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Safety evaluation of the food enzyme glucan 1,4-α-glucosidase from the genetically modified *Aspergillus niger* strain NZYM-BE

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Abstract

The food enzyme glucan 1,4-α-glucosidase (4-α-D-glucan glucohydrolase EC 3.2.1.3) is produced with the genetically modified *Aspergillus niger* strain NZYM-BE by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme was free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in six food manufacturing processes, namely starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, brewing processes, baking processes, cereal-based processes, and fruit and vegetable processing for juice production. Since residual amounts of total organic solids (TOS) are removed by distillation and by the purification steps applied to produce glucose syrups, dietary exposure was not calculated for these two food processes. For the remaining four processes, dietary exposure to the food enzyme–TOS was estimated to be up to 7.7 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise 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 3,795 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure above 490. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched for and one match found. The Panel considered that, under the intended conditions of use (other than distilled alcohol production) the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be 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: food enzyme, glucan 1,4-α-glucosidase, 4-α-D-glucan glucohydrolase, EC 3.2.1.3, glucomylase, *Aspergillus niger*, genetically modified microorganism

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

Three applications have been introduced by the companies DSM Food Specialties B.V, Novozymes A/S and Kerry Ingredients & Flavours for the authorisation of the food enzymes asparaginase from a genetically modified strain of *Aspergillus niger* (strain DS 53180), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BE) and peroxidase obtained from soy bean hulls, 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 three applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter 11 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 asparaginase from a genetically modified strain of *Aspergillus niger*

[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.

(strain DS 53180), glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BE) and peroxidase obtained from soy bean hulls 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 glucoamylase from a genetically modified *Aspergillus niger* (strain NZYM-BE).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glucoamylase from a genetically modified *Aspergillus niger* (strain NZYM-BE).

Additional information was requested from the applicant during the assessment process on 4 June 2015 and 15 February 2021 and was consequently provided (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's of EFSA Scientific Committees.

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, 2021a).

3. Assessment

IUBMB nomenclature	Glucan 1,4-α-glucosidase
Systematic name	4-α-D-glucan glucohydrolase
Synonyms	glucoamylase; amyloglucosidase; exo-1,4-α-glucosidase
IUBMB No	EC 3.2.1.3
CAS No	9,032-08-0
EINECS No	232–877-2

Glucan 1,4-α-glucosidases catalyse the hydrolysis of terminal (1–4)-linked α-D-glucose residues successively from non-reducing ends of amylopectin and amylose with the release of glucose. The enzyme is intended to be used in six food manufacturing processes, namely starch processing for the production of glucose syrups and other starch hydrolysates, distilled alcohol production, brewing processes, baking processes, cereal-based processes, and fruit and vegetable processing for juice production.

3.1. Source of the food enzyme[4]

The glucan 1,4-α-glucosidase is produced with the genetically modified filamentous fungus *Aspergillus niger* strain NZYM-BE, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with deposit number [redacted].[5]

3.1.1. Characteristics of the parental and recipient microorganisms

[redacted] intermediate strains BO-1 [redacted] were found not to produce ochratoxin A and fumonisin B2 under conditions known to induce mycotoxin production in fungi.[6]

[4] Technical dossier/GMM dossier-Annex 4.
[5] Technical dossier/Annex A4.
[6] Technical dossier/GMM dossier/Annex A3.

[redacted][7]

3.1.2. Characteristics of introduced sequences

[redacted]

3.1.3. Description of the genetic modification process

[redacted][8]

[7] Technical dossier/GMM dossier/Annexes A1 and A2.
[8] Technical dossier/Annex D1.

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

[redacted]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[10]

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. Finally, the food enzyme is stabilised.[11] 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.[12]

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 glucan 1,4-α-glucosidase is a single polypeptide chain of 591 amino acids. The molecular mass, calculated from the amino acid sequence, is 62.8 kDa.[13] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gels showed two major protein bands, one migrating close to 66 kDa (consistent with the expected mass of the enzyme), and the other between 45 and 66 kDa.[14] The food enzyme was tested for α-amylase, lipase, peptidase and xylanase activities, and only α-amylase activity was detected.[15]

The in-house determination of glucan 1,4-α-glucosidase activity is based on the hydrolysis of maltose (reaction conditions: pH 4.3, temperature 37°C, reaction time 6 min). The enzymatic activity is determined by measuring the release of glucose. The glucan 1,4-α-glucosidase activity is quantified relative to an enzyme standard and expressed in Amyloglucosidase Units/g (AGU/g).[16]

The food enzyme has a temperature optimum around 65°C (pH 5.0) and a pH optimum around pH 4.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 5.0). Glucan 1,4-α-glucosidase activity decreased above around 63°C showing no residual activity above 76°C.[17]

[9] 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.
[10] Technical dossier/Annex 5.
[11] Technical dossier/p. 51–58.
[12] Technical dossier/Annexes: 6, 2.06 and Additional information September 2015.
[13] Technical dossier/p. 35/Annex 1.
[14] Technical dossier/p. 37.
[15] Technical dossier/p. 12, p.45/Annexes: 3.02, 3.03, 3.04, 3.05.
[16] Technical dossier/p. 12, p. 40-42/Annex 3.01.
[17] Technical dossier/p. 43–44/Annex 9.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and two batches produced for the toxicological tests (Table 1).[18] The average total organic solids (TOS) of the three food enzyme batches for commercialisation is 9.3% and the average enzyme activity/TOS ratio is 4.4 AGU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches				
		1	2	3	4(a)	5(b)
Glucan 1,4-α-glucosidase activity	AGU/g batch(c)	406	424	380	392	925
Protein	%	7.4	6.9	7.2	7.1	16.1
Ash	%	0.9	0.6	1.0	0.9	0.9
Water	%	89.8	90.0	89.9	89.8	81.5
Total organic solids (TOS)(d)	%	9.3	9.4	9.1	9.3	17.6
Activity/mg TOS	AGU/mg TOS	4.4	4.5	4.2	4.2	5.3

(a): Batch used for the genotoxicity studies.
(b): Batch used for the repeated dose 90-day oral toxicity study in rats.
(c): AGU: Amyloglucosidase Units (see Section 3.3.1).
(d): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the two batches used for toxicological studies was below 0.5 mg/kg[19] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of cadmium and mercury were below the limits of detection (LoDs) of the employed methodologies.[20,21] For arsenic, the average concentration determined in the commercial batches was 0.13 mg/kg.[22] The Panel considered this concentration as not of concern.

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).[22] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[22]

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 fumonisin B2 and ochratoxin A was examined in the three food enzyme batches and both were below the LoD of the applied method.[23,24] Adverse effects due to the possible presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in [redacted][25]

The absence of recombinant DNA in the food enzyme concentrate was demonstrated [redacted][26]

[18] Technical dossier/p. 36, p. 67/Annexes: 7.02, 7.03.
[19] Technical dossier/Additional information August 2021.
[20] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[21] Technical dossier/p. 38/Annex 2.04.
[22] Technical dossier/ Additional information August 2021.
[23] Technical dossier/p Additional information August 2021.
[24] LoDs: ochratoxin A = 0.0003 mg/kg; fumonisin B = 0.0005 mg/kg.
[25] Technical dossier/Annex E1.
[26] Technical dossier/Additional information August 2021/Revised Annex E2.

3.4. Toxicological data

3.4.1. Choice of test item

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. The batches 4 and 5 (Table 1) used in these studies have similar protein pattern and chemical purity as the batches used for commercialisation, and thus are considered suitable as test items.

3.4.2. Genotoxicity

3.4.2.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[27] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the 'treat and plate' assay. Two separate experiments in triplicate were carried out using six concentrations of the food enzyme (156–5,000 μg dry matter/plate, corresponding to 142.2, 285.4, 569.9, 1,139.7, 2,279.4 and 4,558.8 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme there was no increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

3.4.2.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2010) and following GLP.[28] A single experiment was performed in duplicate cultures of human peripheral blood lymphocytes. Cells were exposed to the food enzyme for 3 h in the presence or absence of the S9-mix and harvested 24 h after the beginning of treatment (3 h + 21 h recovery time). Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24 h after removal of the food enzyme (24 + 24 h recovery time). The food enzyme was tested at 3,000, 4,000 and 5,000 μg/mL, corresponding to 279, 372 and 465 μg TOS/mL. No cytotoxicity, evaluated as decrease of proliferation index, was observed after treatments, either in the presence or absence of S9-mix. Statistically significant increases of the frequency of binucleated cells with micronuclei (MNBN) were observed at 3,000 μg/mL following the 3 + 21 h treatment in the presence of S9-mix and at 4,000 μg/mL following the 24 + 24 h treatment in the absence of S9-mix. The Panel noted that these values were within the 95% of the historical control range and therefore were not considered to be biologically relevant. The frequency of MNBN was comparable to the negative controls at all the other concentrations tested and conditions of treatment.

The Panel concluded that the food enzyme glucan 1,4-α-glucosidase did not induce an increase in the frequency of MNBN in cultured human peripheral blood lymphocytes under the test conditions employed in this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[29] Groups of 10 male and 10 female Han Wistar (RccHan™; WIST) rats received daily by gavage the food enzyme in 1,897, 2,846 or 3,795 mg TOS/kg bw per day in 2 administrations. Controls received the vehicle.

No mortality was observed.

The haematological investigation revealed several statistically significant differences relative to the control group. In the treated males, a lower haematocrit (HCT) (−4.6%), a lower erythrocyte count (RBC) (−4.9%) and a higher mean cell haemoglobin concentration (MCHC) (+2.3%) at the high dose, and a lower reticulocyte (Retic) count (−16%, −14.2%) at the mid- and high doses were recorded. In the treated females, a lower HCT (−5.4%, −3.5%) and lower RBC count (−4.8%, −3.1%) in mid-

[27] Technical dossier/Annex 7.01.
[28] Technical dossier/ Annex 7.02.
[29] Technical dossier/Additional information, August 2021.

and high-dose groups, a lower haemoglobin (Hb) concentration (−2.5%, −3.8%, −1.9%) at all doses, a higher MCHC (+2%, +1.7%) at the mid- and high doses, a lower eosinophil (EOS) count (+25%) at the high-dose, a higher platelet (PLT) count at mid- and high doses, and a lower activated partial thromboplastin times (APTT) (−34%, −32%) at mid- and high doses were recorded. The Panel considered these changes as not toxicologically relevant because of a low magnitude of the changes (all parameters except Retic in males and APTT in females), an absence of an apparent dose response (Retic in males; MCHC in males and females; RBC, EOS, PLT and APTT in females), or only observed in one sex (Hb, Retic, EOS, PLT, APTT). The Panel further noted that some of the haematological parameters in the concurrent control were above (HCT in males; HCT and RBC count in females) or below (MCHC in females) the relevant historical control ranges in the laboratory, which could add to the recorded differences between the concurrent control and the treated groups.

The clinical chemistry investigation revealed several statistically significant differences relative to the control group. In the treated males, a dose-related increase in blood urea nitrogen (BUN) (+18%, +20%, +26%) and in urea concentrations (+18%, +26%, +26%) at all doses, an increase in creatinine (+25%, +25%) at mid- and high doses, and a decrease in total cholesterol (T-chol) (−20%, −16%) and of high-density lipoprotein (HDL) (−23%, −20%) concentrations at mid- and high doses were recorded. In the treated females, a reduction of albumin to globulin ratio (A/G ratio) (−6%, −6%) at mid- and high doses was observed. The Panel considered the changes as not toxicologically relevant because of the absence of a dose–response relationship (creatinine, T-chol, HDL, A/G ratio), the changes were only observed in one sex (all parameters), the magnitude of changes was low (A/G ratio), there were no changes in other plasma biomarkers for renal toxicity (e.g. albumin and total protein) or liver toxicity (e.g ALT, AST, ALP), no changes in the kidney or liver weights, no histopathological changes in the liver and kidneys, and the changes were within the relevant historical control ranges in the laboratory.

Thyroid hormone analysis revealed a statistically significant increase in the mean serum thyroid stimulating hormone (TSH) concentration (2-fold) in high-dose males. The Panel considered this difference as not toxicologically relevant because of absence of corroborative changes in the serum triiodothyronine (T3) and thyroxine (T4) concentrations in these animals, changes were seen only in one sex and in addition there were no histopathological changes in the thyroid gland.

Statistically significant changes in organ weights included an increase in mean body weight adjusted pituitary weights (+25%, +25%) in mid- and high-dose females, lower adjusted brain weight (−4%, −4%) in mid- and high-dose males, lower heart weight (−6%, −9%, −4%) in all treated males and lower adjusted liver weights (−7%) in low-dose females. The Panel considered these changes as not toxicologically relevant because the magnitude of the changes was small (brain, heart, liver), there was no apparent dose–response relationship (all parameters), the changes were only observed in one sex, there were no histopathological changes in these organs and the values were within the relevant historical control ranges in the laboratory.

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

The Panel identified the no observed adverse effect level (NOAEL) of 3,795 mg TOS/kg bw per day, the highest dose tested.

3.4.4. 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 glucan 1,4-α-glucosidase produced with the genetically modified *A. niger* strain NZYM-BE 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, one match was found.[30] The matching allergen was Sch c 1, a glucan 1,4-α-glucosidase produced by *Schizophyllum commune*.

Glucan 1,4-α-glucosidase from *S. commune* (Toyotome et al., 2014) is known as an occupational respiratory allergen associated with baker's asthma. However, several studies have shown that adults with occupational asthma caused by an enzyme (as described for α-amylase from *A. oryzae*) can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Cullinan et al., 1997;

[30] Technical dossier/p. 74-76/Annexes: 8.01, 8.02.

Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004). Such information has not been reported for glucan 1,4-α-glucosidase.

No information is available on oral and respiratory sensitisation or elicitation reactions of this glucan 1,4-α-glucosidase.

According to the information provided, substances or products that may cause allergies or intolerances ([redacted]) are used as raw materials 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 potentially allergenic residues of these foods employed as protein sources are not expected to be present.

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme can be excluded for distilled alcohol production. The risk cannot be excluded for the rest of the processes, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in six food manufacturing processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[(d)]

Food manufacturing process[(a)]	Raw material (RM)	Recommended dosage of the food enzyme (mg TOS/kg RM)[(b),(c)]
Starch processing for the production of glucose syrups and other starch hydrolysates	Starch	69.6–174
Distilled alcohol production	Starch	69.6–174
Brewing processes	Cereals	150.8–**761**
Baking processes	Flour	127.6–**649.7**
Cereal-based processes	Flour	58–**204.2**
Fruit and vegetable processing for juice production	Fruits or vegetables	12.8–**26.7**

TOS: total organic solids.
(a): The description provided by the applicant 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): Based on 4.3 AGU/mg TOS.
(c): The numbers in bold were used for calculation.
(d): Additional information August 2021.

In starch processing for the production of glucose syrups, the food enzyme is added during the saccharification step, where it hydrolyses starch polysaccharides to glucose.[31] The food enzyme–TOS is removed from the final glucose syrups by treatment with activated charcoal or similar, and with ion-exchange resins. This conclusion is extended to other starch hydrolysates (EFSA CEP Panel, 2021a).

In distilled alcohol production, the food enzyme is added during the pre-saccharification and fermentation steps.[32] It converts liquefied starch into a glucose-rich solution, increasing the amounts of fermentable sugars to produce alcohol. The food enzyme TOS is not carried over with the distilled alcohols (EFSA CEP Panel, 2021a).

[31] Technical dossier/p. 85.
[32] Technical dossier/p. 87.

In brewing processes, the food enzyme is added during the mashing and fermentation steps, where it will hydrolyse the starchy content of the mash to release glucose for fermentation.[33] The food enzyme TOS remains in the beers.

In baking processes and cereal-based processes, the food enzyme is added to flour during the preparation of the dough or batter to release glucose from starch, which may be fermented by yeast.[33] The food enzyme TOS remains in the final bakery and cereal-based foods.

In fruit and vegetable processing for juice production, the food enzyme is added to the crushed fruits or vegetables during mash treatment and again to the raw juices.[34] The food enzyme TOS remains in the juices.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the glucan 1,4-α-glucosidase is inactivated during all 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, namely baking processes, brewing processes, cereal-based processes, and fruit and vegetable processing for juice production.

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level provided by the applicant with the individual data from the EFSA Comprehensive European Food Consumption Database. The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEF Panel, 2021b). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only one day 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 average 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 41 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 at the 95th percentile was estimated to be 7.737 mg TOS/kg bw per day in infants.

Table 3: Summary of 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)	0.259–2.084 (11)	1.635–4.431 (15)	2.015–4.245 (19)	1.113–2.438 (21)	0.848–2.240 (22)	0.786–1.530 (22)
Min–max 95th percentile (number of surveys)	1.095–7.737 (9)	3.981–7.553 (13)	3.503–7.580 (19)	2.227–4.996 (20)	1.909–5.646 (22)	1.599–2.979 (21)

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.

[33] Technical dossier/p. 88.
[34] Technical dossier/p. 91.

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 the following processes from the exposure assessment – Starch processing for the production of glucose syrups and other starch hydrolysates – 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 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 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

A comparison of the NOAEL (3,795 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.259–4.431 mg TOS/kg bw per day at the mean and from 1.095 to 7.737 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of 491.

4. Conclusions

Based on the data provided, the removal of TOS during alcohol distillation and starch processing for the production of glucose syrups and the derived MoE for baking and brewing processes, cereal-based processes, and fruit and vegetable processing for juice production, the Panel concluded that the food enzyme glucan 1,4-α-glucosidase produced with the genetically modified *A. niger* strain NZYM-BE does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

Glucoamylase from a genetically modified strain of *Aspergillus niger* (strain NZYM-BE). November 2013. Submitted by Novozymes A/S.

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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
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
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.2022.7374#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 one 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
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, 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, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, 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, Netherlands, Portugal, Romania, 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).