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SCIENTIFIC OPINION

Safety evaluation of the food enzyme bacillolysin from the non-genetically modified *Bacillus amyloliquefaciens* strain HPN 131

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 | Lieve Herman | Jaime Aguilera | Magdalena Andryszkiewicz | Cristina Fernandez-Fraguas | Yi Liu | Giulio diPiazza | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme bacillolysin (EC 3.4.24.28) is produced with the non-genetically modified *Bacillus amyloliquefaciens* strain HPN 131 by ENMEX SA de CV. The production strain qualifies for the qualified presumption of safety (QPS) approach to safety assessment. The food enzyme under assessment is intended to be used in seven food manufacturing processes: processing of cereals and other grains for the production of baked products, brewed products and distilled alcohol; processing of dairy products for the production of modified milk proteins; processing of meat and fish products for the production of protein hydrolysates; processing of plant- and fungal-derived products for the production of protein hydrolysates; processing of yeasts and yeast products. Since residual amounts of total organic solids (TOS) are not carried over to distilled alcohol, a dietary exposure was estimated only for the remaining six food manufacturing processes. Exposure was estimated to be up to 8.302 mg TOS/kg body weight (bw) per day in European populations. As the production strain qualifies for the QPS status and no issue of concern arose from the production process of the food enzyme, the Panel considered that no toxicological studies other than the assessment of allergenicity were necessary. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no matches were found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded (except for distilled alcohol production), 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

Bacillolysin, *Bacillus* metalloendopeptidase, *Bacillus subtilis,* EC 3.4.24.28, food enzyme

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 Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

An application has been introduced by the applicant "Birkegaard Consult" for the authorisation of the food enzyme Bacillolysin from *Bacillus subtilis* (strain HPN 131). 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: Bacillolysin from *Bacillus subtilis* (strain HPN 131) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.3 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme Bacillolysin from *B. subtilis* strain HPN 131. Recent data identified the production microorganism as *B. amyloliquefaciens* (Section 3.1). Therefore, this name will be used in this opinion instead of *B. subtilis*.

[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.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme Bacillolysin from *B. subtilis* (strain HPN 131).

Additional information was requested from the applicant during the assessment process on 12 September 2022 and was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on March 2023, EFSA requested a clarification teleconference on 31 May 2023, after which the applicant provided additional data on 15 June 2023.

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) 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 with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

3 | ASSESSMENT

IUBMB nomenclature	Bacillolysin
Systematic name	
Synonyms	*Bacillus* metallo endopeptidase; *Bacillus subtilis* neutral proteinase
IUBMB No	EC 3.4.24.28
CAS No	9080-56-2
EINECS No	232-991-2

Bacillolysins catalyse the hydrolysis of peptide bonds of proteins with broad specificity, releasing peptides and amino acids. The food enzyme under assessment is intended to be used in seven food manufacturing processes: processing of cereals and other grains for the production of baked products, brewed products and distilled alcohol; processing of dairy products for the production of modified milk proteins; processing of meat and fish products for the production of protein hydrolysates, processing of plant- and fungal-derived products for the production of protein hydrolysates; and processing of yeast and yeast products.

3.1 | Source of the food enzyme

The bacillolysin is produced with the non-genetically modified bacterium *B. amyloliquefaciens* strain HPN 131 (MC 131), which is deposited at the Belgian Co-ordinated Collections of Micro-organisms/University of Ghent (BCCM/LMG, Belgium) with the deposition number [redacted].[4] The production strain was identified as *B. amyloliquefaciens* by whole genome sequence (WGS) analysis, showing an average nucleotide identity [redacted].[5] *B. amyloliquefaciens* HPN 131 was obtained from *B. amyloliquefaciens* PRC49 (known as *B. subtilis* PRC49) by chemical mutagenesis and selection for high bacillolysin production.

The species *B. amyloliquefaciens* 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 cytotoxic activity are verified for the specific strain used (EFSA BIOHAZ Panel, 2020). *B. amyloliquefaciens* HPN 131 did not show toxicity on VERO cells using lactate dehydrogenase activity.[6] The WGS of the production strain was interrogated for the presence of antimicrobial resistance genes, [redacted] No genes of concern were detected. Therefore, the production strain is considered to qualify for the QPS approach to safety assessment.[5]

[4]Technical dossier/Additional data March 2023/Annex I.

[5]**Technical dossier/Additional data March 2023/Annex J.**

[6]Technical dossier/Additional data March 2023/Annex K.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[7], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[8]

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. 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 is concentrated by evaporation prior to analysis.[9] 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.[10]

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 bacillolysin is a single polypeptide chain of 521 amino acids. The molecular mass of the mature protein, estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, is 32.7 kDa.[11] The identity of the enzyme was confirmed by liquid chromatography with tandem mass spectrometry after tryptic digestion of the excised protein band. Amylase and subtilisin were also identified on the gels. No other enzymatic activities were reported.

The in-house determination of bacillolysin activity is based on the hydrolysis of casein (reaction conditions: pH 7.4, 40°C, 35 min). After precipitation of remaining intact casein, the enzymatic activity is determined by measuring the soluble hydrolysis products colourimetrically at 660 nm. The enzyme activity is expressed in Northrop Units/g (NU/g). One NU is defined as the amount of enzyme which hydrolyses 40% of the casein substrate under the conditions of the assay.[12]

The food enzyme has a temperature optimum around 50°C (pH 7) and a pH optimum around pH 8.[13] Thermostability was tested after a pre-incubation of the food enzyme for 20 min at different temperatures. The bacillolysin activity decreased above 50°C showing no residual activity after 80°C pre-incubation.[14]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation (Table 1).[15] The mean total organic solids (TOS) of the three food enzyme batches was 62.4% and the mean enzyme activity/TOS ratio was 6.4 NU/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches		
Parameters	**Unit**	**1**	**2**	**3**
Bacillolysin activity	NU/g[a]	3535	4814	3635
Protein	%	52.4	54.7	52.8
Ash	%	32.3	31.8	30.0
Water	%	6.7	6.3	5.7
Total organic solids (TOS)[b]	%	61.0	61.9	64.3
Activity/TOS ratio	NU/mg TOS	5.8	7.8	5.7

[a]NU: Northrop Unit (see Section 3.3.1).

[b]TOS calculated as 100% – % water – % ash.

[7]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.

[8]Technical dossier/Annex K.

[9]Technical dossier/Annex J.

[10]Technical dossier/Annex L and Additional data March 2023/Annex N.

[11]Technical dossier/Additional data March 2023/Annex E.

[12]Technical dossier/Annex A.

[13]Technical dossier/Additional data March 2023/Annex G.

[14]Technical dossier/Additional data March 2023/Additional package of information on Bacillolysin from *Bacillus* amyloliquefaciens/p. 40.

[15]Technical dossier/Annex E.

3.3.3 | Purity

The lead content in the three batches was below 1 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[13,16]

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). No antimicrobial activity was detected in any of the tested batches.[13]

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 carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the bacillolysin produced with the non-genetically modified *B.amyloliquefaciens* strain HPN 131 KY 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.[17]

No information was available on oral and respiratory sensitisation or elicitation reactions of this enzyme. The Panel was not aware of reports of allergic reactions after oral exposure to bacillolysins in the literature.

Soybean flour or meal, products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[18]), are used as raw material. In addition, corn steep liquor, a known source of allergens, is 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 potentially allergenic residues from these sources are not expected to be present in the food enzyme.

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 seven food processes at the recommended use levels summarised in Table 2.

[16]LoD: 0.029 mg/kg.

[17]Technical dossier/Additional data March 2023/ Annex Q.

[18]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.

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 cereals and other grains		
Production of baked products	Flour	250–**500**
Production of brewed products	Cereals, malt	1000–**1500**
Production of distilled alcohol	Cereals	1000–1500
Processing of dairy products		
Production of modified milk proteins	Whey protein	160–**1600**
Processing of meat and fish products		
Production of protein hydrolysates from meat & fish protein isolates	Soluble animal proteins from beef, lamb, poultry, crustaceans, etc	160–1600
	Insoluble side-stream animal products (e.g. skin, bone, viscera)	160–**1600**
Processing of plant- and fungal-derived products		
Production of protein hydrolysates from plants and fungi	Protein isolated from plants	160–**1600**
	Chlorella protein, microalgae (*Spirulina*, etc) protein, mycoprotein (Quorn)	160–1600
Processing of yeast and yeast products	Yeast, yeast extract, cell walls	160–**1600**

Abbreviation: TOS, total organic solids.

[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 were used for calculation.

[c]Technical dossier/Additional data March 2023/Technical dossier/pp. 63–64, Additional data June 2023.

In baking processes, the food enzyme is added to flour during the preparation of the dough or batter.[19] The bacillolysin cleaves the peptide bonds in the gluten network, thus, improving the rheology of the dough. The food enzyme–TOS remains in the baked products (e.g. bread, biscuits).

In brewing processes, the food enzyme is added to cereals during the mashing step.[20] The bacillolysin hydrolyses proteins in the cereals to release free amino nitrogen for the optimal growth of the brewer's yeast during fermentation. In addition, the partial degradation of protein ensures the clarity of beer. The food enzyme–TOS remains in the beer.

In distilled alcohol production, the food enzyme is added during the liquefaction and fermentation steps and may also be added during slurry mixing and pre-saccharification.[21] The bacillolysin may be used to improve the yield and to enhance the access of amylolytic enzymes to the starch granules, facilitating the degradation of starch and non-starch polysaccharides into fermentable sugars. The food enzyme–TOS is not carried over to the final processed foods (EFSA CEP Panel, 2023).

To manufacture protein hydrolysates, the food enzyme is used to treat proteins isolated from milk (e.g. whey proteins, caseins), plant (e.g. soy, wheat, maize), fungal or animal sources (e.g. meat, fish, collagen, gelatin).[22] The 'animal protein hydrolysates' may undergo a second processing step of thermal treatment in the presence of added carbohydrates and amino acids to generate meat-based flavouring.[23] The food enzyme–TOS remains in the final protein hydrolysates, which are subsequently used as an ingredients in a variety of foods, including infant formula, follow-on formula and foods for special medical purposes.

In yeast processing, the food enzyme is added to yeast cultures, yeast extracts or yeast cell walls during different stages of the process.[24] The bacillolysin is used to hydrolyse the insoluble proteins, optimising the extraction process and improving the functional properties of the yeast products. The food enzyme–TOS remains in yeast products.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing steps applied in the food manufacturing processes, it is expected that this bacillolysin will be inactivated or removed in all the food manufacturing processes listed in Table 2.

[19]Technical dossier/Additional data March 2023/Technical dossier/p. 58.

[20]Technical dossier/Additional data March 2023/Technical dossier/p. 59.

[21]Technical dossier/Additional data March 2023/Technical dossier/p. 60.

[22]Technical dossier/Additional data March 2023/Technical dossier/p. 61.

[23]Technical dossier/Additional data June 2023.

[24]Technical dossier/Additional data March 2023/Technical dossier/p. 62 and Annex S.

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: processing of cereals and other grains for the production of baked products and brewed products; processing of dairy products for the production of modified milk proteins; processing of meat and fish products for the production of protein hydrolysates; processing of plant- and fungal-derived products for the production of protein hydrolysates; and processing of yeast and yeast products.

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 (bw). 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 8.302 mg TOS/kg bw per day in adults 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)	0.611–2.100 (12)	1.292–3.070 (15)	0.267–2.742 (19)	0.073–1.632 (21)	0.712–2.538 (22)	0.651–1.508 (23)
Min–max 95th percentile (number of surveys)	2.133–4.700 (11)	2.964–4.936 (14)	0.664–5.325 (19)	0.196–3.328 (20)	1.769–8.302 (22)	1.539–4.164 (22)

Abbreviation: TOS, total organic solids.

3.5.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 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	
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 estimation: - production of distilled alcohol	–

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

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

The exclusion of one food manufacturing process from the exposure assessment was based on > 99% TOS removal during the process and was 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, the margin of exposure was not calculated.

4 | CONCLUSIONS

Based on the data provided, the QPS status of the production strain and the absence of issues of concern arising from the production process of the food enzyme, the Panel concluded that the food enzyme bacillolysin produced with the non-genetically modified *B. amyloliquefaciens* strain HPN 131 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Food enzyme application for Bacillolysin from *Bacillus subtilis* (strain HPN 131). June 2017. Submitted by Enmex SA de CV.

Additional data. March 2023. Submitted by Enmex SA de CV., a Kerry Company.

Additional data. June 2023. Submitted by Kerry Ingredients & Flavours Ltd.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
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	kilo Dalton
LoD	limit of detection
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
WHO	World Health Organization

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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, 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, the 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, the 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], the 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], the 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], the Netherlands, Portugal, Romania, Serbia[a], Slovenia, Spain, Sweden

[a] Consumption data from these pre-accession countries are included 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).