).

In the production of distilled alcohol, the food enzyme is added to starch during the slurry mixing, the liquefaction, the pre-saccharification and the distillation steps.[43] The *α*-amylase increases the amount of fermentable sugars for higher alcohol yields. The food enzyme-TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2023).

In refined sugar production, the food enzyme is added to the raw juice during affination and/or clarifying steps to hydrolyse starch from sugar cane or sugar beet.[44] Raw sugar can be additionally treated during melting.[45] The hydrolytic action of the α-amylase increases solubility and facilitates sugar crystallisation. The food enzyme-TOS is not carried over with the crystalised refined sugar, but remains in molasses as a by-product (EFSA CEP Panel, 2023).

In the production of plant-based analogues of milk and milk products, the food enzyme is added to a variety of plant materials together with water during saccharification.[46] The *α*-amylase hydrolyses the gelatinised starch to reduce the viscosity of the slurry, allowing higher inclusion of plant materials in the plant-based beverages and the corresponding fermented semi-solid foods. The food enzyme-TOS remains in the final foods.

The applicant provided a flowchart to represent the production of enzymatically hydrolysed fibres from cereals.[47] Upon request for clarifications, the applicant stated that the food enzyme is added to the dried flour from cereals.[48] The applicant then introduced the production of partially hydrolysed guar gum[49] and analysed the amount of proteins in purified guar gum after treatment with a mannanase (which is not subject of this assessment).[50] The Panel considered that the additional data did not clarify the information provided in the initially submitted technical dossier; on the contrary, reference to guar gum introduced further confusion. Based on this unclear information and considering the composition of the guar bean, the panel is not in the position to properly classify the use of α-amylase in the production of enzymatically hydrolysed fibres from cereals. Therefore, this use is not included in this opinion.

3.5.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), a dietary exposure was calculated only for the six food manufacturing 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 one day

[35]Technical dossier/Annex 11/p. 1.
[36]Technical dossier/p. 64.
[37]Technical dossier/Annex 11/p. 2.
[38]Technical dossier/Annex 11/p. 3.
[39]Technical dossier/Annex 11/p. 4.
[40]Technical dossier/Annex 11/pp. 5–6.
[41]Technical dossier/p. 65.
[42]Additional data December 2023/Answer to question 4.
[43]Technical dossier /Annex 11/p. 11.
[44]Technical dossier /Annex 11/pp. 9–10.
[45]Technical dossier /Annex 11/p. 10.
[46]Technical dossier /Annex 11/p. 12.
[47]Technical dossier /Annex 11/p. 8.
[48]Additional data December 2023/Answer 6a.
[49]Additional data December 2023/Answer 6c.
[50]Additional data December 2023/Annex 3.

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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.056 mg TOS/kg body weight per day in infants at the 95th percentile.

TABLE 3 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)	0.002–0.016 (12)	0.010–0.020 (15)	0.006–0.017 (19)	0.002–0.012 (21)	0.004–0.012 (22)	0.003–0.007 (23)
Min–max 95th percentile (number of surveys)	0.009–0.056 (11)	0.023–0.042 (14)	0.013–0.036 (19)	0.004–0.027 (20)	0.007–0.043 (22)	0.006–0.020 (22)

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.

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	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Exposure from The calculation used the higher use level from the two provided for the production of cereal-based products other than baked. was calculated using the TOS indicated for infant cereals	+
Minor FoodEx categories found to only sporadically contain molasses were excluded from the exposure assessment	–
'Brown sugar' produced through use of cane molasses or caramelised sugar syrup was excluded, due to it being a niche product on the European market	–
The transfer of food enzyme-TOS into cane and beet molasses/syrups was assumed to be 100%	+
No distinction was made between beet molasses and cane syrups used as ingredients in foods	+/–
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of two processes from the exposure estimation: – Production of glucose syrups and other starch hydrolysates – Production of distilled alcohol	–

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

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

The exclusion of two food manufacturing processes 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

Since no toxicological assessment was considered necessary by the Panel, a margin of exposure was not calculated.

4 | CONCLUSIONS

Based on the data provided, the QPS status of the production strain and the absence of concerns arising from the production process, the Panel concluded that the food enzyme α-amylase produced with the non-genetically modified *Bacillus licheniformis* strain AE-TA does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA (IF APPROPRIATE)

"Application for authorisation of Alpha-amylase from Bacillus licheniformis AE-TA". September 2022. Submitted by Amano Enzyme Inc.

Additional information. January 2024. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

AMR	Antimicrobial resistance
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
GMM	genetically modified microorganism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
QPS	qualified presumption of safety
TOS	total organic solids
WGS	whole genome sequence
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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PANEL MEMBERS

José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, and Holger Zorn.

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, Spain
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, Republic of North Macedonia*, Serbia*, 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, Republic of North Macedonia*, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, 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, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion, however, they are included in Appendix B for testing purpose.

[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).