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Safety evaluation of the food enzyme cellulase from the non-genetically modified *Aspergillus niger* strain 294

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Abstract

The food enzyme cellulase (4-(1,3;1,4)-β-D-glucan-4-glucanohydrolase; EC 3.1.2.4) is produced with the non-genetically modified *Aspergillus niger* strain 294 by Kerry Ingredients & Flavours Ltd. The food enzyme is considered free from viable cells of the production organism. The enzyme is intended to be used in eight food manufacturing processes: baking processes, cereal-based processes, brewing processes, grain treatment for the production of starch and gluten fractions, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices, distilled alcohol production and wine and wine vinegar production. Since residual amounts of total organic solids (TOS) are removed during distilled alcohol production and grain treatment for the production of starch and gluten fractions, dietary exposure was only calculated for the remaining six food manufacturing processes. It was estimated to be up to 5.706 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 794 mg TOS/kg bw per day, the highest dose tested. The calculated margin of exposure for each age group was 184 (infants), 146 (toddlers), 139 (children), 219 (adolescents), 305 (adults) and 441 (the elderly). A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and four matches were found. The Panel considered that, under the intended conditions of use (other than distilled alcohol production), the risk of allergic reactions by 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 for adolescents, adults and the elderly.

Keywords: food enzyme, cellulase, 4-(1,3;1,4)-β-D-glucan-4-glucanohydrolase, EC 3.1.2.4, β-1-4-glucanase, *Aspergillus niger*

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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 micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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.

Four applications have been introduced by the companies 'Puratos NV sa.', 'Novozymes A/S.', 'Meito Sangyo Co., Ltd' and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzymes Inulinase from a genetically modified strain of *Aspergillus oryzae* (strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase 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 four 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 Inulinase from a genetically modified strain of *Aspergillus oryzae*

[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, 11.03.2011, pp. 15–24.

(strain MUCL 44346), Trypsin from porcine pancreatic glands, Triacylglycerol lipase from *Candida cylindracea*, and Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger* in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

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 Cellulase, Glucanase and Hemicellulase covering Xylanase and Mannanase from *Aspergillus niger*.

The application was submitted initially as a joint dossier and identified as the EFSA-Q-2015-00340, EFSA-Q-2018-01034 and EFSA-Q-2018-01035. During a meeting between EFSA, the European Commission and AMFEP,[4] it was agreed that joint dossiers will be split into individual data packages.

The current opinion addresses one data package originating from the former joint dossier. This data package is identified as EFSA-Q-2021-00693 and concerns the food enzyme cellulase produced from the *A. niger* strain 294 and submitted by Kerry Ingredients & Flavours Ltd.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme cellulase from *A. niger* strain 294.

Additional information was requested from the applicant during the assessment process on 14 February 2022 and 21 March 2022, and was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data by EFSA on 22 September 2022, EFSA requested a clarification teleconference on 18 January 2023, after which the applicant provided additional data on 3 February 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 existing guidance documents of EFSA Scientific Committee.

The current 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) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the 'CEF Panel Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature	Cellulase
Systematic name	4-(1,3;1,4)-β-D-glucan-4-glucanohydrolase
Synonyms	Carboxymethyl cellulase; β-1-4-glucanase
IUBMB No	3.2.1.4
CAS No	9012-54-8
EINECS No	232-734-4

Cellulases catalyse the hydrolysis of 1–4-β-glycosidic linkages in cellulose and other β-glucans resulting in the generation of shorter β-D-glucan chains. The enzyme is intended to be used in eight food manufacturing processes: baking processes, cereal-based processes, brewing processes, grain treatment for the production of starch and gluten fractions, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices, distilled alcohol production, wine and wine vinegar production.

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

3.1. Source of the food enzyme

The cellulase is produced with the non-genetically modified filamentous fungus *A. niger* strain 294, which is deposited at the Westerdijk Fingal Biodiversity Institute culture collection (the Netherlands), with the deposit number [redacted].[5] The production strain was identified as *A. niger* by phylogenomic analysis using whole genome sequence (WGS) data.[6]

The genome of the production strain was searched for gene clusters with known functions and no cluster was found involved in the synthesis of compounds with known toxicity.[6]

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, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is stabilised and 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.[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 cellulase is a single polypeptide chain of [redacted] amino acids.[11] The molecular mass of the mature protein, calculated from the amino acid sequence, is 36.5 kDa.[12] The food enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.[13] A consistent protein pattern was observed across all batches. The gels showed a protein migrating close to the marker protein of 40 kDa, consistent with the calculated mass of the enzyme. The protein profile also included two bands of around [redacted] and around [redacted] kDa that, according to the applicant, corresponded to mannanase and xylanase, and other bands of lesser staining intensity. The food enzyme was tested for endo-1,3(4)-β-glucanase, mannan endo-1,4-β-mannosidase and endo-1,4-β-xylanase activities and all were detected. No other enzyme activities were reported.[14]

The in-house determination of cellulase activity is based on hydrolysis of an azurine-cross-linked hydroxyethylcellulose (reaction conditions: pH 4.5, 40°C, 20 min). The enzymatic activity is determined by measuring the release of water-soluble dyed fragments of the substrate spectrophotometrically at 590 nm. The cellulase activity is expressed in Units/mL (U/mL). One Unit is defined as the amount of enzyme required to release one μmol of glucose equivalents per minute under the assay conditions.[14]

The food enzyme has a temperature optimum around 55°C (pH 4.5) and a pH optimum around pH 4.5 (40°C).[15] No data on thermostability were provided.[16]

[5] Technical dosser/Risk assessment data/Annex 3.2.1.2.4.1.
[6] Technical dosser/Risk assessment data/Annex 3.2.1.2.4.2.
[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 dosser/Risk assessment data/p. 22.
[9] Technical dossier/Risk assessment data/p. 24–30.
[10] Technical dossier/Risk assessment data/Annex 3.2.1.2.5.3.
[11] Technical dossier/Additional information September 2022/ Annex 3.2.2.2.2 v2.
[12] Technical dossier/Additional information September 2022/Annex 3.2.1.1.1.v2.
[13] Technical dossier/Additional data clarification teleconferece/Annex_3.2.1.1.8_v2.
[14] Technical dossier/Risk assessment data/Annex 3.2.1.1.4.
[15] Technical dossier/Risk assessment data/Risk assessment pp. 18–19.
[16] Data onnthermostability were requested by EFSA (Ref. VC/ac (2022) – out-25839550).

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (batches 1–3) and four batches (batches 4–7) produced for the toxicological tests (Table 1).[17] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 19.8% and the mean enzyme activity/TOS ratio was 25.2 U/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches						
		1	2	3	4[(a)]	5[(b)]	6[(c)]	7[(d)]
Cellulase activity	U/mL[(e)]	5,330	4,662	4,888	23,420	20,058	13,458	4,158
Protein	%	13.7	14.4	14.4	7.5	8.6	NA[(f)]	15.0
Ash	%	0.3	0.2	0.2	4.0	4.9	4.4	0.2
Water	%	80.0	80.0	80.0	7.7	8.8	7.5	80.4
Carrier (gum arabic)	%	0.0	0.0	0.0	8.9	9.0	8.5	0.0
Total organic solids (TOS)[(g)]	%	19.7	19.8	19.8	79.4	77.3	79.6	19.4
Activity/TOS	U/mg TOS	27.1	23.5	24.9	29.5	25.9	16.9	21.4

(a): Batch used for the reverse mutation assay and the repeated dose 90-day oral toxicity study in rats.
(b): Batch used for *in vitro* chromosomal aberration test in human lymphocytes.
(c): Batch used for *in vivo* micronucleus test in mice.
(d): Batch used for *in vitro* micronucleus test.
(e): U: Unit (see Section 3.3.1).
(f): NA: not analysed.
(g): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the four batches used for toxicological studies was below 5 mg/kg[18,19] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[20]

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).[18] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, total deoxynivalenol, fumonisin B1 and B2, ochratoxin and zearalenone was examined in the three food enzyme batches and all were below the limit of detection (LoD) of the applied methods.[22,23] Adverse effects caused by the possible presence of other secondary metabolites were addressed by the toxicological examination of the food enzyme–TOS.

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

[17] Technical dossier/Risk assessment data/Annexes 3.2.1.1.2 and 3.2.2.1.4; Additional information September 2022/Annexes 3.2.2.1.5 and 3.2.2.1.3a; Additional Data clarification teleconference/ Clarification Conf 2021–00693 Additional data request Jan23.
[18] Technical dossier/Risk assessment data/Annexes 3.2.1.1.2 and 3.2.2.1–4.
[19] LoD: Pb = 0.005 mg/kg.
[20] Technical dossier/Risk assessment/Risk assessment p. 17.
[21] Technical dossier/Risk assessment data/Annexes 3.2.1.1.2, 3.2.1.1.6a-b, and 3.2.2.1–4.
[22] Technical dossier/Risk assessment data/Annex 3.2.1.1.2 and 3.2.1.1.5a-b.
[23] LoD: aflatoxins = 1 μg/kg; deoxynivalenol = 100 μg/kg; fumonisins B1 and B2 = 5 μg/kg each; ochratoxin A = 1 μg/kg; zearalenone = 5 μg/kg.

pad, the pad was deposited onto a non-selective agar plate and incubated at 30°C for 5 days. No colonies were produced. A positive control was included.[24]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test, an *in vivo* micronucleus test, an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats, were provided. The batches 4, 5, 6 and 7 (Table 1) used in these studies have similar activity/TOS values as the batches used for commercialisation and are considered suitable as test items.

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, 1997a) and following Good Laboratory Practice (GLP).[25]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2*uvrA*- were used with or without metabolic activation (S9-mix), applying the standard plate incorporation method. Based on the results of a range-finding study, two separate experiments were carried out in triplicate, using five concentrations of the food enzyme ranging from 50 to 5,000 μg/plate, corresponding to 40 to 3,975 μg TOS/plate. No cytotoxicity was observed at any concentration of the food enzyme tested. 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 cellulase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[26]

Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix). In a range-finding test, cytotoxicity of 50% was only seen at 1,250 μg TOS/mL and above in a short-term treatment with metabolic activation (S9-mix).

In the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 156, 313 and 625 μg TOS/mL, in a short-term treatment (4 h exposure and 20 h recovery period) either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 39, 78 and 156 μg TOS/mL, in a short-term treatment (4 h exposure and 20 h recovery period) with S9-mix and at concentrations of 39, 78, 156 and 313 μg TOS/mL in a long-term treatment (24 h exposure without recovery period) without S9-mix.

In the short-term treatment, cytotoxicity of 50% was observed at concentration of 625 μg TOS/mL, with or without S9-mix. In the long-term treatment, cytotoxicity of 59% was observed at 156 μg TOS/mL. The frequency of structural and numerical chromosomal aberrations was not statistically significantly different to the negative controls at any concentrations tested, in neither of the experiments. All results were within the 95% of the historical control range.

The Panel concluded that the food enzyme cellulase did not induce an increase in the frequency of chromosomal aberrations under the test conditions applied in this study.

3.4.1.3. *In vivo* erythrocyte micronucleus test

The *in vivo* micronucleus test was carried out according to OECD Test Guideline 474 (OECD, 1997c) and following GLP.[27]

[24] Technical dossier/risk assessment data/Annex 3_2_1_2_4_4.
[25] Technical dossier/Risk assessment data/Annex 3.2.2.1.1.
[26] Technical dossier/Risk assessment data/Annex 3.2.2.1.2.
[27] Technical dossier/Risk assessment data/Annex 3.2.2.1.3.

The food enzyme was tested for its ability to induce micronuclei in the polychromatic erythrocytes (PCEs) of the bone marrow of treated albino Hsd: ICR (CD-1®) mice. In a range finding study, where mice were treated with a single dose of 2,000 mg/kg food enzyme, no clinical signs of toxicity were observed. In the micronucleus test, a single dose of the food enzyme was given to groups of seven male mice by gavage, in doses corresponding to 500, 1,000 and 2,000 mg/kg bw. Controls received the vehicle (distilled water). The mice were killed 24 h after dosing and an extra group of seven mice treated at the high dose were killed 48 h after treatment.

Mice treated with the food enzyme exhibited %PCE values and mean frequencies of MNPCE that were similar to and not statistically different from those seen in the concurrent vehicle control or in historical control data for all dose groups.

No significant increase in MNPCE in dosed groups compared to the vehicle control was observed. However, the range of doses tested did not reach the limit dose and no data on bone marrow exposure were provided. Therefore, the Panel considered this study as inconclusive.

3.4.1.4. *In vitro* mammalian cell micronucleus test

The *in vitro* mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.[28]

In a cytotoxicity test where cultures of Chinese hamster ovary cells (CHO-K1) were treated with the food enzyme up to 5,000 μg TOS/mL, cytotoxicity of more than 50% was observed at concentrations of 2,500 μg TOS/mL and higher in short-term treatments (4 h treatment followed by 20 h recovery) with or without metabolic activation (S9-mix), and at 156 μg TOS/mL without S9-mix in a long-term treatment (24 h treatment without recovery).

In the main experiment, duplicate cultures of CHO cells were treated with the food enzyme and scored for binucleated cells with micronuclei (MNBN) at concentrations of 313, 625 and 1,250 μg TOS/mL in the short-term treatments with or without S9-mix, and at 25, 50 or 100 μg TOS/mL in the long-term treatment without S9-mix. The frequency of MNBN was not statistically significantly different from the negative controls at any concentrations tested in the short-term or long-term treatments and all results were within the 95% historical control range.

The Panel concluded that the food enzyme cellulase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed following GLP[29] and in accordance with OECD Test Guideline 408 (OECD, 1998) with the following deviations: detailed clinical observations were not made and blood urea nitrogen was not examined. The Panel considered that these deviations are minor and do not impact on the evaluation of the study.

Groups of 10 male and 10 female Wistar (Wistar Han™, HsdRccHan™) rats received by gavage the food enzyme in doses of 20, 250 or 1,000 mg/kg bw per day corresponding to 16, 199 or 794 mg TOS/kg body weight (bw) per day. Controls received the vehicle (distilled water).

No mortality was observed.

In the functional observations, a statistically significant decrease in the overall activity was observed in all male groups (−38%, −34%, −2%, from low to high dose, respectively) and in mean hind limb grip strength in high-dose females (−24%). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters), there was no dose–response relationship (overall activity in males) and the changes were only observed in one of the functional performance tests for each sex (both parameters).

Haematological investigations revealed a statistically significant increase in the eosinophil count (+690%) in low-dose males, in haemoglobin (Hb, +5% and +9%, respectively) and haematocrit (Hct, +6% and +10%, respectively) in mid- and high-dose females and in the erythrocyte count (RBC count, +10%) in high-dose females, and a decrease in the clotting time (−8%) in high-dose females. The Panel considered the changes as not toxicologically relevant as they were only recorded sporadically (eosinophil count), they were only observed in one sex (all parameters), there was no dose–response relationship (eosinophil count), the changes were small (all parameters in females), there were no changes in other relevant parameters (for eosinophil count, in the total white blood cell

[28] Technical dossier/Additional information September 2022/Annex 3.2.2.1.3b.
[29] Technical dossier/Risk assessment data/Annex 3.2.2.1.4.

(WBC) count and in counts of other WBC populations; for clotting time, in the activated partial thromboplastin time and platelet count), and small increases in the red blood cell parameters (RBC count, Hb, Hct) are generally not considered as adverse effects.

Clinical chemistry investigations revealed a statistically significant increase in the total cholesterol concentration (+26%) in low-dose males and a decrease in creatinine levels in mid- and high-dose males (−8% and −10%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (both parameters), there was no dose–response relationship (total cholesterol) and the change was small (creatinine).

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 794 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of the cellulase produced with the genetically modified *A. niger* strain 294 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.[11]

No information is available on oral and respiratory sensitisation or elicitation reactions of this cellulase.

Several cases of respiratory allergy following occupational inhalation of cellulases have been reported (Merget et al., 2001; Elms et al., 2003; Martel et al., 2010). Studies have shown that adults respiratorily sensitised to respiratory allergens can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Indeed, no allergic reactions upon dietary exposure to any cellulase have been reported in the literature.

[redacted], a product that may cause allergies or intolerances (Regulation (EU) No 1169/2011[30]) is used as a raw material. In addition, [redacted] and [redacted], known sources of allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the 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, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded (other than 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.[31]

[30] 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.

[31] Technical dossier/Additional Data September 2022/Answers 6, 7, 8 and 9; Additional Data clarification teleconference/Clarification Conf 2021–00693 Additional data request Jan23, Table 5.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Baking processes	Flour	0.15–**55**
Cereal-based processes	Flour	0.15–**55**
Brewing processes	Cereals	0.5–**69**
Grain treatment for the production of starch and gluten fractions	Cereals	0.5–50
Fruit and vegetable processing for juice production	Fruits and vegetables	0.6–**123**
Fruit and vegetable processing for products other than juices	Fruits and vegetables	0.6–**123**
Distilled alcohol production	Cereals/Grist	0.5–188
Wine and wine vinegar production	Grapes	3.8–**38**

TOS: total organic solids.
(a): The name has been harmonised according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculation.

In baking and cereal-based processes, the food enzyme is added to the flour during the preparation of dough or batter.[32] The cellulase hydrolyses β-D-glucans, reducing the viscosity of the dough. The food enzyme–TOS remains in the final food products.

In brewing processes, the food enzyme is added to cereals during the mashing step. It can be added also to the wort during fermentation.[32] The enzymatic treatment can increase the choice of raw material, facilitates filtration by reducing viscosity and reduces haze and turbidity. The food enzyme–TOS remains in the beer.

In grain treatment for the production of starch and gluten fractions, cellulase is added to grain during multiple steps (steeping with water, dough preparation and agglomeration).[32] The enzymatic reaction reduces viscosity and increases yield. The food enzyme–TOS is removed from the gluten and starch fractions by repeated washing (EFSA CEP Panel, 2021b).

In fruit and vegetable processing for juice production, cellulase is added during mash treatment.[32] The enzymatic treatment decreases viscosity, thereby easing pressing and releasing of cell contents. The food enzyme remains in the juices.

For the production of other fruit and vegetable products, the food enzyme is added to the sliced fruit or milled vegetables.[32] The cellulase degrades cell walls, reducing viscosity and releasing colour and flavour compounds. The food enzyme remains in the final food products (e.g. potato flakes).

In the production of distilled alcohol, cellulase is added to the cereals during mixing and liquefaction before the fermentation.[32] The enzymatic treatment decreases viscosity, improves processing and increases yield. The food enzyme is not carried over with the distilled alcohols (EFSA CEP Panel, 2021b).

For wine and wine vinegar production, the food enzyme can be added to grapes at several stages, i.e. crushing and maceration, pressing, clarification, fermentation, vinification and filtration.[33] The cellulase degrades cell walls, releasing colour or flavour compounds and increasing yield. The food enzyme–TOS remains in wine and wine vinegar.

Because no data on thermostability were provided by the applicant, the Panel is not in the position to assess whether the food enzyme will be inactivated during the food manufacturing processes.

3.5.2. Dietary exposure estimation

A dietary exposure was calculated only for food manufacturing processes where the food enzyme–TOS remains in the final foods: baking processes, cereal-based processes, brewing processes, fruit and vegetable processing for juice production, fruit and vegetable processing for products other than juices, wine and wine vinegar production.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021a). The estimation

[32] Technical dossier/Additional Data clarification teleconference/Annex 3.2.1.3.1 v3.
[33] Technical dossier/Risk assessment data/Annex 3.2.1.3.1.

involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for 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 different 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 5.706 mg TOS/kg bw per day in children.

Table 3: Summary of the estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.233–1.765 (12)	0.721–3.784 (15)	0.472–2.125 (19)	0.253–1.220 (21)	0.203–0.820 (22)	0.136–0.592 (23)
Min–max 95th percentile (number of surveys)	0.596–4.317 (11)	2.244–5.424 (14)	1.031–5.706 (19)	0.671–3.620 (20)	0.588–2.595 (22)	0.366–1.797 (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	
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 – Grain treatment for the production of starch and gluten fractions – distilled alcohol production	–

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

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

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

3.6. Margin of exposure

Using the highest derived exposure estimates at the 95th percentile value for each age group, in comparison with the NOAEL (794 mg TOS/kg bw per day) from the 90-day rat study, resulted in margins of exposure (MoE) for infants, toddlers, children, adolescents, adults and the elderly, of 184, 146, 139, 219, 305 and 441, respectively.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production and grain treatment for the production of starch and gluten fractions, and the derived margin of exposure for the remaining six food manufacturing processes, the Panel concludes that the food enzyme cellulase produced with *A. niger* strain 294 does not give rise to safety concern under the intended conditions of use for adolescents, adults and the elderly. For younger age groups, the calculated MoE do not allow a conclusion on safety.

5. Documentation as provided to EFSA

Application for authorisation of use of cellulase preparation produced by *Aspergillus niger* (strain 294). November 2021. Submitted by Kerry Ingredients & Flavours Ltd.

Additional data. September 2022. Submitted by Kerry Ingredients & Flavours Ltd.

Additional data clarification teleconference. February 2023. Submitted by Kerry Ingredients & Flavours Ltd.

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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 Cooperation and Development

TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization

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.2023.8098#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, Netherlands, Portugal, Republic of North Macedonia*, Serbia*, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia, Serbia*, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina*, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina*, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro*, Netherlands, Portugal, Romania, Serbia*, Slovenia, Spain, Sweden

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

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