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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme AMP deaminase from non-genetically modified *Aspergillus* sp. strain DEA 56-111

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 | Lieve Herman | Yrjö Roos | Magdalena Andryszkiewicz | Daniele Cavanna | Natália Kovalkovičová | Yi Liu | Simone Lunardi | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme AMP deaminase (AMP aminohydrolase; EC 3.5.4.6) is produced with the non-genetically modified microorganism *Aspergillus* sp. strain DEA 56-111 by Shin Nihon Chemical Co., Ltd. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in the processing of yeast and yeast products. Dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.005 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The Panel identified a no observed adverse effect level of 1984 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 396,800. 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 the risk of allergic reactions upon 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

AMP aminohydrolase; EC 3.5.4.6, AMP deaminase, *Aspergillus* sp., food enzyme, non-genetically modified microorganism

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[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.

Three applications have been introduced by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzyme Alpha-amylase from *Bacillus amyloliquefaciens* and the company 'Intertek Scientific & Regulatory Consultancy' for the authorisation of the food enzymes *Aspergillus* nuclease S_1 (the applicant has named the enzyme as Nuclease P1) from *Penicillium citrinum* (strain NP 11–15) and AMP deaminase from *Aspergillus oryzae* (strain DEA 262).

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 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 *Aspergillus* nuclease S_1 from *Penicillium citrinum* (strain NP 11–15), Alpha-amylase from *Bacillus amyloliquefaciens* and AMP deaminase from *Aspergillus oryzae* (strain DEA 262) in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] 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, 11.03.2011, 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 AMP deaminase from the non-genetically modified *Aspergillus oryzae* strain DEA 262.

Recent data identified the production microorganism as *Aspergillus* sp. strain DEA 56-111 (see Section 3.1).[4] Therefore, this name will be used in this opinion instead of *Aspergillus oryzae* strain DEA 262.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme AMP deaminase from non-genetically modified *Aspergillus oryzae* strain DEA 262.

Additional information was requested from the applicant during the assessment process on 28 June 2023 and received on 25 September 2023 (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 the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application. Additional information was requested in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021) and the guidance on 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	AMP deaminase
Systematic name	AMP aminohydrolase
Synonyms	AMP aminase; adenylic acid deaminase; adenylate deaminase
IUBMB No	EC 3.5.4.6
CAS No	9025-10-9
EINECS No	–

AMP deaminases catalyse the deamination of adenosine 5′-monophosphate (AMP) to produce 5′-inosinic acid. The enzyme under assessment is intended to be used in the processing of yeast and yeast products.[5]

3.1 | Source of the food enzyme[6]

The AMP deaminase is produced with the non-genetically modified filamentous fungus *Aspergillus* sp. strain DEA 56-111 (formerly *Aspergillus oryzae* strain DEA 262), which is deposited in [redacted] with the deposit number [redacted].[7]

The identification of the production strain was based on [redacted]. This approach assigned strain DEA 56-111 to [redacted],[8] but it did not allow an unequivocal identification of the production strain at the species level. As a consequence, in this opinion, the production strain is described as *Aspergillus* sp.

[4]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 1; Attachment 2.
[5]Technical dossier/Additional data, 25 September 2023/Annex.
[6]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 1; Attachment 2.
[7]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 2.
[8]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 1.

3.2 | Production of the food enzyme[9]

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 [redacted] fermentation system with conventional process controls in place. After completion of the fermentation, the food enzyme is extracted from the fermentation medium, and then, the solid biomass is removed from the extract by [redacted]. The filtrate containing the enzyme is further [redacted] 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 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 AMP deaminase is a single polypeptide chain of [redacted] amino acids.[14] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[15] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches.[16] No other enzyme activities were reported.[17]

The in-house determination of AMP deaminase activity is based on [redacted]. The enzyme activity is expressed in unit (U)/g. One unit is the amount of enzyme which produces [redacted] under the conditions of the assay.[18]

The food enzyme has a temperature optimum around 50°C (pH 5.5) and a pH optimum around pH 5.5 (30°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 5.5). The enzyme activity decreased above 60°C, showing no residual activity above 65°C.[19]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation and one batch produced for the toxicological tests (Table 1).[20] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 18.7% and the mean enzyme activity/TOS ratio was 14.0 U/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	Batches			
		1	2	3	4[a]
AMP deaminase activity	U/g[b]	3150	2250	2440	2540
Protein	%	9.7	9.9	9.3	10.2
Ash	%	0.8	0.8	0.8	0.9
Water	%	80.4	79.9	81.2	79.3

(Continues)

[9]Technical dossier/Additional data, 25 September 2023/Annex.
[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. 26, 31, 36; Technical dossier/Additional data, 25 September 2023/Annex.
[12]Technical dossier/p. 26–29, 31–34; Technical dossier/Annex III.
[13]Technical dossier/p. 31; Technical dossier/Annex III; Technical dossier/Additional data, 25 September 2023/Annex.
[14]Technical dossier/p. 13; Technical dossier/Annex VII.
[15]Technical dossier/Additional data, 25 September 2023/Annex.
[16]Technical dossier/p. 16.
[17]Technical dossier/p. 23.
[18]Technical dossier/p. 20; Technical dossier/Annex II.1.
[19]Technical dossier/p. 20–23.
[20]Technical dossier/p. 34–35, 48–49; Technical dossier/Annex I, Annex II, Annex IV, Annex V.

TABLE 1 (Continued)

Parameters	Unit	Batches 1	2	3	4[a]
Total organic solids (TOS)[c]	%	18.8	19.3	18.0	19.8
Activity/TOS ratio	U/mg TOS	16.8	11.7	13.6	12.8

[a]Batch used for the toxicological studies.
[b]U: Unit (see Section 3.3.1).
[c]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity[21]

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg,[22] 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 arsenic content was below the limits of detection/quantification (LoD/LoQ) of the employed methods.[23,24]

The food enzyme preparation complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[25] No antimicrobial activity was detected in any of the tested batches.[26]

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 ochratoxin A, aflatoxin B1, B2, G1 and G2, zearalenone, sterigmatocystin and T-2 toxin was examined in the food enzyme batches used for commercialisation and all were below the LoD of the applied methods.[27,28] Adverse effects caused by the possible presence of other secondary metabolites was addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4 | Viable cells of the production strain[29]

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. A positive control was included.

3.4 | Toxicological data[30]

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, has been provided. The batch 4 (Table 1) used in these studies 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).[31]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2*uvrA* were used with or without metabolic activation (S9-mix), applying the pre-incubation method (in the preliminary, dose-finding and first main experiments) and 'treat and wash' assay (in the second main and confirmatory experiments).

[21]Technical dossier/Annex IV; Technical dossier/Additional data, 25 September 2023/Annex/Attachment 3.
[22]Technical dossier/p. 34, 49; Technical dossier/Annex IV.
[23]Technical dossier/p. 34, 49; Technical dossier/Annex IV.
[24]Technical dossier/Additional data, 25 September 2023/Annex: LoQs/LoDs: Pb = 0.05 mg/kg; As = 0.1 mg/kg.
[25]Technical dossier/p. 34, 49; Technical dossier/Annex IV.
[26]Technical dossier/p. 34, 49; Technical dossier/Annex IV.
[27]Technical dossier/p. 35; Technical dossier/Annex I, Annex IV.
[28]Technical dossier/Annex IV; LoDs: ochratoxin A, aflatoxins (B1, B2, G1, G2) = 0.5 µg/kg each; zearalenone = 50 µg/kg; sterigmatocystin = 20 µg/kg; T-2 toxin = 0.1 mg/kg.
[29]Technical dossier/Additional data, 25 September 2023/Annex.
[30]Technical dossier/p. 44–50; Technical dossier/Annex IV– Certificates of analysis/2. Antibacterial activity, Annex VI.
[31]Technical dossier/Annex VI – toxicology study reports/1. Ames test.

Based on the results of a preliminary experiment, the dose-finding experiment using pre-incubation method was carried out in triplicate, using six concentrations of the food enzyme ranging from 1.05 to 254 U/plate, corresponding to 82–19,844 µg TOS/plate in the presence of S9-mix and using eight concentrations of the food enzyme ranging from 0.116 to 254 U/plate, corresponding to 9.1–19,844 µg TOS/plate in the absence of S9-mix. Toxic effects, evident as a reduction in the number of revertant colonies, occurred in *E. coli* WP2*uvrA* at 254 U/plate with and without S9-mix. Growth stimulation, as indicated by the thickening of the background bacterial lawn, was observed at middle and higher concentrations in all *S*. Typhimurium strains in the presence and absence of S9-mix. Upon treatment with the food enzyme, there was a twofold increase in revertant colony numbers above the control values in *S*. Typhimurium TA100 and TA1535 strains with or without S9-mix and in *S*. Typhimurium TA98 without S9-mix.

The first main experiment using the pre-incubation method was carried out in triplicate in *S*. Typhimurium TA1537 and *E. coli* WP2*uvrA* in the absence of S9-mix and *S*. Typhimurium strains TA1537 and TA98 and *E. coli* WP2*uvrA* in the presence of S9-mix. Six concentrations of the food enzyme, ranging from 7.94 to 254 U/plate, were used, corresponding to 620.3–19,844 µg TOS/plate. Toxic effects, evident as a reduction in the number of revertant colonies, occurred in *E. coli* WP2*uvrA* at 254 U/plate with and without S9-mix. Growth stimulation, as indicated by the thickening of the background bacterial lawn, was observed at middle and higher concentrations in all strains in the presence and absence of S9-mix. Upon treatment with the food enzyme, there was a twofold increase in revertant colony numbers above the control values at 63.5 U/plate and above in *S*. Typhimurium TA98 strain with S9-mix.

The study author considered that the increase in revertant colony numbers observed in the preliminary, dose-finding and first main experiment using the pre-incubation method was caused by free amino acids present in the test item.

The second main experiment using the 'treat and wash' assay was carried out in triplicate in *S*. Typhimurium TA100, TA1535 and TA98 strains in the absence and presence of S9-mix, using six concentrations of the food enzyme ranging from 7.94 to 254 U/plate, corresponding to 620.3–19,844 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 confirmatory experiment using the 'treat and wash' assay was carried out in triplicate in *S*. Typhimurium TA100, TA1535 and TA98 strains in the absence and presence of S9-mix, using five different concentrations of the food enzyme ranging from 15.9 to 254 U/plate, corresponding to 1242–19,844 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 AMP deaminase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | *In vitro mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[32] An experiment was performed with duplicate cultures of the Chinese hamster lung fibroblast cell line (CHL/IU). The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

In the cell growth inhibition test, cytotoxicity was observed and the 50% inhibition concentrations were calculated to be 19 U/mL in the short-term treatment without S9-mix, 15.9 U/mL in the short-term treatment with S9-mix and 1.01 U/mL in the long-term treatment without S9-mix, respectively.

Based on these results, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 8.54, 17.4 and 35.6 U/mL (corresponding to 670, 1360 and 2780 µg TOS/mL) in a short-term treatment (6-h exposure and 18-h recovery period) either with or without S9-mix and at concentrations of 0.345, 0.703 and 1.43 U/mL (corresponding to 27, 55 and 112 µg TOS/mL) in a long-term treatment (24 h exposure without recovery period) without S9-mix.

Cytotoxicity of 42%, 47% and 48% was reported in the short-term treatment without S9-mix, with S9-mix and in the long-term treatment, respectively. The frequency of structural and numerical aberrations was not statistically significantly different from the negative controls at any of the concentrations tested.

The Panel concluded that the food enzyme AMP deaminase 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 rodents

The repeated dose 90-day oral toxicity study followed the Guidelines for designation of food additives and for the revision of standards for use of food additives, Notification No. 29 of the Environmental Health Bureau, Japanese Ministry of Health and Welfare (1996), OECD Test Guideline 408 (OECD, 1998) and GLP[33] with the following deviation: The functional observations were not performed. The Panel considered that this deviation was minor and did not impact on the evaluation of the study.

[32]Technical dossier/Annex VI – toxicology study reports/2. Chromosomal aberration test.

[33]Technical dossier/Annex VI – toxicology study reports/90-Day study; Technical dossier/Additional data, 25 September 2023/Annex/Attachment 4.

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) [SPF] rats received by gavage the food enzyme in doses of 254, 2540 and 25,400 U/kg body weight (bw) per day, corresponding to 19.8, 198 or 1984 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

Haematological investigation revealed a statistically significant increase in mean corpuscular volume (MCV) in high-dose males (+ 4%), an increase in mean corpuscular haemoglobin (MCH) in high-dose males (+ 5%) and an increase in neutrophils in low- and mid-dose females (+ 46%, + 122%, 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 (neutrophils), there were no changes in other relevant parameters (haemoglobin concentration, white blood cell count) and the changes were within the historical control values.[34]

Urinalysis revealed a statistically significant increase in sodium concentration (+ 58%) and total sodium excretion (+ 59%) in high-dose males. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex, there were no histopathological changes in kidneys and the changes were within the historical control values.[35]

The microscopic examination revealed mixed glioma (low grade of malignancy) in the brain in one high-dose female. Although the tumour was found in the treated female, the Panel considered this isolated finding as incidental and not test item related, as a spontaneous glioma was previously observed in this strain of rats at the relatively early age (Son & Gopinath, 2004).

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

The Panel identified a no observed adverse effect level (NOAEL) of 1984 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity[36]

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

The potential allergenicity of the AMP deaminase produced with *Aspergillus* sp. strain DEA 56-111 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.[37]

No information was available on oral and respiratory sensitisation or elicitation reactions of this AMP deaminase. No allergic reactions after ingestion of AMP deaminases have been reported.

Aspergillus is a known source of respiratory allergens. However, several studies have shown that adults sensitised to respiratory allergens can ingest the allergens without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004).

[redacted], a product that may cause allergies (listed in the Regulation (EU) No 1169/2011[38]), is used as raw material. In addition, [redacted], a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from these sources are present in the food enzyme.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme[39]

The food enzyme is intended to be used in the processing of yeast and yeast products at a recommended use level of 85.6 mg TOS/kg yeast cells.[40]

[34]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 4.

[35]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 4.

[36]Technical dossier/Additional data, 25 September 2023/Annex.

[37]Technical dossier/p. 50–51; Technical dossier/Annex VII; Technical dossier/Additional data, 25 September 2023/Annex.

[38]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.

[39]Technical dossier/Additional data, 25 September 2023/Annex/Attachment 5.

[40]Technical dossier/Additional data, 25 September 2023/Response 8 and Table 3.

In yeast processing, following cell lysis and RNA hydrolysis, the food enzyme is added to the yeast biomass to convert the AMP to 5′-inosinic acid.[41] The enzymatic conversion improves the sensory property of the yeast extract, which is then used (in paste or powder form) as an ingredient to enhance the umami taste in a wide range of foods, such as soups and savoury sauces.[42,43] The food enzyme–TOS remains in yeast extracts and the final foods.

Based on data provided on thermostability (see Section 3.3.1), it was expected that the food enzyme is inactivated during the processing of yeast and yeast products.

3.5.2 | Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). 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 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 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 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 0.005 mg TOS/kg bw per day in children at the 95th percentile.

TABLE 2 Summary of the estimated dietary exposure to food enzyme–TOS in six population groups.

	Estimated exposure (mg TOS/kg body weight per day)					
Population group	**Infants**	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0–0.001 (12)	0–0.002 (15)	0–0.002 (19)	0–0.001 (21)	0 (22)	0–0.001 (23)
Min–max 95th percentile (number of surveys)	0–0.003 (11)	0–0.003 (14)	0–0.005 (19)	0–0.002 (20)	0–0.002 (22)	0–0.002 (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 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	
Although only yeast extracts are produced by the enzymatic treatment, food categories chosen for calculation included foods relevant not only to yeast extract, but also to yeast autolysates and yeast cell wall.	+
Exposure to food enzyme–TOS 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	+/–

+: uncertainty with potential to cause overestimation of exposure.

–: uncertainty with potential to cause underestimation of exposure.

[41]Technical dossier/Additional data, 25 September 2023/Attachment 5.

[42]Technical dossier/p. 38–39.

[43]Technical dossier/Additional data, 25 September 2023/Table 3.

The conservative approach applied to estimate the 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

A comparison of the NOAEL (1984 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.002 mg TOS/kg bw per day at the mean and of 0–0.005 mg TOS/kg bw per day at the 95th percentile resulted in margin of exposure of at least 396,800.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme AMP deaminase produced with the non-genetically modified *Aspergillus* sp. strain DEA 56-111 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier 'Application for the Authorisation of AMP Deaminase from *Aspergillus oryzae* strain DEA 262 as a food enzyme in the European Union'. 3 March 2015. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. 25 September 2023. Submitted by Shin Nihon Chemical Co., Ltd.

ABBREVIATIONS

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
AMP	adenosine 5′-monophosphate
bw	body weight
CAS	Chemical Abstracts Service
CHL/IU	Chinese hamster lung fibroblast cell line
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
FoodEx	standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
[redacted]	[redacted]
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
LoQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
[redacted]	[redacted]
non-GM	non-genetically modified
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
RNA	ribonucleic acid
SPF	Specific pathogen free
TOS	total organic solids
U	unit
WHO	World Health Organization

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CONFLICT OF INTEREST

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

REQUESTOR

European Commission

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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.

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SUPPORTING INFORMATION

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

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APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

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, 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).