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SCIENTIFIC OPINION

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Safety evaluation of the food enzyme oryzin from the non-genetically modified *Aspergillus ochraceus* strain AE-P

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Abstract

The food enzyme oryzin (EC 3.4.21.63) is produced with the non-genetically modified *Aspergillus ochraceus* strain AE-P by Amano Enzyme Inc. The food enzyme was considered free from viable cells of the production organism. It is intended to be used in nine food manufacturing processes. The dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.1 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1862 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 18,620. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and 31 matches were found, including one food allergen (melon). The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to melon, cannot be excluded, but would not exceed the risk from consumption of this food. 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

aspergillopeptidase B, *Aspergillus ochraceus*, EC 3.4.21.63, food enzyme, oryzin

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; and
- its use does not mislead the consumer.

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

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

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

1.1.1 | Background as provided by the European Commission

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

Five applications have been introduced by the companies "Amano Enzyme Inc." and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorization of food enzymes Ribonuclease P from *Penicillium citrinum* (strain AE-RP), Glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), Oryzin from *Aspergillus melleus* (strain AE-P), Triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and Glucoamylase from *Aspergillus niger* respectively.

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the five 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 requested EFSA to carry out the safety assessments of the food enzymes Ribonuclease P from *Penicillium citrinum* (strain AE-RP), Glutaminase from *Bacillus amyloliquefaciens* (strain AE-GT), Oryzin from *Aspergillus melleus* (strain AE-P), Triacylglycerol lipase from *Candida rugosa* (strain AE-LAY) and Glucoamylase from *Aspergillus niger* 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, 11.03.2011, 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 food enzyme oryzin from *A. melleus* strain AE-P.

Recent data identified the production microorganism as *A. ochraceus* (see Section 3.1). Therefore, this name will be used in this opinion instead of *A. melleus*.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for the authorisation of the food enzyme oryzin from *Aspergillus melleus* (strain AE-P). The dossier was updated in September 2015.

Additional information was requested from the applicant during the assessment process on 29 September 2021 and on 20 September 2023, and received on 30 August 2022 and 11 October 2023 respectively (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 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009b) 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 the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

3 | ASSESSMENT

IUBMB nomenclature	Oryzin
Systematic name	–
Synonyms	Aspergillopeptidase B; aspergillopepsin B; aspergillopepsin F
IUBMB No	EC. 3.4.21.63
CAS No	9074-07-1
EINECS No	232-977-6

Oryzins catalyse the hydrolysis of proteins with broad specificity, resulting in the generation of peptides and amino acids. The food enzyme under assessment is intended to be used in nine food manufacturing processes as described in the EFSA guidance (EFSA CEP Panel, 2023): (1) processing of cereals and other grains for the production of baked products; (2) processing of eggs and egg products; processing of dairy products for the production of (3) flavouring preparations and (4) modified milk proteins; processing of meat and fish products for the production of (5) modified meat and fish products and (6) protein hydrolysates; processing of plant- and fungal-derived products for the production of (7) plant-based analogues of milk and milk products and (8) protein hydrolysates and (9) processing of yeast and yeast products.

3.1 | Source of the food enzyme

The oryzin is produced with the non-genetically modified filamentous fungus *Aspergillus ochraceus* strain AE-P, which is deposited at the National Institute for Technological Evaluation (NITE) Biological Resource Center (Japan), with the deposit number [redacted].[4] The production strain was identified as *A. ochraceus* by [redacted].[5]

The production strain *A. ochraceus* AE-P was derived from the parental strain [redacted].[6]

[4]Additional data August 2022/Annex 2.

[5]Additional data August 2022/Annex 1.

[6]Additional data August 2022/p. 34.

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004, with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[7]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch fermentation system with conventional process controls in place. After the completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration, leaving a filtrate containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[8] 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.[9]

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

Oryzin is a single polypeptide chain of [redacted] amino acids.[10] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[11] The food enzyme was analysed by size exclusion chromatography.[12] The chromatograms of the three food enzyme batches for commercialisation showed a consistent pattern. No other enzyme activities were reported.[13]

The determination of oryzin activity is based on the hydrolysis of casein (reaction conditions: pH 7.0, 37°C, 60 min). The enzymatic activity is determined by measuring the release of amino acids, which react with Folin's test solution and are detected spectrophotometrically at 660 nm. The enzyme activity is expressed in units (U)/g. One unit is the amount of enzyme that produces an absorbance equivalent to 1 µg of tyrosine per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 45°C (pH 8.0) and a pH optimum around pH 7.0 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 60 min at different temperatures (pH 8.0). The oryzin activity decreased above 45°C, showing no residual activity above 60°C.[15]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for six batches used for commercialisation and three batches produced for the toxicological tests (Table 1).[16] The mean total organic solids (TOS) of the six food enzyme batches for commercialisation was 88.2% and the mean enzyme activity/TOS ratio was 1235 U/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches used for commercialisation		Batches used for toxicological tests		
Parameters	**Unit**	**Mean**	**Minimum–maximum**	**4[a]**	**5[b]**	**6[c]**
Oryzin activity	U/g[d]	1,098,000	808,000–1,380,000	759,000	999,000	1,300,000
Protein	%	62.0	57.3–69.5	44.8	50.5	63.4
Ash	%	5.8	2.1–9.4	8.2	9.5	2.3
Water	%	6.0	5.6–6.4	5.0	6.8	4.6
[redacted] (excipient)	%	0	0	20.0	20.0	0
Total organic solids (TOS)[e]	%	88.2	84.4–92.1	66.8	63.7	93.1
Activity/TOS ratio	U/mg TOS	1235	954–1498	1136	1568	1396

[a]Batch used for the Ames test.

[b]Batch used for chromosomal aberration assay.

[c]Batch used for the repeated dose 90-day oral toxicity study in rats.

[d]UNIT: U/g (see Section 3.3.1).

[e]TOS calculated as 100% – % water – % ash – % excipient.

[7]Technical dossier/2nd submission/Annexes: 4.1, Annex 4.2.

[8]Technical dossier/2nd submission/pp. 36-43/Annex 5.

[9]Technical dossier/2nd submission/pp. 36-43/Annex 6; Additional data August 2022.

[10]Technical dossier/2nd submission/pp. 29.

[11]Technical dossier/2nd submission/pp. 29.

[12]Technical dossier/2nd submission/pp. 28.

[13]Technical dossier/2nd submission/pp. 30.

[14]Technical dossier/2nd submission/Annex 2.

[15]Technical dossier/2nd submission/pp. 31–32.

[16]Technical dossier/2nd submission/pp. 27, pp. 61-62/Annex 3.1, Annex 3.2; Additional data August 2022/Annex 4, Annex 5.

3.3.3 | Purity

The mean lead content in the commercial batches was 0.03 mg/kg,[17,18] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[19] No antimicrobial activity was detected in any of the tested batches.[20]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins (B1, B2, G1 and G2), ochratoxin A, sterigmatocystin, HT-2 toxin, T-2 toxin, deoxynivalenol and zearalenone was examined in the food enzyme batches used for commercialisation and all were below the limit of quantification (LoQ) of the applied method.[21,22] 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 was sufficient.

3.3.4 | Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in [redacted]. No colonies of the production strain were produced. [redacted].[23]

3.4 | Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an in vitro mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided.

Batches 4, 5 and 6 in Table 1 had a similar activity/TOS ratio as the batches intended for commercialisation and were considered as suitable test items.

3.4.1 | Genotoxicity

3.4.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation assay (Ames test) was performed according to the 'Guidelines for *in vitro* mutagenicity testing' (Japan, 1985) and 'Guidebook of mutagenicity study using bacteria' (Japan, 1986).[24]

Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 *uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the pre-incubation method. Based on a preliminary test, five concentrations of the food enzyme (313, 625, 1250, 2500 and 5000 µg/plate, corresponding to 209, 418, 835, 1670 and 3340 µg TOS/plate, respectively) were used in the two main experiments with triplicate plating.

No cytotoxicity was observed at any concentration level of the food enzyme. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

3.4.1.2 | *In vitro mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out in Chinese hamster lung cells in accordance with the 'Guidelines for toxicity studies required to manufacture (import) drugs' ('Japan 1989'), the Organisation for Economic

[17]Technical dossier/2nd submission/Annex 3.1; Additional data August 2022/Annex 4.

[18]Limit of detection: Pb = 0.01 mg/kg.

[19]Technical dossier/2nd submission/Annex 3.1; Additional data August 2022/Annex 4.

[20]Technical dossier/2nd submission/Annex 3.3; Additional data August 2022/Annex 4.

[21]Technical dossier/2nd submission/Annex 3.1; Additional data August 2022/Annex 4.

[22]LoQs: aflatoxins (B1, B2, G1 and G2) = 0.2 µg/kg each; HT-2 toxin, T-2 toxin, sterigmatocystin, zearalenone = 10 µg/kg each; ochratoxin A = 0.5 µg/kg; deoxynivalenol = 20 µg/kg.

[23]Additional data August 2022/Annex 3.

[24]Technical dossier/2nd submission/Annex 8.

Co-operation and Development (OECD) Guidelines for the testing of chemicals ('OECD, 1983') and the 'Food Laboratory Practice Regulations' ('Japan 1982').[25]

Based on the results of a dose-finding study, the cells were exposed to four concentrations of the food enzyme in six separate experiments: 6 h + 16 h recovery period with or without S9-mix (78, 156, 313 and 625 µg/mL, corresponding to 50, 100, 199 and 398 µg TOS/mL, respectively); 6 h + 40 h recovery period with or without S9-mix (312.5, 625, 1250 and 2500 µg/mL, corresponding to 199, 398, 796 and 1592 µg TOS/mL, respectively); 22 h continuous treatment without S9-mix (19.5, 39, 78 and 156 µg/mL, corresponding to 12, 25, 50 and 100 µg TOS/mL, respectively); 46 h continuous treatment without S9-mix (4.9, 9.8, 19.5 and 39 µg/mL corresponding to 3, 6, 12 and 25 µg TOS/mL, respectively). The frequencies of structural and numerical chromosomal aberrations in treated cultures were comparable to the values observed in negative controls.

The Panel concluded that the food enzyme oryzin did not induce chromosomal aberrations under the test conditions employed for this study.

3.4.1.3 | *Repeated dose 90-day oral toxicity study in rodents*

The repeated dose 90-day oral toxicity study followed Good Laboratory Practice[19] and the OECD Test Guideline 408 (OECD, 2018), but the vaginal smears were not examined. The Panel considered that this omission did not impact on the evaluation of the study. Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 500, 1000 or 2000 mg/kg body weight (bw) per day, corresponding to 466, 931 or 1862 mg TOS/kg bw per day respectively. Controls received the vehicle (water for injection).

No mortality was observed.

Clinical observations revealed a statistically significant increase in rearing count in low-dose males in Week 9 (+67%) and in mid-dose males in Week 6 (+100%). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically, they were only observed in one sex and there was no dose–response relationship.

Haematological investigations revealed a statistically significant increase in eosinophil count (EOS) in high-dose males (+73%), an increase in mean corpuscular volume (MCV, +4%) and a decrease in mean corpuscular haemoglobin concentration (MCHC, −2%) in high-dose females. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), the changes were small (all parameters), there were no changes in other relevant parameters (for EOS in total count of white blood cells, for MCV and MCHC in total count of red blood cells and haemoglobin concentration) and the changes in MCV and MCHC were within the historical control values.

The macroscopic examination showed raised foci in the forestomach in 1/10 and 3/10 mid- and high-dose males and in 4/10 high-dose females. These changes correlated with the microscopic changes in the forestomach of focal hyperplasia of squamous cells, which was minimal in 1/10 mid-dose male and in 1/10 high-dose female, and mild in 3/10 high-dose males and in 3/10 high-dose females. Furthermore, a minimal hyperplasia of squamous cells was also recorded in the limiting ridge in 1/10, 5/10 and 9/10 males in the low-, mid- and high-dose groups and in 6/10 high-dose females. These changes were not present in the control group. The Panel considered these changes as test item related. However, considering the minimal to mild severity of the effects, the nature of the enzyme and the bolus effect of the gavage, possibly causing an irritation, the Panel did not judge these changes as adverse.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1862 mg TOS/kg bw per day, the highest dose tested.

3.4.2 | Allergenicity

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

The potential allergenicity of the oryzin produced with the *Aspergillus ochraceus* strain AE-P 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, 31 matches were found.[26] The matching allergens were 26 respiratory allergens, four contact allergens and one food allergen (Cuc m 1, alkaline serine protease (cucumisin) from *Cucumis melo* (melon)).[27]

No information was available on the oral and respiratory sensitisation or elicitation reactions of this oryzin.

Reports identifying oryzin of fungal origin as inhalation and contact allergens can be found in the literature (Matsumura, 2012; Simon-Nobbe et al., 2008).[28] However, contact allergy follows a different mechanism than oral allergy to food allergens. Concerning respiratory allergens, to which specific IgE is formed, several studies have shown that adults respiratorily sensitised to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Cullinan et al., 1997; Poulsen, 2004).

[25]Technical dossier/2nd submission/Annex 9.

[26]Technical dossier/2nd submission/pp. 63–64/Annex 11; Additional data October 2023/Annex 1.

[27]AllergenOnline database ver 22, 25 May 2023.

[28]Additional data August 2022.

Allergic reactions to *Cucumis melo* have been reported (Cuesta-Herranz et al., 2003; Hassan & Venkatesh, 2015; Neeharika & Sunkar, 2021).

[redacted], [redacted] and [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]), are used as raw materials. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and the 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 a risk of allergic reactions upon dietary exposure to this food enzyme, particularly in individuals sensitised to melon, cannot be excluded, but it would not exceed the risk from consumption of this food.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

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

TABLE 2 Intended uses and use levels of the food enzyme as provided by the applicant.[30]

Food manufacturing process[a]	Raw material (RM)	Use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
• Production of baked products	Flour	**5.3**
Processing of eggs and egg products	Eggs	**5.3**
Processing of dairy products		
• Production of flavouring preparations from dairy products	Cheese, cream, butter, etc.	**5.3**
• Production of modified milk proteins	Milk proteins	**26.4**
Processing of meat and fish products		
• Production of modified meat and fish products	Raw meat and fish	**5.3**
• Production of protein hydrolysates from meat and fish proteins	Meat, fish, egg, etc.	**26.4**
Processing of plant- and fungal-derived products		
• Production of plant-based analogues of milk and milk products	Cereals, pulses, legumes, oil seeds, nuts	**10.6**
• Production of protein hydrolysates from plants and fungi	Soybean, pea and cereal, etc.	**26.4**
Processing of yeast and yeast products	Dried yeast	**5.3**

[a]The name has been harmonised by EFSA according to the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The numbers in bold represent the maximum use levels and were used for calculation.

In the production of baked products, the food enzyme is added to flour during the preparation of dough.[31] The hydrolysis by oryzin partially degrades the gluten network, modifying the rheological properties of the dough.[32] The food enzyme remains in the dough.

In the processing of eggs and egg products, after the breaking of the eggs, the food enzyme is added to treat the whole egg, or egg white or yolk.[33] The hydrolysis by oryzin enhances the sensory properties of the final products.[34] The food enzyme–TOS remains in these enzyme-modified egg products, which are ingredients used in a variety of final foods (e.g. prepared foods, mayonnaise, dressings, sauces and pastries).

[29]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.

[30]Spontaneous additional data December 2023.

[31]Technical dossier/2nd submission/p. 45.

[32]Technical dossier/2nd submission/p. 72.

[33]Technical dossier/2nd submission/p. 47.

[34]Technical dossier/2nd submission/p. 75.

In the production of flavouring preparations from dairy products, the food enzyme is added to curd (together with lipases) to hydrolyse proteins during the incubation step,[35] giving a savoury flavour to the resulting enzyme-modified dairy ingredients (EMDI).[36] The food enzyme–TOS remains in the EMDI, which is an ingredient of a variety of final foods (e.g. processed cheese, soups, snacks, dressings and sauces).[37]

In the production of modified meat and fish products, the food enzyme is added to the broth to obtain meat and fish extracts.[38] The action of oryzin reduces viscosity and enhances the flavour of these extracts.[39] The food enzyme–TOS remains in the final foods.

In the production of protein hydrolysates, the food enzyme is added to a variety of protein-rich materials from plants and animals (e.g. whey protein, caseins, collagen, corn protein and soybean protein) during hydrolysis.[40] This improves the yield. The food enzyme–TOS remains in the final protein hydrolysates, which are used as ingredients in a variety of final foods,[41] excluding infant formulae, but including follow-on formulae and food for special medical purposes.[42]

In the production of plant-based analogues of milk and milk products, the food enzyme is added to plant materials (e.g. cereals, pulses, legumes and nuts) to enrich the flavour of the final foods.[43] The food enzyme–TOS remains in the final foods (e.g. plant-based beverages and their fermented products).

In the processing of yeast and yeast products, the food enzyme is added to the yeast culture during the lysis step or directly to yeast extract.[44] The enzyme is used to enrich the savoury taste of the yeast products that are used (in paste or powder form) as an ingredient in a wide range of foods (e.g. filled pastas, sausages, soups and dressings). The food enzyme–TOS remains in the yeast products.

Based on data provided on thermostability (see Section 3.3.1) and the downstream processing step applied in the food manufacturing processes, it is expected that the enzyme is inactivated in all of the food manufacturing processes listed in Table 2.

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 the 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 the 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.100 mg TOS/kg bw per day in toddlers at the 95th percentile.

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

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.007–0.040 (12)	0.014–0.055 (15)	0.020–0.051 (19)	0.009–0.029 (21)	0.010–0.020 (22)	0.008–0.016 (23)
Min–max 95th percentile (number of surveys)	0.022–0.077 (11)	0.040–0.100 (14)	0.043–0.094 (19)	0.020–0.060 (20)	0.021–0.040 (22)	0.017–0.030 (22)

[35]Technical dossier/2nd submission/p. 46.
[36]Technical dossier/2nd submission/p. 73.
[37]Additional data August 2022/Answer 16.
[38]Technical dossier/2nd submission/p. 48.
[39]Technical dossier/2nd submission/p. 76.
[40]Technical dossier/2nd submission/p. 49.
[41]Additional data August 2022/Answers 14, 17 and 18.
[42]Spontaneous additional data December 2023.
[43]Additional data August 2022/Answer 15.
[44]Technical dossier/2nd submission/p. 50.

3.5.3 | Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 4.

TABLE 4 Qualitative evaluation of the influence of uncertainties on the refined dietary exposure estimate.

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Exposure to food enzyme–TOS was calculated based on the actual use level	+/–
Selection of broad FoodEx categories for the exposure assessment	+
In the absence of analytical data to demonstrate the removal of the food enzyme–TOS in follow-on formulae and foods for special medical purposes,[a] these highly regulated formulae were included in the calculation.	+/–
For yeast processing, although only yeast extract is produced,[b] the food categories chosen for calculation cover also those containing mannoproteins resulting from the treatment of yeast cell walls.	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

Note: +: uncertainty with potential to cause overestimation of exposure. –: uncertainty with potential to cause underestimation of exposure.

[a]Additional data August 2022/p. 13.

[b]Additional data August 2022/Answer 12.

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 (1862 mg TOS/kg bw per day) identified from the 90-day rat study with the exposure estimates of 0.007–0.055 mg TOS/kg bw per day at the mean and from 0.017 to 0.100 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 18,620.

4 | CONCLUSIONS

Based on the data provided and the derived MoE, the Panel concluded that the food enzyme oryzin produced with the non-genetically modified *Aspergillus ochraceus* strain AE-P does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of Oryzin (Protease) from *Aspergillus melleus* AE-P. January 2015. Submitted by Amano Enzyme Inc. The dossier was updated in September 2015.

Additional information. August 2022. Submitted by Amano Enzyme Inc.

Spontaneous data. December 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
EMDI	enzyme-modified dairy ingredients
IUBMB	International Union of Biochemistry and Molecular Biology
LoQ	limit of quantification
MoE	margin of exposure

OECD	Organisation for Economic Co-operation and Development
TOS	total organic solids
WHO	World Health Organization

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.

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European Commission

QUESTION NUMBER

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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 up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia,* Serbia,* Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Republic of North Macedonia,* Serbia,* Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina,* Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro,* Netherlands, Portugal, Romania, Serbia,* Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina,* Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro,* Netherlands, Portugal, Romania, Serbia,* Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro,* Netherlands, Portugal, Romania, Serbia,* Slovenia, Spain, Sweden

*Consumption data from these pre-accession countries are not reported in Table 3 of this opinion; however, they are included in Appendix 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).