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SCIENTIFIC OPINION

Safety evaluation of the food enzyme glutaminase from the non-genetically modified *Bacillus amyloliquefaciens* strain AE-GT

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Abstract

The food enzyme glutaminase (L-glutamine amidohydrolase; EC 3.5.1.2) is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain AE-GT by Amano Enzyme Inc. The production strain met the requirements for the qualified presumption of safety (QPS) approach. The food enzyme is intended to be used in five food manufacturing processes. Dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.462 mg TOS/kg body weight per day in European populations. Given the QPS status of the production strain and the absence of concerns resulting from the food enzyme's manufacturing process, toxicity tests were considered unnecessary by the Panel. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that a risk of allergic reactions upon dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

Bacillus amyloliquefaciens, EC 3.5.1.2, food enzyme, glutaminase, L-glutaminase, L-glutamine amidohydrolase

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 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.

Five applications have been introduced by the companies 'Amano Enzyme Inc.' and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorization of food enzymes Ribonuclease P from *Penicillium citrinum* (strain AE-RP), Glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), Oryzin from *Aspergillus melleus* (strain AE-P), Triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and Glucoamylase from *Aspergillus niger*, respectively.

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 five applications fall within the scope of the food enzyme Regulation and contain 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 assessments of the food enzymes Ribonuclease P from *Penicillium citrinum* (strain AE-RP), Glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), Oryzin from *Aspergillus melleus* (strain AE-P), Triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and Glucoamylase from *Aspergillus niger* in accordance with Article 17.3 of Regulation (EC) No 1332/2008 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 glutaminase from *B. amyloliquefaciens* strain AE-GT.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glutaminase *B. amyloliquefaciens* strain AE-GT. The dossier was updated on 30 January 2014.

Additional information was requested from the applicant during the assessment process on 29 September 2021 and on 5 June 2023, and received on 17 March 2022 and on 25 July 2023, respectively (see 'Documentation provided to EFSA').

2.2 | Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023) have been followed for the evaluation of the application.

3 | ASSESSMENT

IUBMB nomenclature	Glutaminase
Systematic name	L-glutamine amidohydrolase
Synonyms	Glutaminase I; L-glutaminase; glutamine aminohydrolase
IUBMB no	EC 3.5.1.2
CAS no	9001-47-2
EINECS no	618–332-5

Glutaminases catalyse the hydrolysis of the carboxamide group of L-glutamine, releasing L-glutamic acid and ammonia. The food enzyme under assessment is intended to be used in five food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) processing of dairy products for the production of flavouring preparations; (2) processing of eggs and egg products; (3) processing of meat and fish products for the production of protein hydrolysate; (4) processing of plant- and fungal-derived products for the production of protein hydrolysates; and (5) processing of yeast and yeast products.

3.1 | Source of the food enzyme

The glutaminase is produced with the non-genetically modified bacterium *Bacillus amyloliquefaciens* strain AE-GT, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan), with the deposit number [redacted].[4] The production strain was identified as *B. amyloliquefaciens* [redacted].[5]

The production strain *B. amyloliquefaciens* AE-GT was derived from the parental strain [redacted].

The species *B. amyloliquefaciens* is included in the list of organisms for which the qualified presumption of safety (QPS) approach to safety assessment may be applied, provided that the absence of acquired antimicrobial resistance genes and

[4]Additional data March 2022/Annex 2.
[5]Additional data March 2022/Annex 1.

toxigenic activity are verified for the specific strain used.[6] The strain AE-GT showed no cytotoxic activity [redacted][7] [redacted]. No genes of concern were identified [redacted].

Consequently, the production strain met the requirements of the QPS approach.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[8] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, 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, leaving a filtrate containing the food enzyme. 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 weight material passes the filtration membrane and is discarded.[10] 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.[11]

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 glutaminase is a single polypeptide chain of [redacted] amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[13] The food enzyme was analysed by size exclusion chromatography. The chromatograms of the three food enzyme batches for commercialisation showed a consistent pattern.[14] No other enzyme activities were reported.[15]

The in-house determination of glutaminase activity is based on the hydrolysis of L-glutamine (reaction conditions: pH 6.0, 37°C, 20 min). The enzyme activity is determined by measuring the release of L-glutamic acid using a commercial colorimetric assay measured spectrophotometrically at 600 nm. The enzyme activity is expressed in units/g. One unit is defined as the quantity of enzyme that releases 1 µmol of L-glutamic acid per minute under the conditions of the assay.[16]

The food enzyme has a temperature optimum around 60°C (pH 6.0) and a pH optimum around pH 6.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 60 min at different temperatures (pH 6.0) in the presence and absence of L-glutamine. Glutaminase activity decreased above 65°C (in the presence of L-glutamine) and above 55°C (in the absence of L-glutamine), showing no residual activity above 70°C in both cases.[17]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme preparation were provided for three batches used for commercialisation (Table 1).[18] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 65.7% and the average enzyme activity/TOS ratio was 3.3 U/mg TOS.

[6] https://zenodo.org/record/3336268#.X8pXR2hKiUn
[7] Additional data March 2022/Annex 3.
[8] 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.
[9] Technical dossier/Annex 4.1, Annex 4.2.
[10] Technical dossier/pp. 41–48/Annex 5.
[11] Technical dossier/Annex 6.
[12] Technical dossier/p. 30/Annex 8.
[13] Technical dossier/p. 30.
[14] Technical dossier/p. 28.
[15] Technical dossier/p. 31.
[16] Technical dossier/Annex 2.
[17] Technical dossier/pp. 31–32.
[18] Technical dossier/p. 28/Annex 1, Annex 3; Additional data March 2022.

TABLE 1 Composition of the food enzyme preparation.

Parameters	Unit	Batches		
		1	2	3
Glutaminase activity	U/g[a]	2300	1860	2230
Protein	%	53.9	54.4	52.8
Ash	%	3.5	3.8	3.6
Water	%	4.4	4.0	4.2
Diluent (dextrin)	%	25.8	25.8	27.7
Total organic solids (TOS)[b]	%	66.3	66.4	64.5
Activity/TOS ratio	U/mg TOS	3.5	2.8	3.5

[a]UNIT: U/g (see Section 3.3.1).

[b]TOS calculated as 100%−% water−% ash−% diluent.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg,[19] 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 level of mercury was below the limit of quantification (LoQ) of the employed method. For arsenic, the average concentration determined in the commercial batches was 0.13 mg/kg and for cadmium was 0.1 mg/kg.[20,21] The Panel considered these concentrations 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).[22] No antimicrobial activity was detected.[23]

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

3.4 | Toxicological data

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

3.4.1 | Allergenicity

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

The potential allergenicity of the glutaminase produced with the *B. amyloliquefaciens* strain AE-GT was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[24]

No information was available on oral and respiratory sensitisation or elicitation reactions of this glutaminase.

Epitopes on glutaminases have been identified that may indicate a potential for allergenicity (Parmar et al., 2022). In addition, two cases have been reported of hypersensitivity reactions to glutaminase used therapeutically as an antileukemic infusion (Harrison & Lilleyman, 1978). The Panel noted that IgE binding was not investigated in either of these studies. The Panel is not aware of allergic reactions after oral exposure to glutaminases.

[19]Technical dossier/Annex 3.

[20]LoQs: Pb = 0.005 mg/kg; As = 0.002 mg/kg; Cd and Hg = 0.001 mg/kg each.

[21]Technical dossier/Annex 3.

[22]Technical dossier/Annex 3.

[23]Technical dossier/Annex 3.

[24]Technical dossier/p. 60/Annex 8.

[redacted] and [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[25]), are used as raw materials. In addition, [redacted] and [redacted], known 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 a 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 five 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.[c]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of dairy products		
• Production of flavouring preparations from dairy products	Cheese[d]	8–**33**
Processing of eggs and egg products	Whole egg, egg yolk and egg white	41–**203**
Processing of meat and fish products		
• Production of protein hydrolysates from meat and fish proteins[e]	Meat and fish proteins	17–**33**
Processing of plant- and fungal-derived products		
• Production of protein hydrolysates from plants and fungi[e]	Plant and vegetable proteins	17–**33**
Processing of yeast and yeast products	Yeast extract, autolysed yeast[f]	59–**590**

[a]The name has been harmonised by EFSA according to 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.

[c]Technical dossier/p. 55; Additional data March 2022/Answers 9, 10, 11.

[d]Additional data March 2022/Answer 9.

[e]Additional data March 2022/Answer 10.

[f]Additional data March 2022/Answer 11.

In all of the food manufacturing processes, the release of L-glutamic acid catalysed by glutaminase contributes to the improvement of the sensory properties of the final foods.[26]

In the production of flavouring preparations from dairy products, the food enzyme is added to cheese slurry after the heat treatment.[27] The food enzyme-TOS remains in the final foods. This glutaminase is used to treat only cheese and cheese slurry, but no other dairy ingredients, such as cream or butter.[28]

In the processing of eggs and egg products, after the breaking of the eggs, the food enzyme is added to treat the whole egg or egg white and yolk.[29] The food enzyme-TOS remains in the final foods.

In the production of protein hydrolysates, the food enzyme is added to a variety of plant and animal protein-rich materials (e.g. meat extract, corn protein, soybean protein) during hydrolysis.[30] The food enzyme-TOS remains in the final protein hydrolysates, which is an ingredient in a variety of final foods, including infant formulae and follow-on formulae.

In the processing of yeast and yeast products, glutaminase may be added to autolysed yeast or directly to the yeast extract.[31] The food enzyme-TOS remains in the final yeast extracts.

[25]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.

[26]Technical Dossier/p. 68; Additional data March 2022/Answer 12.

[27]Technical Dossier/p. 50.

[28]Additional data March 2022/Answer 9.

[29]Technical Dossier/p. 51.

[30]Technical Dossier/p. 52.

[31]Technical Dossier/p. 53.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing steps applied in the food processes, it is expected that the food enzyme is inactivated by heat in all the food manufacturing processes listed in Table 2.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme-TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 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 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 0.462 mg TOS/kg body weight (bw) per day in toddlers 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)	0.001–0.039 (12)	0.015–0.159 (15)	0.049–0.169 (19)	0.015–0.083 (21)	0.011–0.047 (22)	0.006–0.048 (23)
Min–max 95th percentile (number of surveys)	0.002–0.234 (11)	0.086–0.462 (14)	0.131–0.421 (19)	0.058–0.225 (20)	0.035–0.136 (22)	0.036–0.134 (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	
To estimate from the production of flavouring preparations from dairy products, cheese is the only raw material indicated by the applicant, but the calculation included all types of enzyme modified dairy ingredients	+
Although the food enzyme under application is not used to treat the yeast cell wall,[a] food categories chosen for calculation are broader than yeast extract and include also those relevant to yeast autolysates and yeast cell wall	+
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	+/–

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

[a]Additional data March 2022/Answer 11.

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.

3.6 | Margin of exposure

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

4 | CONCLUSION

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

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of Glutaminase from *Bacillus amyloliquefaciens* AE-GT. January 2015. Submitted by Amano Enzyme Inc.

Additional information. March 2023. Submitted by Amano Enzyme Inc.

Additional information. July 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
TOS	total organic solids
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

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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.

REFERENCES

EFSA (European Food Safety Authority). (2006). Opinion of the scientific committee related to uncertainties in dietary exposure assessment. *EFSA Journal*, *5*(1), 438. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority). (2009a). Guidance of EFSA prepared by the scientific panel of food contact material, enzymes, Flavourings and processing aids on the submission of a dossier on food enzymes. *EFSA Journal*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority). (2009b). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General Principles. *EFSA Journal*, *7*(5), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority). (2011). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Zorn, H., Glandorf, B., … Rainieri Sand Chesson, A. (2021). Scientific Guidance for the submission of dossiers on FoodEnzymes. *EFSA Journal*, *19*(10), 6851, 37. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Apergi, K., … Chesson, A. (2023). Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, *21*(7), 8094. https://doi.org/10.2903/j.efsa.2023.8094

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010). Scientific opinion on the assessment of allergenicity of GM plants and micro-organisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011). Scientific opinion on guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. *EFSA Journal*, *9*(6), 2193. https://doi.org/10.2903/j.efsa.2011.2193

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). (2006). General specifications and considerations for enzyme preparations used in food processing in compendium of food additive specifications. 67th meeting. *FAO JECFA Monographs*, *3*, 63–67. https://www.fao.org/3/a-a0675e.pdf

Harrison, J. F., & Lilleyman, J. S. (1978). Toxicity of glutaminase in patients with advanced lymphoblastic leukaemia. *Leukemia Research*, *2*(2), 147–149.

Parmar, J., Tripathi, S., & Kumar, A. (2022). Immunogenicity assessment of antileukemic agent glutaminase from Escherichia coli, pseudomonas sp., and bacillus sp. *Biomedical and Biotechnology Research Journal (BBRJ)*, *6*(1), 138–144. https://doi.org/10.4103/bbrj.bbrj_136_21

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

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[a], Serbia[a], 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[a], Serbia[a], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[a], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden
The elderly[b]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[a], Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden

[a]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.

[b]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).