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Safety evaluation of the food enzyme ribonuclease P from the non-genetically modified *Penicillium citrinum* strain AE-RP-4

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Abstract

The food enzyme ribonuclease P (EC 3.1.26.5) is produced with the non-genetically modified *Penicillium citrinum* strain AE-RP-4 by Amano Enzyme Inc. It is intended to be used in yeast processing only for the production of yeast extract. Dietary exposure to the food enzyme total organic solids (TOS) was estimated to be up to 0.153 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not raise safety concerns. 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 (NOAEL) of 134.7 mg TOS/kg bw per day, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 880. 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, under the intended conditions of use, 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: food enzyme, ribonuclease P, EC 3.1.26.5, RNase P, *Penicillium citrinum*

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1. Introduction

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

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

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

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

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 requests the European Food Safety Authority to carry out the safety assessments of the food enzymes Ribonuclease P from *Penicillium citrinum* (strain AE-RP), Glutaminase

[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.

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.

1.2. 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 ribonuclease P from the *P. citrinum* (strain AE-RP).

Multiple strains were coded under the same name 'AE-RP' in the initially submitted dossier. The applicant clarified that strain AE-RP-4 is the production strain of the food enzyme under this assessment.[4]

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme ribonuclease P from the *P. citrinum* (strain AE-PR). The dossier was updated on 30 January 2015.

Additional information was requested from the applicant during the assessment process on 29 September 2021 and 12 April 2022 and received on 28 February 2022 and on 28 March 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, 2009a) 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	Ribonuclease P
Systematic name	–
Synonyms	RNase P
IUBMB No	EC.3.1.26.5
CAS No	71427-00-4
EINECS No	NA

Ribonucleases P catalyse the end-processing of the tRNA precursor by a single endonucleolytic cleavage that removes the leader sequence from the 5′ side of the tRNA. The enzyme under assessment is intended to be used in yeast processing.

3.1. Source of the food enzyme

The ribonuclease P is produced with the non-genetically modified filamentous fungus *P. citrinum* strain AE-RP-4, which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan) with the deposit number [redacted].[5] The production strain AE-RP-4 was identified as *P. citrinum* by [redacted].[6]

[4] Additional data August 2022/Answer to point 1; Additional data March 2023.
[5] Additional data August 2022/Annex 2.
[6] Technical dossier/pp. 30–31; Additional data February 2022/Annex 3.

The parental strain was obtained [redacted]. The production strain was obtained from the parental strain [redacted].[7]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[9]

The production strain is grown as a pure culture using a typical industrial medium in a submerged (batch or fed-batch) or solid state fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration, leaving a 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.[10] 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.[11]

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 ribonuclease P is a single polypeptide chain of [redacted] amino acids.[12] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[12] The food enzyme was analysed by size exclusion chromatography. The chromatograms of the three food enzyme batches for commercialisation showed a consistent pattern containing one major peak accompanied by some minor peaks.[13] Protease activity was reported.[14]

The in-house determination of ribonuclease P activity is based on hydrolysis of adenosine 3′-monophosphate (reaction conditions: pH 5.0, 70°C, 35 min). The enzymatic activity is determined using a colourimetric assay to measure the release of phosphate spectrophotometrically at 750 nm. The enzyme activity is expressed in units (U)/g. One U is defined as the quantity of enzyme that liberates 1 μmol of phosphoric acid per minute under the conditions of the assay.[15]

The food enzyme has a temperature optimum around 70°C (pH 5.0) and a pH optimum around pH 5.0 (50°C). Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 5.0). The ribonuclease P activity decreased above 70°C, showing no residual activity above 80°C.[16]

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 tests (Table 1).[17] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 36.7% and the mean enzyme activity/TOS ratio was 52.0 U/mg TOS.

[7] Technical dossier/p. 30; Additional data/Annex 1.
[8] 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.
[9] Technical dossier/Annex 4.1, Annex 4.2.
[10] Technical dossier/pp. 33–40, Annex 5.
[11] Additional data August 2022.
[12] Technical dossier/p. 25; Additional data August 2022.
[13] Technical dossier/p. 23.
[14] Technical dossier/p. 27.
[15] Technical dossier/Annex 2.
[16] Technical dossier/pp. 28–29.
[17] Technical dossier/p. 23, p. 50, Annex 3.1, Annex 8, Annex 10.1; Additional data August 2022; Additional data March 2023.

Table 1: Composition of the food enzyme preparation

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Ribonuclease P activity	U/g[(b)]	18,600	19,400	19,300	20,400
Protein	%	36.5	36.1	37.6	44.9
Ash	%	11.7	12.0	12.0	11.0
Water	%	7.0	6.0	6.2	6.0
[redacted] **(excipient)**	%	44.8	45.9	44.2	38.1
Total organic solids (TOS)[(c)]	%	36.5	36.1	37.6	44.9
Activity/TOS	U/mg TOS	51.0	53.7	51.3	45.4

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

3.3.3. Purity

The lead content in the three commercial batches was below 5 mg/kg,[18] 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, arsenic was detected in the commercial batches at an average concentration of 0.32 mg/kg, cadmium at 0.07 mg/kg and mercury at 0.02 mg/kg.[19,20] The Panel considered these concentrations as not of concern.

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[21] No antimicrobial activity was detected in any of the tested batches.[21]

Strains of *Penicillium*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins, deoxynivalenol, HT-2 toxin, T-2 toxin, zearalenone, ochratoxin A and sterigmatocystin was examined in the three food enzyme batches and all were below the limits of quantification (LoQs) of the applied analytical methods.[22] *P. citrinum* is known to produce the toxin citrinin (Park et al., 2008), which was not included in the analysis of secondary metabolites. However, 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

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted]. A positive control was included.[23]

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* micronucleus test and a repeated dose 90-day oral toxicity study in rats, were provided. The batch 4 (Table 1) used in these studies had a lower activity/TOS value than the batches used for commercialisation, and was considered suitable as a test item.

[18] Technical dossier/p. 24/Annex 1, Annex 3.1, Annex 8, Annex 10.1; Additional data August 2022/Annex 5.
[19] Technical dossier/Annex 1, Annex 3.1; Additional data August 2022.
[20] LoQs: Pb = 0.05 mg/kg; As = 0.002 mg/kg; Cd and Hg = 0.001 mg/kg each.
[21] Technical dossier/Annex 1, Annex 3.1.
[22] LoQs: aflatoxins (B1, B2, G1 and G2) = 0.2 μg/kg each; DON = 20 μg/kg; HT-2 toxin = 10 μg/kg; T-2 toxin = 10 μg/kg; ZON = 10 μg/kg; ochratoxin A = 0.5 μg/kg; sterigmatocystin = 10 μg/kg.
[23] Additional data February 2022/Annex 4.

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, 2020) and following Good Laboratory Practice (GLP).

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used with or without metabolic activation (S9-mix), applying the pre-incubation method. A range finding test in triplicate and two main experiments in duplicate were performed. In the range finding test, carried out at five concentrations from 19.5 to 5,000 μg TOS/plate, precipitates were observed at ≥ 1,250 μg TOS/plate both with and without metabolic activation. Based on these results, the main experiments were carried out with five concentrations of the food enzyme: 78.1, 156, 313, 625 and 1,250 μg TOS/plate.

No cytotoxicity was observed at any concentration of the test substance in any strain with and without S9-mix. 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 ribonuclease P did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* micronucleus test

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2016) and following GLP.

A single experiment was performed with duplicate cultures of TK cell line with and without metabolic activation (S9-mix). Based on the results of a dose finding test, the cells were exposed to the food enzyme and scored for the presence of micronuclei at 1,250, 1,500 and 1,750 μg TOS/mL in a short-term treatment (4 h treatment followed by 20 h recovery period) with S9-mix, at 1,750, 2,000 and 2,250 μg TOS/mL in a short-term treatment without S9-mix and at 800, 900 and 1,000 μg TOS/mL in a long-term treatment (24 h continuous treatment) without S9-mix.

Cytotoxicity, evaluated as relative population doubling (RPD), was observed at the highest concentrations tested: 48%, 46% and 47% in the short-term treatment without S9-mix, with S9-mix and in the long-term treatment, respectively. The frequency of micronucleated cells was not statistically significantly different from the negative controls at any concentrations tested.

The Panel concluded that the food enzyme ribonuclease P did not induce an increase in the frequency of micronucleated cells under the test conditions employed in this study.

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 2018) and following GLP. Groups of 10 male and 10 female Sprague–Dawley (Crj:CD(SD9)) rats received by gavage the food enzyme in doses of 300, 1,000 or 3,000 mg/kg body weight (bw) per day corresponding to 134.7, 449 or 1,347 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

In the functional observations, a statistically significant increase in the grip strength of the hind limb (+25%) in mid-dose females and a statistically significant increase in the motor activity at the 0–10 min interval (+25%) in low-dose females were observed. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship and the change was seen sporadically (motor activity).

Haematological investigations revealed a statistically significant increase in red blood cell count (+7%) and a statistically significant decrease in absolute monocyte count (−65%) in low-dose males. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex, there was no dose–response relationship, the changes were small (both parameters) and there were no changes in other relevant parameters (for monocytes in white blood cells).

Clinical chemistry investigations revealed statistically significant increases in total cholesterol (+37%), phospholipid (+21%) and HDL (+21%) in low-dose females. The Panel considered the changes as not toxicologically relevant as they were only observed in one sex (all parameters) and there was no dose–response relationship (all parameters).

Urinalysis revealed a statistically significant increase in urine volume (+61%) in mid-dose females. The Panel considered the change as not toxicologically relevant as it was only observed in one sex and there was no dose–response relationship.

Statistically significant changes in organ weights were limited to an increase in the absolute salivary gland weight (+13%) in mid-dose females. The Panel considered the change as not toxicologically relevant as was only observed in one sex and there was no dose–response relationship.

The microscopic examination revealed minimal hyperplasia of the squamous cells in the limiting ridge of the stomach in mid- and high-dose males (3/10 and 6/10, respectively vs. 0/10 in the control group) and in high-dose females (3/10 vs. 0/10 in the control group). The authors suggested that this change was caused by protease activity present in the food enzyme based on their previous studies with protease, which have shown similar histopathological changes in the stomach. The Panel considered the explanation as plausible.

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

The Panel identified a no observed adverse effect level (NOAEL) of 134.7 mg TOS/kg bw per day based on the squamous cell hyperplasia in the limiting ridge of the stomach.

3.4.3. Allergenicity

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

The potential allergenicity of the ribonuclease P produced with the non-genetically modified *P. citrinum* strain AE-RP-4 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.[24]

No information is available on oral and respiratory sensitisation or elicitation reactions of this ribonuclease P. No information on allergy related to ribonucleases P has been reported in the literature.[11]

[redacted], [redacted], [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[25]), are used as raw materials. In addition, [redacted], a known allergen, is also 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, 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 in yeast processing only for the production of yeast extract at the recommended use levels of 10–1,000 mg TOS/kg yeast.[26] The food enzyme under application is not used to treat the yeast cell wall or autolyzed yeast.[27]

[24] Additional data August 2022/Annex 6.
[25] 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.
[26] Technical dossier/p. 44.
[27] Additional data August 2022/Answer to point 12 and 14.

The food enzyme is added to yeast during the lysis step in the production of yeast extract.[28] The ribonuclease P hydrolyses ribonucleic acid (RNA), releasing nucleotides that enhance the umami taste.[29] Yeast extract (liquid or powder form) is an ingredient found in a wide range of food products, used in concentrations ranging between 0.001% and 5%.[30] The food enzyme–TOS remains in yeast extracts.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the ribonuclease P is inactivated during the yeast processing.

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, 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 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 41 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 0.153 mg TOS/kg bw per day in toddlers at the 95th percentile.

Table 2: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0–0.022 (11)	0.003–0.032 (15)	0.002–0.035 (19)	0.001–0.018 (21)	0.001–0.009 (22)	0–0.009 (22)
Min–max 95th (number of surveys)	0.002–0.040 (9)	0.005–0.153 (13)	0.007–0.135 (19)	0.003–0.071 (20)	0.002–0.046 (22)	0.001–0.032 (21)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 3.

[28] Technical dossier/p. 60; Additional data August 2022/Answer to point 12.
[29] Technical dossier/pp. 58–59.
[30] Additional data August 2022/Answer to point 12 and 13.

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	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Food categories chosen for calculation are broader than yeast extract. Also those relevant to yeast autolysates and yeast cell wall were included.	+
Use of recipe fractions in disaggregation FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

TOS: total organic solids.
+: uncertainty with potential to cause overestimation of exposure.
–: uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (134.7 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.035 mg TOS/kg bw per day at the mean and from 0.001–0.153 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 880.

4. Conclusion

Based on the data provided, and the derived margin of exposure, the Panel concluded that the food enzyme ribonuclease P produced with the non-genetically modified *P. citrinum* strain AE-RP-4 does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Application for authorisation of Ribonuclease P from *Penicillium citrinum* AE-RP. January 2015. Submitted by Amano Enzyme Inc.

Additional information August 2022. Submitted by Amano Enzyme Inc.

Additional data March 2023. Submitted by Amano Enzyme Inc.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoQ	limit of quantification
MoE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.8153#support-information-section).

The file contains two sheets, corresponding to two tables.

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

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

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 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
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).