SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 March 2022

doi: 10.2903/j.efsa.2022.7264

Safety evaluation of the food enzyme mannan endo-1,4-β-mannosidase from the genetically modified *Aspergillus niger* strain NZYM-NM

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Abstract

The food enzyme mannan endo-1,4-β-mannosidase (1,4-β-D-mannan mannanohydrolase, EC 3.2.1.78) is produced with the genetically modified *Aspergillus niger* strain NZYM-NM by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is considered free from viable cells of the production organism and its DNA. The food enzyme is intended to be used in coffee processing. Based on the maximum use levels, dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 0.956 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 1,151.7 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of more than 1,200. A search for similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, under the intended conditions of use 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: mannanase, mannan endo-1,4-β-mannosidase, 1,4-β-D-mannan mannanohydrolase, EC 3.2.1.78, *Aspergillus niger*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2021-00123

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Legal notice: 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.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output: Jaime Aguilera, Erik Boinowitz, Ana Gomes, Simone Lunardi, Sandra Rainieri, Helen Regina.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Andryszkiewicz M, Arcella D, Kovalkovičová N, Liu Y and Chesson A, 2022. Scientific opinion on the safety evaluation of the food enzyme mannan endo-1,4-β-mannosidase from the genetically modified *Aspergillus niger* strain NZYM-NM. EFSA Journal 2022;20(4):7264, 16 pp. https://doi.org/10.2903/j.efsa.2022.7264

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

† Deceased.

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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[1] 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 CEF Panel, 2009) 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[1] on food enzymes.

An application has been introduced by the applicant "Novozymes A/S" for the authorisation of the food enzyme mannanase from a genetically modified strain of *Aspergillus niger* (strain NZYM-NM).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[4], the European Commission asks the European Food Safety Authority to carry out the safety assessment on the following food enzyme: mannanase from a genetically modified strain of *Aspergillus niger* (strain NZYM-NM), in

[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.

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–44.

accordance with Regulation (EC) No 1331/2008[2] establishing a common authorisation procedure for food additives and food flavourings.

1.1.3. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of food enzyme mannan endo-1,4-β-mannosidase from a genetically modified *A. niger* (strain NZYM-NM).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme mannanase from the genetically modified *A. niger* (strain NZYM-NM). The dossier was updated on 12 May 2021.

Additional information was requested from the applicant during the assessment process on 15 October 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, 2009) and following the relevant guidance documents of EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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 to the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment[5]

IUBMB nomenclature	Mannan endo-1,4-β-mannosidase
Systematic name	1,4-β-D-mannan mannanohydrolase
Synonyms	endo-1,4-β-mannanase; β-mannanase; endo-β-mannanase; β-D-mannanase
IUBMB No	EC 3.2.1.78
CAS No	37288-54-3
EINECS No	253-446-5

Mannan endo-1,4-β-mannosidases catalyse the random hydrolysis of 1,4-β-mannosidic linkages of mannans, galactomannans and related polysaccharides. The enzyme is intended to be used in coffee processing.[6]

3.1. Source of the food enzyme[7]

The mannan endo-1,4-β-mannosidase is produced with the genetically modified filamentous fungus *A. niger* strain NZYM-NM, which is deposited [redacted] with deposit number [redacted].[8] The production strain NZYM-NM was identified as *A. niger* [redacted]

[5] Technical dossier Version 2/2nd submission/p. 6–7, 11, 49, 63–64.
[6] Technical dossier Version 2/2nd submission/p. 7, 13, 49.
[7] Technical dossier Version 2/2nd submission/p. 6, 11, 36–41; Technical dossier/2nd submission/Annex 4: GMM dossier Version 2; Technical dossier/Additional information, 13.1.2022.
[8] Technical dossier/2nd submission/Annex A4.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted][9]

The recipient strain [redacted]

3.1.2. Characteristics of introduced sequences

[redacted][10]

[redacted][11]

3.1.3. Description of the genetic modification process

[redacted]

[9] Technical dossier/2nd submission/Annexes A1 and A2.
[10] Technical dossier/Additional information, 13.1.2022.
[11] Technical dossier/2nd submission/Annex 4: GMM dossier Version 2/p. 18–20 and Annex C1.

[redacted][12]

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.

The production strain *A. niger* strain NZYM-NM differs from the recipient strain in its capacity to produce the mannan endo-1,4-β-mannosidase [redacted]

The absence of the antimicrobial resistance genes [redacted] was confirmed by [redacted][13]

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

3.2. Production of the food enzyme[14]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[15], with food safety procedures based on hazard analysis and critical control points, and in accordance with current good manufacturing practice.[16]

The production strain is grown as a pure culture using a typical industrial medium in a [redacted] with conventional process controls in place. After completion of the fermentation, [redacted] 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.[17]

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[18]

The mannan endo-1,4-β-mannosidase is a single polypeptide chain of [redacted] amino acids.[19] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[19] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[20] A consistent protein pattern was observed across all batches. The gels showed a protein migrating between the marker proteins of 45 and 66 kDa in all batches, consistent with the expected mass of the enzyme. The protein profile also included bands of lesser staining intensity.[20] The food enzyme was tested for α-amylase, amyloglucosidase and lipase activities and none were detected. No other enzymatic activities were reported by the applicant.[21]

The in-house determination of mannan endo-1,4-β-mannosidase activity[22] is based on the hydrolysis of galactomannan (reaction conditions: pH 5.0, 50°C, 20 min). The reducing carbohydrates released by the enzyme react with *p*-hydroxybenzoic acid hydrazide to generate a yellow complex that

[12] Technical dossier/2nd submission/Annex 4: GMM dossier Version 2/p. 21–22.
[13] Technical dossier/2nd submission /Annex D1.
[14] Technical dossier Version 2/2nd submission/p. 12, 41–47; Technical dossier/2nd submission/Annex 5 and Annex 6.
[15] 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.
[16] Technical dossier Version 2/2nd submission/p. 12.
[17] Technical dossier Version 2/2nd submission/p. 41–47; Technical dossier/2nd submission/Annex 6.
[18] Technical dossier Version 2/2nd submission/p. 28; Technical dossier/2nd submission/Annex 1 and Annex 9.
[19] Technical dossier Version 2/2nd submission/p. 28; Technical dossier/2nd submission/Annex 1.
[20] Technical dossier Version 2/2nd submission/p. 30.
[21] Technical dossier Version 2/2nd submission/p. 11, 35, 47; Technical dossier/2nd submission/Annex 3.
[22] Technical dossier Version 2/2nd submission/Annex 3.01.

is detected spectrophotometrically at 405 nm. The enzyme activity is quantified relative to an internal enzyme standard and expressed in Acidic Mannanase Novozymes Units (AMNU)/g.

The mannan endo-1,4-β-mannosidase has a temperature optimum of 80°C (pH 5.0) and a pH optimum around pH 4.0 (at 37°C).[23] Preincubation of the food enzyme for 30 min at different temperatures (pH 5.0)[24] showed that mannan endo-1,4-β-mannosidase activity is stable up to 70°C, but the enzyme showed no residual activity at 87°C and above.[25]

3.3.2. Chemical parameters[26]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[27] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation is 11.9% and the mean enzyme activity/mg TOS ratio is 227.2 AMNU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Mannan endo-1,4-β-mannosidase activity	AMNU/g batch[(b)]	27,900	25,700	27,200	16,700
Protein	%	8.6	7.8	7.6	5.7
Ash	%	0.6	0.5	0.4	1.2
Water	%	86.7	88.2	88.0	87.8
Total organic solids (TOS)[(c)]	%	12.7	11.3	11.6	11.0
Activity/mg TOS	AMNU/mg TOS	219.7	227.4	234.5	151.8

(a): Batch used for the toxicological studies.
(b): AMNU: Acidic Mannanase Novozymes Unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity[28]

The lead content[29] in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[30] In addition, the levels of arsenic, cadmium and mercury were below the limits of detection (LODs) of the employed methodologies.[31,32]

The food enzyme complies with the microbiological criteria (for total coliforms, *E. coli* and *Salmonella*)[33] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[34] No antimicrobial activity was detected in any of the tested batches.[35]

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.[35,36] Adverse effects caused by the possible presence of other secondary metabolites are 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.

[23] Technical dossier Version 2/2nd submission/p. 11, 34; 64–65; Technical dossier/2nd submission/Annex 9.
[24] Technical dossier/2nd submission/Annex 9.
[25] Technical dossier Version 2/2nd submission/p. 65–66; Technical dossier/2nd submission/Annex 9.
[26] Technical dossier/2nd submission/Annex 2.01, Annex 2.02, Annex 2.03, Annex 2.06, Annex 3.01, Annex 10 and Annex 9.
[27] Technical dossier Version 2/2nd submission/p. 29, 52; Technical dossier/2nd submission/Annex 10.
[28] Technical dossier Version 2/2nd submission/p. 11, 63; Technical dossier/2nd submission/Annex 10, Annex 2.04, Annex 2.05, Annex 2.07, Annex 2.08, Annex 2.09, Annex 2.10, Annex 2.11.
[29] Technical dossier Version 2/2nd submission/p. 11, 63; Technical dossier/2nd submission/Annex 2.04.
[30] Technical dossier Version 2/2nd submission/p. 31, 52; 63; Technical dossier/2nd submission/Annex 10.
[31] Technical dossier Version 2/2nd submission/p. 52; LODs: Pb = 5 mg/kg; As = 0.3 mg/kg; Cd = 0.05 mg/kg; Hg = 0.05 mg/kg.
[32] Technical dossier Version 2/2nd submission/p. 31, 52; 63; Technical dossier/2nd submission/Annex 2.04 and Annex 10.
[33] Technical dossier Version 2/2nd submission/p. 11, 32, 52, 63; Technical dossier/2nd submission/Annex 10, Annex 2.08, Annex 2.09, Annex 2.10, Annex 2.11.
[34] Technical dossier Version 2/2nd submission/p. 11, 32, 52; 63; Technical dossier/2nd submission/Annex 10.
[35] Technical dossier Version 2/2nd submission/p. 11, 31, 52; 63; Technical dossier/2nd submission/Annex 10.
[36] Technical dossier Version 2/2nd submission/p. 52; Technical dossier/2nd submission/Annex 2.05, Annex 10; LOD: fumonisin B2=0.0003 mg/kg, ochratoxin A = 0.0003 mg/kg.

3.3.4. Viable cells and DNA of the production strain[37]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced.[38]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] of three batches in triplicate. No DNA was detected [redacted] with a LOD of 10 ng spiked DNA/g food enzyme.[37]

3.4. Toxicological data[39]

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian cell micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 4 (Table 1) used in these studies has lower purity than the commercial batches and therefore is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. 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).[40] 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 (16, 50, 160, 500, 1,600 and 5,000 μg TOS/mL in the first experiment and 160, 300, 625, 1,250, 2,500 and 5,000 μg TOS/mL in the second experiment). No cytotoxicity was observed at any concentration of the food enzyme. In the second experiment, in the absence of S9-mix an increase in revertant colony numbers above the control values was observed in *S*. Typhimurium strain TA1535 at 300 and 1,250 μg TOS/mL (2.3- and 2.5-fold increase) and in strain TA1537 at 160 and 625 μg TOS/mL (2.3- and 2.0-fold increase). The increases were not concentration dependent, not reproducible and the values were within the normal variability of historical control ranges in the laboratory. Consequently, they were considered by the Panel as not biologically relevant. Upon treatment with the food enzyme there was no biologically relevant increase in the number of revertant colonies above the control values in any other strain tested, with or without S9-mix.

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

3.4.1.2. *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.[41] The experiment was performed in duplicate cultures of human peripheral whole blood lymphocytes. The food enzyme was tested at 3,000, 4,000 and 5,000 μg TOS/mL. Cells cultures were treated with the food enzyme for 3 h in the presence or absence of S9-mix and harvested 24 h after the beginning of treatment (3 + 21 h recovery time). Additionally, a continuous 24-h treatment without S9-mix was included with harvesting 24 h after removal of the test substance (24 + 24 h recovery time). No cytotoxicity was seen either in the short-term with or without S9-mix or in the long-term treatment. The frequency of binucleated cells with micronuclei (MNBN) was not statistically significantly different to the negative controls at all concentrations tested.

The Panel concluded that the food enzyme mannan endo-1,4-β-mannosidase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes, under the test conditions employed in this study.

[37] Technical dossier/Additional information, 13.1.2022/Annex E2 Version 2.
[38] Technical dossier/2nd submission/Annex E1.
[39] Technical dossier Version 2/2nd submission/p. 14–15; 52–57; Technical dossier/2nd submission/Annex 7.01, Annex 7.02 and Annex 7.03.
[40] Technical dossier/2nd submission/Annex 7.01.
[41] Technical dossier/2nd submission/Annex 7.02.

3.4.2. 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.[42] Groups of 10 male and 10 female Han Wistar RccHan™:WIST rats received by gavage the food enzyme in doses of 115.17, 380.06 and 1,151.7 mg TOS/kg body weight (bw) per day. Controls received the vehicle (reverse osmosis-purified water).

No mortality was observed.

In the functional observations, a statistically significant increase in motor activity (high and low beam levels) after 6 minutes in high-dose males (+42% and +47%, respectively) was observed. The Panel considered this change as incidental.

The haematological investigation revealed a statistically significant decrease in total white blood cell numbers (−20%) and platelet numbers (−11%) in high-dose males, a decrease in large unstained cell (LUC) numbers in mid- and high-dose males (−20% and −20%) and in high-dose females (−50%), and reduced prothrombin times (−9.8%) in mid-dose females. The Panel considered the differences as not toxicologically relevant because the changes were small (all parameters except of the LUC number in high-dose females), there was no apparent dose-response relationship (all parameters) or as they were only observed in one sex (all parameters except of the LUC). The Panel noted that all changes were within the range of historical control values.

The clinical chemistry investigation revealed a statistically significant increase in plasma aspartate aminotransferase activity (AST) (+22%) in high-dose males, an increase in plasma creatinine concentration in low-, mid- and high-dose males (+16%, +9.7% and +9.7%), an increase in plasma alkaline phosphatase (ALP) activity (+20%) in high-dose females and an increase in creatinine values (+15%) in mid-dose females. The Panel considered the differences as not toxicologically relevant because the changes were small (all parameters), there was no apparent dose–response relationship (creatinine) or they were only observed in one sex (AST, ALP). The Panel noted that all values were within the laboratory historical control values.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1,151.7 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity[43]

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 mannan endo-1,4-β-mannosidase produced with the genetically modified *A. niger* strain NZYM-NM 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.[10]

No information is available on oral and respiratory sensitisation or elicitation reactions of this mannan endo-1,4-β-mannosidase.

Welter et al. (2013) and Endo et al. (2019) identified a mannan endo-1,4-β-mannosidase as a putative tomato allergen, but since no match with known allergens was identified, it is likely that the putative tomato allergen is different from the mannan endo-1,4-β-mannosidase under evaluation.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[44]) are used as raw materials ([redacted]). 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

[42] Technical dossier/2nd submission/Annex 7.03.

[43] Technical dossier Version 2/2nd submission/p. 57–59; Technical dossier/2nd submission/Annex 1 and Annex 8; Technical dossier/Additional information, 13.1.2022.

[44] 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.

processing, the Panel considered that potentially allergenic residues of these materials employed as protein sources are not expected to be present in the food enzyme.

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 cannot be excluded, 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 coffee processing at a recommended use level of 30–150 AMNU/kg demucilaged coffee beans, corresponding to 0.13–0.66 mg TOS/kg demucilaged coffee beans.[45]

In coffee processing, the food enzyme is added to the demucilaged coffee beans during the extraction step.[46] Mannan endo-1,4-β-mannosidase degrades mannans and related polysaccharides randomly into oligomers and monomers of mannose.

The food enzyme–TOS remains in coffee products. Based on data provided on thermostability (see Section 3.3.1), it is expected that the mannan endo-1,4-β-mannosidase is inactivated during coffee processes.

3.5.2. Dietary exposure estimation

Chronic exposure 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 CEP 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 2 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 41 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure at the 95th percentile was estimated to be 0.931 and 0.956 mg TOS/kg bw per day in adults and elderly people, respectively.

Table 2: 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)	0–0.002 (12)	0–0.016 (17)	0–0.034 (20)	0.001–0.032 (22)	0.024–0.357 (23)	0.019–0.426 (23)
Min–max 95th percentile (number of surveys)	0–0.001 (10)	0–0.138 (15)	0.001–0.104 (20)	0.001–0.195 (21)	0.129–0.931 (23)	0.113–0.956 (22)

[45] Technical dossier Version 2/2nd submission/p. 49.
[46] Technical dossier Version 2/2nd submission/p. 66–67.

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 3.

Table 3: 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 to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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.

3.6. Margin of exposure

A comparison of the NOAEL (1,151.7 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.426 mg TOS/kg bw per day at the mean and from 0–0.956 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) of at least 1,205.

4. Conclusions

Based on the data provided and the derived margin of exposure for coffee processing, the Panel concluded that the food enzyme mannan endo-1,4-β-mannosidase produced with the genetically modified *A. niger* strain NZYM-NM 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.

5. Documentation as provided to EFSA

Technical dossier `Mannanase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-NM), version 2'. 12 May 2021. Submitted by Novozymes A/S.

Additional information. 13 January 2022. Submitted by Novozymes A/S.

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Abbreviations

ALP	alkaline phosphatase
AMNU	Acidic Mannanase Novozymes Units
AST	aspartate amino-transferase
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
DNA	deoxyribonucleic acid
[redacted]	[redacted]
EFSA CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids

EFSA CEP Panel	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
[redacted]	[redacted]
FoodEx	food classification and description system
[redacted]	[redacted]
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
[redacted]	[redacted]
IUBMB	International Union of Biochemistry and Molecular Biology
[redacted]	[redacted]
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LUC	large unstained cells
MOE	margin of exposure
MNBN	binucleated cells with micronuclei
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
[redacted]	[redacted]
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
[redacted]	[redacted]
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.2022.7264#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).