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SCIENTIFIC OPINION

Safety evaluation of the food enzyme triacylglycerol lipase from the non-genetically modified *Penicillium caseifulvum* strain AE-LRF

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) is produced with the non-genetically modified *Penicillium caseifulvum* strain AE-LRF by Amano Enzyme Inc. The food enzyme was free from viable cells of the production organism. It is intended to be used in four food manufacturing processes. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.013 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 69 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 5308. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. However, the Panel noted that traces of [redacted], used in the manufacture of the triacylglycerol lipase, may be found in the food enzyme. The Panel considered that the risk of allergic reactions upon dietary exposure could not be excluded, particularly in individuals sensitised to fish. 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

EC 3.1.1.3, EFSA-Q-2014-00545, food enzyme, non-genetically modified microorganism, *Penicillium caseifulvum*, *Penicillium roqueforti*, triacylglycerol acylhydrolase, triacylglycerol lipase

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1 | INTRODUCTION

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

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

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008[1] on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

All food enzymes currently on the 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 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.

Two applications have been introduced by the company Amano Enzyme Inc. for the authorisation of the food enzymes alpha-amylase from *Microbacterium imperial* strain AE-AMT and triacylglycerol lipase from *Penicillium roqueforti* strain AE-LRF.

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

1.1.2 | Terms of Reference

The European Commission (EC) requests the European Food Safety Authority (EFSA) to carry out the safety assessments on the following food enzymes alpha-amylase from *Microbacterium imperial* strain AE-AMT and triacylglycerol lipase from *Penicillium roqueforti* strain AE-LRF in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] 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 triacylglycerol lipase from a non-genetically modified *Penicillium roqueforti* strain AE-LRF.

[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.

Recent data identified the production microorganism as *Penicillium caseifulvum* (Section 3.1). Therefore, this name will be used in this opinion instead of *Penicillium roqueforti*.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme triacylglycerol lipase from a non-genetically modified *Penicillium roqueforti* strain AE-LRF.

Additional information was requested from the applicant during the assessment process on 8 October 2020 and received on 25 April 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 EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance 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[4]

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase; triglyceride lipase; glycerol ester hydrolase
IUBMB no	EC 3.1.1.3
CAS no	9001-62-1
EINECS no	232-619-9

Triacylglycerol lipases catalyse, in the presence of water, the hydrolysis of the ester linkages in triacylglycerols, resulting in the generation of glycerols, fatty acids, diacylglycerols and monoacylglycerols. At very low concentrations of water, interesterification, i.e. the exchange of free fatty acids between two or more triacylglycerols, may occur.

The food enzyme under assessment is intended to be used in four 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 dairy products for the production of flavouring preparations; (3) processing of plant- and fungal-derived products for the production of plant-based analogues of milk and milk products and (4) processing of fats and oils for the production of modified fats and oils by interesterification.[5]

3.1 | Source of the food enzyme[6]

The triacylglycerol lipase is produced with the non-genetically modified filamentous fungus *Penicillium caseifulvum* (notified as *Penicillium roqueforti*) strain AE-LRF, which is deposited [redacted], with the deposit number [redacted].[7]

The production strain AE-LRF was obtained [redacted].[8] The production strain was identified as *Penicillium caseifulvum* [redacted].[9]

[4]Technical dossier/p. 6, 22, 58.
[5]Technical dossier/Additional data, 25 April 2023.
[6]Technical dossier/p. 28–31, 58.
[7]Technical dossier/Additional data, 25 April 2023/Annex 2.
[8]Technical dossier/Additional data, 25 April 2023.
[9]Technical dossier/Additional data, 25 April 2023/Annex 3–1; Annex 3–2.

3.2 | Production of the food enzyme[10]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[11] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[12]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including ultrafiltration in which enzyme protein is retained while most of the low molecular mass material passes the filtration membrane and is discarded.[13] 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.[14]

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[15]

The triacylglycerol lipase is a single polypeptide chain of [redacted] amino acids.[16] The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[17] The food enzyme was analysed by size exclusion chromatography. A consistent protein profile was observed across all batches.[18] No other enzyme activities were reported.[19]

The in-house determination of triacylglycerol lipase activity is based on the titration of fatty acids released by the hydrolysis of acylglycerols present in olive oil (reaction conditions: [redacted]). The enzyme activity is expressed in Unit/g or mL. One Unit is defined as the quantity of enzyme that will liberate 1 µmol of fatty acids per minute under the conditions of the assay.[20]

The food enzyme has a temperature optimum around 40°C (pH 7.0) and a pH optimum between pH 6 and 7 (30°C).[21] Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 7.0). The enzyme activity decreased above 30°C, showing no residual activity after pre-incubation above 45°C.[22]

3.3.2 | Chemical parameters[23]

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialization, of which one (Batch 1) was used for the toxicological tests (Table 1).[24] The mean total organic solids (TOS) of the three food enzyme batches was 2.5% and the mean enzyme activity/TOS ratio was 839 U/mg TOS.

[10]Technical dossier/p. 10–11, 31–38; Technical dossier/Annex 5; Annex 6.
[11]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.
[12]Technical dossier/p. 31–32; Technical dossier/Annex 4.
[13]Technical dossier/p. 31–38; Technical dossier/Annex 5.
[14]Technical dossier/Annex 6; Technical dossier/Additional data, 25 April 2023.
[15]Technical dossier/p. 10, 23, 25.
[16]Technical dossier/p. 25; Technical dossier/Additional data, 25 April 2023/Annex 8.
[17]Technical dossier/p. 25.
[18]Technical dossier/p. 23.
[19]Technical dossier/p. 26.
[20]Technical dossier/Annex 2.
[21]Technical dossier/p. 10, 26–27.
[22]Technical dossier/p. 27.
[23]Technical dossier/p. 23; Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 25 April 2023.
[24]Technical dossier/p. 23, 51; Technical dossier/Annex 3.

TABLE 1 Compositional data of the food enzyme preparation.[25]

Parameters	Unit	Batches		
		1[a]	2	3
Lipase activity	U/g[b]	16,500	27,200	20,200
Protein	%	2.2	2.6	2.8
Ash	%	0.3	0.3	0.3
Water	%	5.5	3.7	3.9
[redacted] (excipient)	%	92.0	93.4	93.0
Total organic solids (TOS)[c]	%	2.2	2.6	2.8
Activity/TOS ratio	U/mg TOS	750	1046	721

[a]Batch used for the toxicological studies.

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

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

3.3.3 | Purity[26]

The lead content[27] in the three commercial batches was below 5 mg/kg which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme preparation complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*,[28] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of the tested batches.[29]

Strains of *Penicillium*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The presence of ochratoxin A, citrinin, cyclopiazonic acid, ochratoxin B, mycophenolic acid, penicilic acid and patulin[30] was examined in the three food enzyme batches, and all were below the limits of quantification (LoQs) of the applied methods.[31] 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[32]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted]. In two samples, colonies were produced which were confirmed to be different from the production strain based on [redacted]. A positive control was included.

3.4 | Toxicological data[33]

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

Batch 1 (Table 1) is one of the food enzyme preparations intended for commercialisation and was considered acceptable as a test item.

[25]Technical dossier/Additional data, 25 April 2023.

[26]Technical dossier/p. 8–9, 24, 51, 58; Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 25 April 2023/Annex 1.

[27]Technical dossier/p. 9, 24, 51; Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 25 April 2023/Annex 1.

[28]Technical dossier/Additional data, 25 April 2023/Annex 1; Technical dossier/p. 9, 24, 51; Technical dossier/Annex 1; Annex 3.

[29]Technical dossier/Additional data, 25 April 2023/Annex 1; Technical dossier/p. 9, 24; Technical dossier/Annex 1; Annex 3.

[30]Technical dossier/p. 9, 24; Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 25 April 2023/Annex 1.

[31]Technical dossier/Additional data, 25 April 2023/Annex 1: LoQ: ochratoxin A = 0.005mg/kg; citrinin = 0.05 mg/kg, cyclopiazonic acid = 0.05 mg/kg, ochratoxin B = 0.005 mg/kg, mycophenolic acid = 0.05 mg/kg, penicilic acid = 0.2 mg/kg; patulin = 0.05 mg/kg.

[32]Technical dossier/Additional data, 25 April 2023/Annex 4.

[33]Technical dossier/Additional data, 25 April 2023.

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).[34] 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.

A range-finding experiment was carried out in duplicate, using five concentrations of the food enzyme ranging from 117 to 30,000 µg/plate, corresponding to 2.7 to 690 µg TOS/plate. No cytotoxicity was observed at any concentration of the test substance.

Two main experiments were carried out in triplicate, using five concentrations of the food enzyme ranging from 1875 to 30,000 µg/plate, corresponding to 43.1, 86.3, 172.5, 345 and 690 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 triacylglycerol lipase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2 | *In vitro mammalian cell micronucleus test*

The in vitro mammalian cell micronucleus test was carried out according to OECD Test Guideline 487 (OECD, 2016) and following GLP.[35] An experiment was performed with duplicate cultures of human lymphoblastoid TK6 cells. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

In the range-finding test, cells were exposed to the food enzyme at ten concentrations from 1.35 to 690 µg TOS/mL in a short-term treatment (4 h exposure and 20 h recovery period) either with or without S9-mix, and in a long-term treatment (24 h exposure without recovery period) without S9-mix. No cytotoxicity (cell growth inhibition) above 50% was seen at any concentration tested up to 690 µg TOS/mL in the short-term treatment with S9-mix. Cytotoxicity of 50% or more was seen at 690 µg TOS/mL in the short-term treatment without S9-mix, and at 345 µg TOS/mL and above in the long-term treatment without S9-mix. The 50% cell-growth inhibition concentration (IC_{50}) was 438 µg TOS/mL in the short-term treatment without S9-mix and 320 µg TOS/mL in the long-term treatment, respectively.

Based on these results, in the main experiment cells were exposed to the food enzyme and scored for the frequency of cells with micronuclei at concentrations of 350, 400, 450, 500 and 550 µg TOS/mL in a short-term treatment without S9-mix, at concentrations of 173, 345 and 690 µg TOS/mL in a short-term treatment with S9-mix and at concentrations of 300, 350 and 400 µg TOS/mL in a long-term treatment without S9-mix.

Cytotoxicity (cell-growth inhibition) was seen at 500 and 550 µg TOS/mL in the short-term treatment without S9-mix (52% and 57%, respectively) and at 400 µg TOS/mL in the long-term treatment without S9-mix (56%). The frequency of cells with micronuclei was not statistically significantly different to the negative controls at any concentrations tested.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce an increase in the frequency of cells with micronuclei 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 was performed under GLP and according to the OECD Test Guideline 408 (OECD, 2018).[36]

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 300, 1000 and 3000 mg/kg body weight (bw) per day, corresponding to 6.9, 23 and 69 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

The body weight was statistically significantly increased on days 28, 63, 77, 84 and 91 of administration in mid-dose males (+6%, +8%, +9%, +10% and +10%, respectively). The body weight gain was statistically significantly increased throughout the dosing period of administration in mid-dose males (+16%). The Panel considered the changes as not toxicologically relevant, as they were only recorded at single time intervals (body weight), they were only observed in one sex (both parameters), there was no dose–response relationship (both parameters) and the changes were without a statistically significant effect on the final body weight.

The feed consumption was statistically significantly increased on days 63 and 77 of administration in mid-dose males (+11% and +11%, respectively) and decreased on days 21, 35, 70 and 77 in high-dose females (–10%, –9%, –9% and –9%, respectively). The Panel considered the change as not toxicologically relevant, as it was only recorded at single time intervals,

[34]Technical dossier/Additional data, 25 April 2023/Annex 6.

[35]Technical dossier/Additional data, 25 April 2023/Annex 7.

[36]Technical dossier/Additional data, 25 April 2023/Annex 5.

it was only observed in one sex, there was no consistency between the change in males and females, there was no dose–response relationship (males) and there was no statistically significant change in the final feed consumption and body weight.

In the functional observations, a statistically significant increase in the grip strength of hindlimbs was observed in mid- and high-dose males (+25% and +26%, respectively) and in high-dose females (+26%). The Panel considered the change as not toxicologically relevant, as there were no changes in other relevant parameters (grip strength of forelimbs, increased muscle tonus in the detailed clinical observations or functional tests).

The clinical chemistry investigation revealed a statistically significant decrease in γ-glutamyl transpeptidase (γ-GTP) in high-dose males (0 IU/L vs. 1 IU/L in the control) and a decrease in total cholesterol (–19%), phospholipids (–16%) and high-density lipoprotein cholesterol (HDL-cholesterol; –14%) in high-dose males. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (all parameters), there were no changes in other relevant parameters (other liver enzymes), there were no histopathological changes in the liver and the changes were within the historical control values (total cholesterol, phospholipids, HDL-cholesterol).

Statistically significant changes in organ weights detected were an increase in absolute brain weight (+4%) in high-dose males, an increase in the absolute heart weight in mid-dose males (+9%), an increase in the absolute lung weight in mid-dose males (+9%) and a decrease in the relative adrenal gland weight in mid-dose males (–9%). 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 (the absolute heart and lung weights, the relative adrenal gland weight), the changes were small (all parameters) and there were no histopathological changes in brain, heart, lungs and adrenal glands.

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

The Panel identified a no observed adverse effect level (NOAEL) of 69 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity[37]

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

The potential allergenicity of the triacylglycerol lipase produced with the *Penicillium caseifulvum* strain AE-LRF 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.[38]

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

Respiratory allergy following occupational inhalation of triacylglycerol lipase has been reported (Brant et al., 2004; Elms et al., 2003; Martel et al., 2010). *Penicillium* species are known to cause respiratory allergy (Kurup et al., 2000). Several studies have shown that adults respiratorily sensitised may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Cullinan et al., 1997; Poulsen, 2004). Information on adverse reactions upon ingestion of triacylglycerol lipase in individuals sensitised through the respiratory route has not been reported.

[redacted][39] all known sources of allergens, are 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.

[redacted], that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[40]) is used as a production aid in the downstream processing of the food enzyme. Traces of [redacted] could potentially be 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 fish, cannot be excluded.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

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

[37]Technical dossier/p. 13, 52; Technical dossier/Additional data, 25 April 2023/Annex 8; Annex 9.

[38]Technical dossier/p. 13, 52; Technical dossier/Additional data, 25 April 2023/Annex 8; Annex 9.

[39]Technical dossier/Annex 6; Technical dossier/Additional data, 25 April 2023.

[40]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 (Text with EEA relevance). OJ L 304, 22.11.2011. pp. 18–63.

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[41]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[b]
Processing of cereals and other grains		
• Production of baked products	Flour	**0.025**
Processing of dairy products		
• Production of flavouring preparations from dairy products	Dairy materials such as milk, cream and butter	**1.0**
Processing of plant- and fungal-derived products		
• Production of plant-based analogues of milk and milk products	Cereals, legumes, oilseeds, nuts, etc.	**15.2**
Processing of fats and oils		
• Production of modified fats and oils by interesterification	Edible vegetable oils or edible vegetable oil fractions, free fatty acids made from edible vegetable oil	**5.8**

Abbreviation: TOS, total organic solids.

[a]The names have been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in enzyme exposure assessment' (EFSA CEP Panel, 2023).

[b]The numbers in bold were used for calculation.

In the production of baked products, the food enzyme is added to flour during the preparation of dough or batter.[42] The triacylglycerol lipase hydrolyses fats and oils in flour, which improves gas retention and the dough structure. The food enzyme–TOS remain in the bakery products.

To produce flavouring preparation from dairy products, the food enzyme is added to a variety of dairy ingredients such as cheese or cream.[43] The triacylglycerol lipase releases free fatty acids, which contribute to the intensified flavour of enzyme modified dairy ingredients (EMDI) products. The food enzyme–TOS remain in the EMDI.

In the production of plant-based analogues of milk and milk products, the food enzyme is added to the slurry of plant materials to hydrolyse fats.[44] The hydrolysis increases the amount of medium-chain fatty acids and can improve taste. The food enzyme–TOS remain in these plant-based analogues.

In the production of modified fats and oils by interesterification, vegetable oils are treated with the immobilised or non-immobilised food enzyme.[45] Under microaqueous environments, this triacylglycerol lipase catalyses the exchange of fatty acids at the 1- and 3-position of the triglycerides, modifying the properties of the resulting triglycerides (e.g. 2-palmitic acid enriched vegetable oils). The modified fats are further incorporated into many foods as ingredients, e.g. infant formulae, croissants, doughnuts, biscuits, crackers. No information or analytical data was provided to establish whether the food enzyme–TOS were removed in the interesterified fats/oil;[46] as a result, the Panel decided to proceed with the dietary exposure assessment by considering that the full amount of the food enzyme–TOS remain in the modified fats.

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 triacylglycerol lipase is inactivated in 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 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 one day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 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

[41]Technical dossier/Additional data, 25 April 2023/Answer 5.1.

[42]Technical dossier/p. 41.

[43]Technical dossier/p. 42; Technical dossier/Additional information, 25 April 2023/Answer 5.1.

[44]Technical dossier/p. 43–44; Technical dossier/Additional information, 25 April 2023/p. 8–9 and Answer 5.4.

[45]Technical dossier/Additional information, 25 April 2023/p. 9–10.

[46]Technical dossier/Additional information, 25 April 2023/Answer 5.3.

in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.013 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.

	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)	0–0.003 (12)	0.001–0.008 (15)	0.001–0.003 (19)	0.001–0.002 (21)	0–0.001 (22)	0–0.001 (23)
Min–max 95th percentile (number of surveys)	0–0.009 (11)	0.004–0.013 (14)	0.003–0.010 (19)	0.002–0.005 (20)	0.001–0.004 (22)	0.001–0.005 (22)

Abbreviation: 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 4.

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

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
Selection of broad FoodEx categories for the exposure assessment	+
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
Use of recipe fractions to disaggregate FoodEx categories	+/–
Use of technical factors in the exposure model	+/–

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

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 (69 mg TOS/kg bw per day) identified from the 90-day rat study with the derived exposure estimates of 0–0.008 mg TOS/kg bw per day at the mean and from 0 to 0.013 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 5308.

4 | CONCLUSION

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

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier "Application for authorisation of triacylglycerol lipase from *Penicillium roqueforti* AE-LRF in accordance Regulation (EC) No 1331/2008". 7 July 2014. Submitted by Amano Enzyme Inc.

Additional information. 25 April 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
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
EMDI	enzyme-modified dairy ingredients
FAO	Food and Agricultural Organization of the United Nations
FoodEx	a standardised food classification and description system
γ-GTP	γ-glutamyl transpeptidase
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
HDL-cholesterol	high-density lipoprotein cholesterol
IC_{50}	50% cell-growth inhibition concentration
IU	International Unit
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoQ	limit of quantification
MoE	margin of exposure
[redacted]	[redacted]
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OECD	Organisation for Economic Cooperation and Development
[redacted]	[redacted]
RM	raw material
TK6	human lymphoblastoid cells
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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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, 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).