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Safety evaluation of the food enzyme triacylglycerol lipase from the non-genetically modified *Mucor circinelloides* strain AE-LMH

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) is produced with the non-genetically modified *Mucor circinelloides* strain AE-LMH by Amano Enzyme Inc. The food enzyme is considered free from viable cells of the production organism. The food enzyme is intended to be used in baking processes, egg processing and the manufacture of enzyme-modified dairy ingredients (EMDI). Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.242 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 784 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 3,240. 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 by dietary exposure cannot be excluded, but the likelihood of such reactions 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.

Requestor: European Commission

Question number: EFSA-Q-2015-00692

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

Five applications have been introduced by the company 'Amano Enzyme Inc.' for the authorisation of the food enzymes Beta-amylase from *Bacillus flexus* (strain AE-BAF), Triacylglycerol lipase from *Mucor javanicus* (strain AE-LM), Beta-glucanase from *Cellulosimicrobium cellulans* (strain AE-TN), Laccase from *Trametes hirusta* (strain AE-OR) and Protein-glutaminase from *Chryseobacterium proteolyticum* (strain AE-PG).

Following the requirements of Article 12.1 of Commission Regulation (EU) 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 a safety assessments of the food enzymes Beta-amylase from *Bacillus flexus* (strain AE-BAF), Triacylglycerol

[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, p. 15–24.

lipase from *Mucor javanicus* (strain AE-LM), Beta-glucanase from *Cellulosimicrobium cellulans* (strain AE-TN), Laccase from *Trametes hirusta* (strain AE-OR) and Protein-glutaminase from *Chryseobacterium proteolyticum* (strain AE-PG) 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 triacylglycerol lipase from a non-genetically modified *Mucor javanicus* strain AE-LM. Following a clarification,[4] the applicant renamed the production strain AE-LM(B) as AE-LMH.

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 *Mucor javanicus* (strain AE-LM).

Additional information was requested from the applicant during the assessment process on 22 October 2021, on 11 April 2022 and on 11 October 2022, and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant existing guidance documents of the EFSA Scientific Committee.

The 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021a).

3. Assessment

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase, glycerol ester hydrolase, triacylglycerol ester hydrolase
IUBMB No	EC 3.1.1.3
CAS No	9,001-62-1
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 glycerol, free fatty acids, diacylglycerols and monoacylglycerols. The food enzyme is intended to be used in baking processes, egg processing and the manufacture of enzyme-modified dairy ingredients.

3.1. Source of the food enzyme

The food enzyme is produced with a non-genetically modified filamentous fungus *Mucor circinelloides* strain AE-LMH,[5] which is deposited at the National Institute for Technology and Evaluation (NITE) Biological Resource Center (Japan), with the deposit number [redacted].[6]

The production strain AE-LMH was obtained from the parental [redacted].[7] The production strain was identified as *M. circinelloides* [redacted]

[4] Additional data July 2022/Answer to point 1.
[5] Additional data October 2022.
[6] Technical dossier/Additional data April 2021/Annex 3/Additional data July 2022/Annex 1.
[7] Technical dossier/Additional data April 2021/Additional information April 2021.

[redacted].[8]

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[9] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current Good Manufacturing Practice.[10]

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 completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a filtrate containing the food enzyme. This filtrate 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. Finally, the food enzyme is spray-dried prior to analysis.[11] 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.[12]

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 triacylglycerol lipase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, was [redacted] kDa.[13] The food enzyme was analysed by size exclusion chromatography. The chromatograms of three food enzyme batches showed a consistent pattern with two major peaks accompanied by some minor peaks.[14] No other enzymatic activities were reported by the applicant.[15]

The in-house determination of triacylglycerol lipase activity is based on the hydrolysis of triglycerides in olive oil. The enzymatic activity is determined by the back titration of the released free fatty acids from the olive oil emulsion with hydrochloric acid in an excess of sodium hydroxide (reaction conditions: pH 7.0, 37°C, 10 min). The enzyme activity is expressed in Unit/g or Unit/mL. One unit is defined as the amount of enzyme required to liberate 1 μmol of fatty acids per min under the conditions of the assay.[16]

The food enzyme has a temperature optimum between 35°C and 40°C (pH 7.0) and a pH optimum between pH 7.0 and pH 8.0 (at 37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures at pH 7.0. The enzyme activity decreased above 30°C, showing no residual activity above 60°C.[17]

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).[18] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 37.9% and the mean enzyme activity/TOS ratio was 82.1 U/mg TOS.[19]

[8] Technical dossier/Additional data April 2021/Annex 4.
[9] 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.
[10] Technical dossier/pg. 34/Annex 4.
[11] Technical dossier/pg. 34-41/Annex 5/Additional data October 2022.
[12] Technical dossier/Annex 6/Additional data April 2021.
[13] Technical dossier/pg. 28.
[14] Technical dossier/pg. 27.
[15] Technical dossier/pg. 29.
[16] Technical dossier/pg. 29/Annex 2.
[17] Technical dossier/pg. 30–31.
[18] Technical dossier/pg. 26, 53/Annexes: 1, 3, 8, 9 and 10/Additional data April 2021/Annexes: 1 and 2.
[19] Technical dossier/Additional data April 2021/Additional data October 2022.

Table 1: Compositional data of the food enzyme preparation

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Triacylglycerol lipase activity	U/g[(b)]	31,100	35,700	26,500	2,550 (U/mL)
Protein	%	38.1	38.5	30.7	3.3
Ash	%	4.1	3.8	2.8	0.7
Water	%	4.5	4.7	5.8	95.4
Excipient (dextrin)	%	51.2	50.7	58.8	—
Total organic solids (TOS)[(c)]	%	40.2	40.8	32.6	3.9
Triacylglycerol lipase activity/TOS	U/mg TOS	77.4	87.5	81.3	65.4

(a): Batch used for toxicological studies.
(b): U: UNIT/g (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 0.1 mg/kg[20,21] 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*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[22] No antimicrobial activity was detected in any of the tested batches.[20]

Strains of *Mucor*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Hollmann et al., 2008). The presence of aflatoxins (B1, B2, G1, G2), ochratoxin A, HT2-toxin, T2-toxin, zearalenone, sterigmatocystin, fumonisins (B1 and B2) was examined in the three food enzyme preparation batches. All were below the reporting limits of the applied analytical methods.[20,23] Adverse effects caused by the potential presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme–TOS.

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

3.3.4. Viable cells of the production strain

The absence of the production strain in the food enzyme was demonstrated [redacted]. No colonies of the production strain were produced [redacted].[24]

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, was provided. The batch 4 (Table 1) used in these studies was prepared without an excipient, and has similar activity/TOS value as the batches used for commercialisation and is considered suitable as a test item.

[20] Technical dossier/Annexes: 1 and 3.
[21] Reporting limit: Pb = 0.01 mg/kg.
[22] Technical dossier/Annexes: 1_1, Annex 1_2, Annex 1_3 and 3.
[23] Reporting limits: aflatoxins (B1, B2, G1, G2) = 0.2 μg/kg each; ochratoxin A = 0.5 μg/kg; HT2-toxin, T2-toxin, zearalenone and, sterigmatocystin = 10 μg/kg each; fumonisins (B1, B2) = 5 μg/kg.
[24] Additional data April 2021/Annex 5.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[25]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the preincubation method. Two separate experiments were carried out in triplicate using five concentrations of the food enzyme (15.2, 61, 244, 975 and 3,900 μg TOS/plate).

Toxic effects, evident as a reduction in the number of revertants, occurred in TA1537 in the presence of S9-mix at 3,900 μg/plate in the first and second experiment, respectively. Upon treatment with the food enzyme, there was no biologically relevant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme 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 on Chinese hamster lung fibroblasts (CHL/IU cell line) according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[26]

A single experiment was carried out in duplicate. Based on the results of a dose-finding test, the cells were exposed to the food enzyme at 1,154, 1,731, 2,601 and 3,900 μg TOS/mL and at 45.24, 67.5, 101.4, 152 and 228 μg TOS/mL in a short-term treatment (6 h followed by 18 h recovery period) without and with metabolic activation (S9-mix) respectively The cells were treated at 1,154, 1,731, 2,601 and 3,900 μg TOS/mL in a continuous treatment (24 h) in the absence of S9-mix.

Cytotoxic effects were observed at the highest concentrations (62% cell growth inhibition at 2,500 μg/mL in the presence of S9-mix in the short-term treatment and 72% in the continuous treatment without S9-mix). The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that the food enzyme did not induce chromosome aberrations under the test conditions employed for 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 the Japanese test guideline (Notification No 29:1996 and No 655_1999) and GLP system of the JMHW, Ordinance No 21 (1997), No 114 (2008) and No 0613007 (2008). The study is in accordance with OECD Test Guideline 408 (OECD, 1998) with the following deviations: detailed clinical observations and functional observations were not performed, urea was not determined, epididymides were not weighed, and only two areas of the brain and one level of the spinal cord were examined in the microscopy. The Panel considered that these deviations were not major and do not impede the evaluation of the study.[27]

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 25, 50 or 100 vol%, corresponding to 196, 392 or 784 mg TOS/kg bw per day. Controls received the vehicle (water for injection).

No mortality was observed.

Statistically significant changes in organ weights included a decrease in the relative heart weight of high-dose males (−7%). The Panel considered the change as not toxicologically relevant as it was small, it was only observed in one sex and there were no histopathological changes in the organ.

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

The Panel identified the no observed adverse effect level (NOAEL) of 784 mg TOS/kg bw per day, the highest dose tested.

[25] Technical dossier/ Annex 8.
[26] Technical dossier/Annex 9.
[27] Technical dossier/Annexes: 10–1, 10–2 and 10–3.

3.4.3. Allergenicity

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 non-genetically modified *Mucor circinelloides* strain AE-LMH 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.[28]

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

Respiratory allergy following occupational inhalation of triacylglycerol lipase have been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma to an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Adverse reactions upon ingestion of triacylglycerol lipase in individuals sensitised through the respiratory route has not been reported. FORMTEXT FORMTEXT FORMTEXT [redacted], products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[29]), are used as raw materials. In addition, [redacted], a known source of allergens, 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 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 three food manufacturing processes. Intended uses and the recommended use levels are summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[30]

Food manufacturing process[(a)]	**Raw material (RM)**	**Recommended dosage of the food enzyme (average – maximum) (mg TOS/kg RM)[(b)]**
Baking processes	Flour	3–**18**
Egg processing	Egg white	3–**12**
Manufacture of EMDI	Various dairy materials (e.g. cheese, butter, cream)	494–**2,203**

(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.
(b): The numbers in bold were used for calculations.

[28] Technical dossier/pg. 54/Annex 11.
[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] Technical dossier/p. 46 & Additional data April 2021/Answers 9, 10 and 11 & Additional data October 2022/Answer 4.

In baking processes, the food enzyme is added to flour during the preparation of dough or batter.[31] The hydrolysis of the triacylglycerol lipase in flour reduces batter viscosity, facilitates the handling of dough, improves the dough structure, thus, contributing to an improved and consistent baking process. The food enzyme–TOS remains in the bakery products.

In egg processing, the food enzyme is added to egg white in the manufacture of egg white powder.[32] The triacylglycerol lipase hydrolyses traces of yolk lipids remaining in the egg white, ensuring its ability to form stable and voluminous foam. The food enzyme–TOS remains in the final foods.

To produce EMDI, the food enzyme is used to treat cheese, cream or other dairy products.[33] The hydrolytic action of the triacylglycerol lipase releases free fatty acids, which contribute to the intensified flavour of EMDI products. The food enzyme remains in the EMDI products.[34]

Based on data provided on thermostability (see Section 3.3.1), it is expected that the triacylglycerol lipase is inactivated by heat during all three food manufacturing processes.

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 (bw). This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 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 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 0.242 mg TOS/kg bw per day in infant at the 95th percentile.

Table 3: 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)	0.009–0.070 (11)	0.046–0.193 (15)	0.053–0.123 (19)	0.029–0.072 (21)	0.020–0.044 (22)	0.020–0.060 (22)
Min–max 95th percentile (number of surveys)	0.049–0.242 (9)	0.119–0.212 (13)	0.100–0.225 (19)	0.058–0.143 (20)	0.042–0.103 (22)	0.041–0.122 (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 4.

[31] Technical dossier/p.43.
[32] Technical dossier/p.45.
[33] Technical dossier/p.44.
[34] Additional data April 2021/Answer 11 and Annex 6.

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	
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Not only egg white was considered in the calculation, but also foods containing egg yolk and whole egg	+
Use of recipe fractions to disaggregate 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 of food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to considerable overestimation of the exposure.

3.6. Margin of exposure

A comparison of the NOAEL (784 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.009–0.193 mg TOS/kg bw per day at the mean and from 0.041 to 0.242 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 3,240.

4. Conclusion

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

5. Documentation as provided to EFSA

Dossier for triacylglycerol lipase from *Mucor javanicus* AE-LM. February 2015. Submitted by Amano Enzyme Inc.

Additional information. April 2021. Submitted by Amano Enzyme Inc.

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

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

References

Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204.

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502.

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α-amylase and hemicellulase in persons sensitized to Aspergillus fumigatus? Allergy, 52, 1997, 346–349.

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

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

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

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

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

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Glandorf B, Herman L, Aguilera J, Andryszkiewicz M, Gomes A, Kovalkovicova N, Liu Y, Rainieri S and Chesson A, 2021a. Scientific Guidance for the submission of dossiers on Food Enzymes. EFSA Journal 2021;19(10):6851, 37 pp. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Processing Aids),Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A, Rivière G, Steffensen I-L, Tlustos C, Van Loveren H, Vernis L, Zorn H, Liu Y and Chesson A, 2021b. Statement on the process-specific technical data used in exposure assessment of food enzymes. EFSA Journal 2021;19(12):7010, 37 pp. https://doi.org/10.2903/j.efsa.2021.7010

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

Elms J, Fishwick D, Walker J, Rawbone R, Jeffrey P, Griffin P, Gibson M and, Curran AD, 2003. Prevalence of sensitisation to cellulase and xylanase in bakery workers. Occupational and Environmentl Medicine 60, 802–804.

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

Hollmann M, Razzazi-Fazeli E, Grajewski J, Twaruzek M, Sulyok M and Böhm J, 2008 Sep. Detection of 3-nitropropionic acid and cytotoxicity inMucor circinelloides. Mycotoxin Research, 24, 140–150. https://doi.org/10.1007/BF03032341 PMID: 23604749

Martel C, Nielsen GD, Mari A, Licht TR and Poulsen LK, 2010. Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. EFSA Supporting Publication 2010;7(9):EN-75, 95 pp. https://doi.org/10.2903/sp.efsa.2010.en-75.

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

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

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

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423.

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
EMDI	enzyme-modified dairy ingredients
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology

JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
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.2023.7755#support-information-section).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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 one 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).