SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 12 September 2023

doi: 10.2903/j.efsa.2023.8254

Safety evaluation of the food enzyme asparaginase from the genetically modified *Aspergillus oryzae* strain NZYM-SP

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Jaime Aguilera, Magdalena Andryszkiewicz, Erik Boinowitz, Ana Gomes, Natalia Kovalkovicova, Yi Liu, Rita Ferreira de Sousa and Andrew Chesson

Abstract

The food enzyme asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is produced with the genetically modified *Aspergillus oryzae* strain NZYM-SP 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. It is intended to be used to prevent acrylamide formation in food processing. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.101 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 880 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 8,713. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that a risk of allergic reactions 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.

Keywords: food enzyme, asparaginase, L-asparagine amidohydrolase, EC 3.5.1.1, α-Asparaginase, *Aspergillus oryzae*, genetically modified microorganism

Requestor: European Commission

Question number: EFSA-Q-2013-00587

Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Aguilera, J., Andryszkiewicz, M. … Chesson, A. 2023. Safety evaluation of the food enzyme asparaginase from the genetically modified *Aspergillus oryzae* strain NZYM-SP. *EFSA Journal*, *21*(10), 1–15. https://doi.org/10.2903/j.efsa.2023.8254

ISSN: 1831-4732

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

Table of contents

1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

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

Two applications have been introduced by the company Novozymes A/S for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AC) and Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-SP).

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 bot applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (strain NZYM-AC) and Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-SP) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[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.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme Asparaginase from the genetically modified *Aspergillus oryzae* strain NZYM-SP.

2. Data and methodologies

2.1. Data

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

Additional information was requested from the applicant during the assessment process on 20 June 2014, 23 September 2022 and 20 February 2023 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 guidance documents of the 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) 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	Asparaginase
Systematic name	L-Asparagine amidohydrolase
Synonyms	L-asparaginase; α-asparaginase
IUBMB No	EC 3.5.1.1
CAS No	9015-68-3
EINECS No	232-765-3

Asparaginases catalyse the hydrolysis of L-asparagine, releasing L-aspartic acid and ammonia. The enzyme under assessment is intended to be used to prevent acrylamide formation during food processing.

3.1. Source of the food enzyme[4]

The enzyme is produced with the genetically modified filamentous fungus *Aspergillus oryzae* strain NZYM-SP, which is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) with the deposit number [redacted]. The production strain was identified as *A. oryzae* [redacted].[5]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is *A. oryzae* strain A1560 [redacted].[6]

The recipient strain BECh2 was developed from the parental strain A1560 [redacted]. The mutagenesis steps resulted in the deletion of gene clusters required for

[4] Technical dossier/GMM dossier/Annex 4.
[5] Technical dossier/Additional data December 2022/Annex A1 Version 2.
[6] Technical dossier/GMM dossier/Annexes A1–A2.

the synthesis of cyclopiazonic acid and aflatoxins, as well as a reduced potential [redacted] to produce kojic acid.[7]

[redacted][8]

[redacted][4]

3.1.2. Characteristics of introduced sequences

[redacted][9]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to overproduce asparaginase.

[redacted][4]

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. oryzae* NZYM-SP differs from the recipient strain in its ability to overproduce asparaginase [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[10], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[11]

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

[7] Technical dossier/GMM dossier/Annexes A3.
[8] Technical dossier/GMM dossier/Annexes B1–B9.
[9] Technical dossier/GMM dossier/Annex C1.
[10] 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.
[11] Technical dossier/p. 21 and Annex 5.

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.[12] 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.[13]

The Panel considered that sufficient information was 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 asparaginase is a single polypeptide chain of 359 amino acids.[14] The molecular mass of the mature protein, calculated from the amino acid sequence, is 37.7 kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about 40 kDa,[15] consistent with the expected mass of the enzyme. The food enzyme was tested for protease, α-amylase, lipase, cellulase, peroxidase and glucoamylase activities, and none were detected.[16] No other enzyme activities were reported.

The in-house determination of asparaginase activity is based on the hydrolysis of L-asparagine (reaction conditions: pH 7.0, 37°C, 1.5 min). The enzymatic activity is determined by measuring the release of ammonia by a coupled reaction that consumes NADH, which is detected spectrophotometrically at 340 nm. The enzyme activity is expressed in Asparaginase Units (ASNU)/g. One ASNU/g is defined as the amount of enzyme that produces 1 μmol of ammonia per minute under the conditions of the assay.[17]

The food enzyme has a temperature optimum around 50°C (pH 7.0) and a pH optimum between pH 6.0 and pH 7.0 (37°C). Thermostability was tested by pre-incubation of the food enzyme for 2 h at different temperatures (pH 7.0). The enzyme activity decreased above 55°C, showing no residual activity above 60°C.[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological studies (Table 1).[19] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 5.9% and the mean enzyme activity/TOS ratio was 76.2 ASNU/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Asparaginase activity	ASNU/g[(b)]	3,990	4,610	4,390	4,440
Protein	%	4.7	5.3	4.7	5.9
Ash	%	1.6	1.9	1.8	2.1
Water	%	94.2	91.6	91.2	89.5
Total organic solids (TOS)[(c)]	%	4.2	6.5	7.0	8.4
Activity/TOS	ASNU/mg TOS	95.0	70.9	62.7	52.9

(a): Batch used for the toxicological studies.
(b): UNIT: Asparaginase Units (ASNU)/g (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

[12] Technical dossier/pp. 21–24, 67–73.
[13] Technical dossier/pp. 70, 72 and Annex 6.
[14] Technical dossier/pp. 12, 52 and Annex 1.
[15] Technical dossier/pp. 12–13, 54–55.
[16] Technical dossier/pp. 17, 61.
[17] Technical dossier/pp. 16–17, 58–59 and Annex 3.
[18] Technical dossier/pp. 17, 59–61, Annex 9 and Additional data December 2022/CoA-production batches NZYM-SP.
[19] Technical dossier/pp. 13, 52–53, 80–81 and Annexes 7.02, 7.03.

3.3.3. Purity

The lead content in all batches was below 0.5 mg/kg,[20] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the concentrations of arsenic, cadmium and mercury were below the limits of detection (LoD) of the employed methods.[20,21]

The food enzyme complied with the microbiological criteria for total coliforms, *E. 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 the tested batches.[23]

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 kojic acid and 3-nitropropionic acid was examined in all food enzyme batches and were below the LoD of the applied methods.[24,25] Adverse effects caused by 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 was sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted].[26]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted].[27]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats was provided. The batch 4 (Table 1) had similar protein pattern as the batches used for commercialisation and a lower activity/TOS value, and was considered suitable as a test item.

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).[28]

Four strains of *S*. Typhimurium (TA98, TA100, TA1535 and TA1537) were used applying the 'treat and plate' assay and *E. coli* WP2uvrA pKM101 applying the plate incorporation method. Three experiments were carried out in triplicate with or without metabolic activation (S9-mix), using six concentrations of the food enzyme ranging from 156 to 5,000 μg dry matter/plate, corresponding to 124.8, 250.4, 500, 1,000, 2,000 and 4,000 μg TOS/plate.

No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme, there was no biologically relevant increase in the number of revertant colonies above the control values in any strain tested with or without S9-mix.

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

[20] Technical dossier/pp. 14, 55–57and Annex 2.04.
[21] LoDs: Pb = 1 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[22] Technical dossier/pp. 15–16, 57 and Annexes 2.8–2.11.
[23] Technical dossier/pp. 15–16, 57 and Annex 2.7.
[24] Technical dossier/pp. 14, 55–56 and Annex 2.05.
[25] LoDs: kojic acid = 1.4 mg/kg; β-nitro propionic acid = 0.8 mg/kg.
[26] Technical dossier/GMM dossier/Annex E1.
[27] Technical dossier/Additional data May 2023/Annex E2, Version2.
[28] Technical dossier/Annex 7.01.

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.[29] 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 the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1,187, 2,813 and 5,000 μg dry matter/mL (corresponding to 950, 2,250 and 4,000 μg TOS/mL) in a short-term treatment (3 h exposure and 17 h recovery period) without S9-mix and at concentrations of 1,582, 2,109 and 5,000 μg dry matter/mL (corresponding to 1,267, 1,687 and 4,000 μg TOS/mL) in a short-term treatment with S9-mix.

In the second experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 426, 839 and 1,311 μg dry matter/mL (corresponding to 344, 671 and 1,049 μg TOS/mL) in a long-term treatment (20 h exposure and no recovery period) without S9-mix and at concentrations of 3,200, 4,000 and 5,000 μg dry matter/mL (corresponding to 2,560, 3,200 and 4,000 μg TOS/mL) in a short-term treatment) with S9-mix.

In the long-term treatment without S9, cytotoxicity of 53% (mitotic inhibition) was observed at 839 and 1,311 μg dry matter/mL (corresponding to 671 and 1,049 μg TOS/mL without S9-mix.

The frequency of structural and numerical aberrations was not statistically significantly different to the negative controls at any concentration tested.

The Panel concluded that the food enzyme asparaginase did not induce an increase in the frequency of structural and numerical aberrations under the test conditions applied in this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[30] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD))IGS BR rats received by gavage the food enzyme in doses of 1, 3.3 and 10 mL/kg body weight (bw) per day, corresponding to 88, 290 and 880 mg TOS/kg bw per day. Controls received the vehicle (purified water obtained by reverse osmosis).

No mortality was observed.

In the functional observations, a statistically significant increase in the forelimb grip strength was observed in high-dose males (+14%). The Panel considered the change as not toxicologically relevant as it was only observed in one sex and the change was within the historical control values.

Haematological investigations revealed a statistically significant decrease in haematocrit in mid- and high-dose males (−4% and −4%, respectively), haemoglobin concentrations in mid- and high-dose males (−4% and −4%, respectively), total white blood cell counts in low-, mid- and high-dose females (−24%, −21% and −24%, respectively), basophil counts in low-, mid- and high-dose females (−55%, −55% and −55%, respectively) and activated partial thromboplastin time in low-, mid- and high-dose females (−15%, −14% and −14%, respectively). The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters), there was no dose–response relationship (all parameters), the changes were small (haematocrit, haemoglobin, basophils), there were no changes in other relevant parameters (red blood cells, platelet count, prothrombin) and the changes were within the historical control values (haematocrit, haemoglobin, total white blood cell counts).

Clinical chemistry investigations revealed a statistically significant increase in plasma potassium concentrations in mid- and high-dose males (+7% and + 9%, respectively) and in high-dose females (+8%), an increase in glucose concentrations in low- and high-dose females (+12% and +19%, respectively), a statistically significant decrease in total protein concentrations in mid-dose males (−4%) and plasma albumin concentrations in mid- and high-dose males (−3% and −3%, respectively). The Panel considered the changes as not toxicologically relevant as they were small (total protein, albumin), they were only observed in one sex (all parameters except potassium), there was no dose–response relationship (total protein, albumin), there were no histopathological changes in kidneys (potassium) and there were no changes in other relevant parameters (albumin/globulin ratio).

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

[29] Technical dossier/Annex 7.02.
[30] Technical dossier/Annex 7.03.

The Panel identified a no observed adverse effect level (NOAEL) of 880 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considered only the food enzyme and not carriers or other excipients, which may be used in the final formulation.

The potential allergenicity of the asparaginase produced with the genetically modified *A. oryzae* strain NZYM-SP 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.[31,32]

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

Allergic reactions have been described when asparaginase is given by injection as part of chemotherapy (Pagani et al., 2021). These have been characterised as cell-mediated type IV delayed hypersensitivity reactions (Scheper et al., 1992; Kimber, 1994). No IgE-dependent reactions have been reported after oral exposure.

[redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product 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 from this source are not expected to be 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, 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 to prevent acrylamide formation in food processing at a recommended use level shown in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[33]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Prevention of acrylamide formation in foods		
Baked products	Flour	2.58–**7.36**
Cereal-based products (e.g. crackers, tortilla chips)	Cereals	1.67–5.91
Coffee and cocoa products	Coffee beans, cocoa beans	0.97–4.60
French fries	Potatoes	0.30–1.31
Potato-based snacks (e.g. sliced crisps)	Potatoes	0.39–1.58
Fruits and vegetable products (e.g. prune puree)	Plums/prunes	0.30–1.31

TOS: total organic solids.
(a): The name has been harmonised by the EFSA's call-for-data on acrylamide reduction process (available online: https://www.efsa.europa.eu/en/call/call-input-data-exposure-assessment-food-enzymes-16th-call).
(b): The number in bold was used for calculation.

The food enzyme can be added to a variety of raw materials before high temperature treatment (e.g. baking, frying, roasting) to prevent the formation of acrylamide. For baked/fried/roasted foods, the food enzyme is added to a variety of starch-rich food commodities (e.g. flour, potato, coffee,

[31] Technical dossier/Annex 8.
[32] Technical dossier/Additional data December 2022/Sequence homology to known allergens_Asparaginase_NZYM-SP.
[33] Technical dossier/Additional data December 2022/Answer 5.

prunes) at various stages. For bread and extruded snacks, it is added to flour or potato flakes during dough making. To coffee and cocoa products, it is added to the green coffee beans prior to roasting. Potato products are dipped into an enzyme solution before frying. Prune products are treated with the food enzyme during hydration or steaming.[34] The asparaginase hydrolyses the free L-asparagine to release L-aspartic acid and ammonia. This enzymatic treatment prevents the formation of acrylamide from asparagine. The food enzyme–TOS remains in the final processed foods.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food processes, it is expected that this asparaginase will be inactivated in the final foods.

3.5.2. Dietary exposure estimation

The 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 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 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be about 0.101 mg TOS/kg bw per day in infants at the 95th percentile.

Table 3: Summary of estimated dietary exposure to the 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)	0.003–0.044 (12)	0.005–0.021 (15)	0.002–0.011 (19)	0.001–0.007 (21)	0.002–0.005 (22)	0.002–0.004 (23)
Min–max 95th percentile (number of surveys)	0.012–0.101 (11)	0.012–0.066 (14)	0.006–0.021 (19)	0.002–0.013 (20)	0.004–0.010 (22)	0.004–0.007 (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.

[34] Technical dossier/pp.95–102.

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	
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	+/–
Although different use levels were provided, the highest value of the recommended maximum use level was used in the calculation	+

+: 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.

3.6. Margin of exposure

The comparison of the NOAEL (880 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.044 mg TOS/kg bw per day at the mean and from 0.002 to 0.101 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 8,713.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme asparaginase produced with the genetically modified *A. oryzae* strain NZYM-SP does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

Application for authorisation. Asparaginase from a genetically modified strain of *Aspergillus oryzae* (strain NZYM-SP). June 2013. Submitted by Novozymes A/S.

Additional information. September 2014. Submitted by Novozymes A/S.

Additional information. December 2022. Submitted by Novozymes A/S.

Additional information. May 2023. Submitted by Novozymes A/S.

References

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

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, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

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, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 38 pp. https://doi.org/10.2903/j.efsa.2021.7010

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs, 3, 63–67. Available online: https://www.fao.org/3/a-a0675e.pdf

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J, 2018. Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus Niger, Aspergillus oryzae*, and *Trichoderma reesei*. Applied Microbiology and Biotechnology, 102, 9481–9515. https://doi.org/10.1007/s00253-018-9354-1

Kimber I, 1994. Cytokines and regulation of allergic sensitization to chemicals. Toxicology, 93, 1–11.

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents 21 September 1998. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test 21 July 1997. 11 pp. Available online: https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1639400502&id=id&accname=guest&checksum=D2E89C16911BE1BEB13BBC206B402098

OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: In vitro mammalian chromosomal aberration test 21 July 1997. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

Pagani M, Bavbek S, Alvarez-Cuesta E, Berna Dursun A, Bonadonna P, Castells M, Cernadas J, Chiriac A, Sahar H, Madrigal-Burgaleta R and Sanchez Sanchez S, 2021. Hypersensitivity reactions to chemotherapy: an EAACI Position Paper. Allergy, 77, 388–403.

Scheper RJ, Mary B and von Blomberg E, 1992. Cellular mechanisms in allergic contact dermatitis. Textbook of Contact Dermatitis, 11–27.

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
OECD	Organisation for Economic Co-operation 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

Appendix A can be found in the online version of this output (in the 'Supporting information' section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Portugal, Republic of North Macedonia*, 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, the 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*, the 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*, the 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*, the 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 B 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).