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SCIENTIFIC OPINION

Safety evaluation of the food enzyme endo-1,4-β-xylanase from the non-genetically modified *Trichoderma citrinoviride* strain 278

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Abstract

The food enzyme endo-1,4-*β*-xylanase (4-*β*-D-xylan xylanohydrolase; EC 3.2.1.8) is produced with the non-genetically modified *Trichoderma citrinoviride* strain 278 by Kerry Ingredients & Flavours Ltd. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in eight food manufacturing processes: processing of cereals and other grains for the production of baked products; production of cereal-based products other than baked, brewed products, starch and gluten fractions, distilled alcohol; processing of fruits and vegetables for the production of juices, wine and wine vinegar and processing of yeast and yeast products. Since residual amounts of total organic solids (TOS) are removed during two processes, dietary exposure was only calculated for the remaining six food manufacturing processes. Exposure was estimated to be up to 4.808 mg TOS/kg body weight (bw) per day in European populations. The Panel was unable to reach a conclusion on genotoxicity and systemic toxicity. 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 (except for distilled alcohol production), but the likelihood is low. In the absence of an acceptable full set of toxicological data, the Panel was unable to complete the safety assessment of the food enzyme.

KEYWORDS

endo-1,4-*β*-, xylanase, 4-*β*-D-xylan xylanohydrolase, EC 3.2.1.8, food enzyme, *Trichoderma citrinoviride*, xylanase

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 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.

The following three applications have been submitted for the authorisation of food enzymes:

1. From "Amano Enzyme Inc." for Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU);
2. From the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for Endo-1,3(4)-beta-glucanase, Endo-1,4-beta-xylanase and Cellulase from *Talaromyces emersonii*;
3. From AMFEP for Cellulase, Endo-1,3(4)-beta-glucanase and Endo-1,4-beta-xylanase obtained from *Trichoderma reesei*.

Following the requirements of Article 12.1 of Regulation (EC) No 234/20113, implementing Regulation (EC) No 1331/2008, the Commission has verified that the three 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 on the food enzymes Alpha-glucosidase from *Aspergillus niger* (strain AE-TGU), Endo-1,3(4)-beta-glucanase, Endo-1,4-beta-xylanase and Cellulase from *Talaromyces emersonii*, and Cellulase, Endo-1,3(4)-beta-glucanase and Endo-1,4-beta-xylanase obtained from *Trichoderma reesei* 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.

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 the food enzyme cellulase, endo-1,3(4)-beta-glucanase and endo-1,4-beta-xylanase obtained from *Trichoderma reesei*. The applicants have submitted 13 independent data packages corresponding to this mandate (former question numbers EFSA-Q-2014-00804 to EFSA-Q-2014-00806). The current opinion addresses the food enzyme produced with strain 278 submitted by Kerry Ingredients & Flavours Ltd, under a new question number (EFSA-Q-2021-00689). Recent data identified the production microorganism as *Trichoderma citrinoviride* (Section 3.1). Therefore, this name will be used in this opinion instead of *T. reesei*.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme endo-1,4-beta-xylanase from *T. citrinoviride*. The dossier was updated on 5 June 2023.

Additional information was requested from the applicant during the assessment process on 28 March 2022 and received on 29 September 2022 (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 existing guidance documents of EFSA Scientific Committee.

A data package originated from a joint dossier should fulfil the data requirements in the 'Submission of a Dossier on Food Enzymes for Safety Evaluation' (EFSA 2009a. During the evaluation, the Panel applied, whenever possible, the updated current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel et al., 2021) and the guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes '(EFSA CEP Panel et al., 2023).

3 | ASSESSMENT

IUBMB nomenclature	Endo-1,4-β-xylanase
Systematic name	4-β-D-xylan xylanohydrolase
Synonyms	4-xylanohydrolase; xylanase; β-1,4-xylanase; β-xylanase
IUBMB No	EC 3.2.1.8
CAS No	9025-57-4
EINECS No	232-800-2

Endo 1,4-β-xylanases catalyse the random hydrolysis of 1,4-β-D-xylosidic linkages in xylans (including arabinoxylans) resulting in the generation of (1–4)-β-D-xylan oligosaccharides. The enzyme under assessment is intended to be used in eight food manufacturing processes: processing of cereals and other grains for the production of baked products, cereal-based products other than baked, brewed products, starch and gluten fractions, distilled alcohol; processing of fruits and vegetables for the production of juices, wine and wine vinegar; and processing of yeast and yeast products.

3.1 | Source of the food enzyme

The endo-1,4-β-xylanase is produced with the non-genetically modified filamentous fungus *T. citrinoviride* strain 278, which is deposited as *Trichoderma* spp at the Westerdijk Fungal Biodiversity Institute culture collection (the Netherlands) with deposit number CBS [redacted].[3] The production strain was identified as *T. citrinoviride* by [redacted].[4]

[3]Technical dossier/Risk assessment data/Annex IX.

[4]Technical dossier/Risk assessment data/Annex XI/11_1.

Its genome was searched for gene clusters coding for toxic compounds. Partial similarity was found with some clusters involved in the synthesis of secondary metabolites with possible toxicity (sorbicillin, tenellin, fumorisone, chaetocin).[4]

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[5], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[6]

The production strain is grown as a pure culture using a typical industrial medium in a submerged ([redacted]) 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 supernatant 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 mass material passes the filtration membrane and is discarded.[7] 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.[8]

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The endo-1,4-β-xylanase is a single polypeptide chain of [redacted] amino acids.[9] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[10] A consistent protein pattern was observed across all batches. The gels showed a major protein band of around [redacted] kDa in all batches, consistent with the calculated mass of the food enzyme. β-Glucanase activity was detected in the food enzyme.[11] No other enzyme activities were reported.

The in-house determination of endo-1,4-β-xylanase activity is based on hydrolysis of arabinoxylan cross-linked to azurien (reaction conditions: pH [redacted], [redacted]°C, 10 min). The enzyme activity is determined by measuring the release of soluble dyed oligomers spectrophotometrically at 590 nm. The endo-1,4-β-xylanase activity is expressed in Units/mL (U/mL). One unit is defined as the amount of enzyme required to release one µmol of reducing sugar equivalents from arabinoxylan per minute under the assay conditions.[12]

The food enzyme has a temperature optimum around [redacted]°C (pH [redacted]) and a pH optimum between pH [redacted] and [redacted] ([redacted]°C).[13] Thermostability was tested after a pre-incubation of the food enzyme for [redacted] at different temperatures (pH [redacted]). The endo-1,4-β-xylanase activity decreased above 50°C, showing no residual activity after pre-incubation at 65°C.[13]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and three batches produced for the toxicological tests (Table 1).[14] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 16.7% and the mean enzyme activity/TOS ratio was 191.8 Unit/mg TOS.

[5]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.
[6]Technical dossier/Risk assessment data/p. 36/Annex XII.
[7]Technical dossier/Risk assessment data/pp. 39–44/Annex XIII.
[8]Technical dossier/Risk assessment data/Annex XIII.
[9]Technical dossier/Additional data September 2022/ Annex XVII Conf_ Amino acid sequence_ and MW_Mar22.
[10]Technical dossier/Additional data September 2022/Annex XVII_ SDS-PAGE gel analysis_ SS enzyme.
[11]Technical dossier/Risk assessment data/Annex I/1_2 and Annex II.
[12]Technical dossier/Risk assessment data/Annex I/ 1_1.
[13]Technical dossier/Risk assessment data/pp. 28–29/Annex VII.
[14]Technical dossier/Risk assessment data/p. 25/Annex II and Annex XIV/14_4; Additional data September 2022/Annexes XXIV and XV; Spontaneous submission June 2023/Annex XXIII.

TABLE 1 Composition of the food enzyme preparation

Parameters	Unit	Batches					
		1	2	3	4[a]	5[b]	6[c]
Endo-1,4-β-xylanase activity	Unit/mL[d]	33,157	31,278	31,629	10,523	25,000	29,976
Protein	%	11.1	11.1	11.6	5.7	5.0	12
Ash	%	0.7	0.7	0.6	NA[e]	10.7	0.9
Water	%	83	83	82	NA	72	82.8
Excipient	%	NA	NA	NA	NA	12	NA
Total organic solids (TOS)[f]	%	16.69	16.43	16.99	NA	5.3	16.3
Activity/TOS ratio	Unit/mg TOS	198.7	190.38	186.16	NA	471.7	183.9

[a]Batch used for the chromosomal aberration test.
[b]Batch used for the repeated dose 90-day oral toxicity study in rats.
[c]Batch used for the Ames test.
[d]U: Unit (see Section 3.3.1).
[e]NA: not analysed.
[f]TOS calculated as 100% – % water – % ash – % excipient.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg,[15,16] 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, arsenic, cadmium and mercury contents were determined in one batch at concentrations below 0.1 mg/kg.[17] The Panel considered these concentrations as not of concern.[17]

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).[18] No antimicrobial activity was detected in any of the tested batches.[19]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, ochratoxin A and zearalenone was examined in four food enzyme batches and all were below the LoD of the applied method[20,21] Deoxynivalenol (DON) was found in all batches tested, with a mean concentration of 40 µg/kg.[17] Given the use level of the food enzyme, the Panel considered this finding of no concern.

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

3.3.4 | Viable cells 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. One hundred mL of product was filtered through a 0.45 µm membrane. The membrane was placed on non-selective agar medium and incubated at 30°C for 5 days. No colonies were produced. A positive control was included.[22]

3.4 | Toxicological data

A battery of toxicological tests including a bacterial reverse mutation test (Ames test; enzyme batch 6), an in vitro mammalian chromosomal aberration test (batch 4) and a repeated dose 90-day oral toxicity study in rats (batch 5) has been provided.

The batch 6 was considered as suitable test-item.

No chemical composition was given for the batch 4. Instead, the mean values of three commercial batches from a later period was submitted.[23] In addition, from the data provided, batch 5 was not considered representative of the commercial

[15]Technical dossier/Risk assessment data/Annexes I/1_11, II and IV.
[16]LoD: Pb = 0.005 mg/kg.
[17]Technical dossier/Risk assessment data/Annex IV.
[18]Technical dossier/Risk assessment data/p. 27 and Annexes I/1_3, 1_4, 1_5 and II; Additional data September 2022/Annex XXII.
[19]Technical dossier/Risk assessment data/Annexes II and III/3_1, 3_2; Additional data September 2022/Annex XXII.
[20]LoDs: aflatoxins: B1, B2, G1, G2 = 0.5 mg/kg each; deoxinyvalenol = not provided, fumonisins: B1, B2 = 20 µg/kg each; ochratoxin A = 1 µg/kg; zearalenone = 10 µg/kg.
[21]Technical dossier/Risk assessment data/Annexes II and IV.
[22]Technical dossier/Risk assessment data/Annex V.
[23]Technical dossier/Spontaneous submission June 2023/Annex XXIII.

batches, as the value for the activity/TOS ratio was substantially higher than that of the commercial batches.[24] Consequently, data on the 90-day study and the chromosomal aberration test were not considered. Therefore, the genotoxic potential and systemic toxicity of the food enzyme could not be fully evaluated.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 2020) and following Good Laboratory Practice (GLP).[25] Five strains of Salmonella Typhimurium (TA98, TA100, TA102, TA1535 and TA1537) were used with or without metabolic activation (S9-mix), applying both the pre-incubation and the standard plate incorporation methods.

Based on the results of a range finding test, the experiments were carried out in triplicate, using five concentrations of the food enzyme at 50, 158, 500, 1580 and 5000 µg TOS/plate. No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme endo-1,4-*β*-xylanase did not induce gene mutations under the test conditions applied in this study.

3.4.2 | 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 endo-1,4-*β*-xylanase produced with the non-genetically modified *T. citrinoviride* strain 278 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.[26]

No information was available on oral and respiratory sensitisation or elicitation reactions of this endo-1,4-*β*-xylanase.

Respiratory allergy following occupational exposure to xylanase, e.g. baker's asthma, has been described in some epidemiological studies (Elms et al., 2003; Martel et al., 2010) and case reports (Baur et al., 1955; Merget et al., 2001). However, several studies have shown that adults with occupational asthma caused by an enzyme can ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Poulsen, 2004).

[redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[27]) are used as raw materials. In addition, [redacted] 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 fungal 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 distilled alcohol production), 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.

[24]Technical dossier/Risk assessment data/Annex XIV/14_4.

[25]Technical dossier/Additional data September 2022/Annex XXIV.

[26]Technical dossier/Risk assessment data/pp. 64–65 and Annex XV/15_1.

[27]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	0.1–**100**
Production of cereal-based products other than baked	Flour	0.1–**100**
Production of brewed products	Cereals	0.1–**100**
Production of starch and gluten fractions	Cereals	1–70
Production of distilled alcohol	Cereals	3–200
Processing of fruits and vegetables		
Production of juices	Fruit and vegetables	5–**100**
Production of wine and wine vinegar	Grape	3–**80**
Processing of yeast and yeast products	Yeast cells, yeast extract and cell walls	10–**250**

Abbreviation: TOS, total organic solids.

[a]The names have been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel et al., 2023).

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

[c]Technical dossier/p. 51; Additional data September 2022/Responses 8 and 9.

For the production of baked products,[28] the food enzyme is added to flour during the preparation of the dough or batter. It hydrolyses (arabino)xylans, increasing the water binding capacity of the dough and reducing viscosity. The food enzyme–TOS remains in the baked products.

For the production of cereal-based products other than baked, the food enzyme is added to cereals and other grains during the preparation of the dough or batter.[29] The endo-1,4-β-xylanase hydrolyses (arabino)xylans, decreasing the water binding capacity of the dough and reducing viscosity. The food enzyme–TOS remains in cereal-based products.

For the production of brewed products, the food enzyme is added to cereals during the mashing step.[29] It degrades cell walls, promoting the release of starch and protein and increasing the brewing yield. The food enzyme is also added during fermentation to aid beer filtration. The food enzyme–TOS remains in beer.

For the production of starch and gluten fractions, the food enzyme can be added to the grain to obtain flour, or to the dough to obtain starch and gluten fractions.[30] Repeated washing steps remove the food enzyme–TOS in the final starch or gluten (EFSA CEP Panel et al., 2023).

For the production of distilled alcohol, the food enzyme is applied during liquefaction and fermentation and may also be added during slurry mixing and pre-saccharification.[30] The food enzyme–TOS is removed in the final processed foods (EFSA CEP Panel et al., 2023).

For the production of juices, the food enzyme is added to fruits or vegetables during the mashing step.[30] The endo-1,4-*β*-xylanase degrades cell walls, reducing cloudiness and turbidity. The food enzyme–TOS remains in juices.

For the production of wine and wine vinegar production, the food enzyme can be added to grapes in several steps: crushing and maceration, pressing, clarification, fermentation, vinification and filtration.[30] The endo-1,4-*β*-xylanase degrades cell walls, releasing colour or flavour compounds and increasing yield. The food enzyme–TOS remains in the wine and the wine vinegars.

In the processing of yeast and yeast products, the food enzyme can be added to yeast cells, yeast extracts or yeast cell walls.[31] The food enzyme–TOS remains in the yeast extract and the yeast cell walls as well as the foods prepared from these materials.[32]

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that the endo-1,4-*β*-xylanase is inactivated during most food manufacturing processes, but may remain active in juices, depending on the pasteurisation conditions.

3.5.2 | Dietary exposure estimation

A dietary exposure was calculated only for the food manufacturing processes where the food enzyme–TOS remains in the final foods: production of baked products, production of cereal-based products other than baked, production of brewed products, production of juices, production of wine and wine vinegar, processing of yeast and yeast products.

[28]Additional data September 2022/Response 8a and Annex XIII v2.

[29]Technical dossier/Annex XIII/13_2.

[30]Additional data September 2022/Response 8b and Annex XIII v2.

[31]Additional data September 2022/Response 10 and Annex XIII v2.

[32]Additional data September 2022/ Annexes XVI and XVII.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel et al., 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel et al., 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 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure to the food enzyme–TOS was estimated to be 4.808 mg TOS/kg 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)	0.107–0.917 (12)	0.492–3.063 (15)	0.424–1.661 (19)	0.342–1.132 (21)	0.243–0.738 (22)	0.209–0.596 (23)
Min–max 95th percentile (number of surveys)	0.387–2.792 (11)	1.376–4.808 (14)	0.807–4.797 (19)	0.669–2.844 (20)	0.625–2.178 (22)	0.537–1.547 (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	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–
Exclusion of other processes from the exposure assessment – Production of starch and gluten fractions – 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 an overestimation of the exposure.

The exclusion of two food manufacturing processes from the exposure assessment was based on > 99% of TOS removal during these processes and was not expected to have an impact on the overall estimate derived.

3.6 | Margin of exposure

In the absence of acceptable toxicological data, no margin of exposure was calculated.

4 | CONCLUSIONS

In the absence of an acceptable full set of toxicological data, the Panel was unable to complete the safety assessment of the food enzyme endo-1,4-β-xylanase produced with the non-genetically modified *T. citrinoviride* strain 278.

5 | DOCUMENTATION AS PROVIDED TO EFSA (IF APPROPRIATE)

Application for authorisation of the food enzyme Endo-1,4-β-xylanase from *T. citrinoviride*. November 2021. Submitted by Kerry Ingredients & Flavours Ltd. The dossier was updated on 05 June 2023.

Additional information. 29 September 2022. 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
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Co-operation and Development
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

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None.

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.

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NOTE

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.8399#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 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[b], Serbia[b], 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, Serbia[b], Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro[b], the Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina[b], Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro[b], the Netherlands, Portugal, Romania, Serbia[b], 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[b], the Netherlands, Portugal, Romania, Serbia[b], Slovenia, Spain, Sweden

[a] The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).

[b] 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.