

Safety evaluation of an extension of use 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. A safety evaluation of this food enzyme was made previously, in which EFSA concluded that this food enzyme did not give rise to safety concerns when used in three food manufacturing processes. Subsequently, the applicant requested to extend its use to include two additional processes. In this assessment, EFSA updated the safety evaluation of this food enzyme when used in a total of five food manufacturing processes. The dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.845 mg TOS/kg body weight (bw) per day in European populations. When combined with the no observed adverse effect level previously reported (784 mg TOS/kg bw per day, the highest dose tested), the Panel derived a margin of exposure of at least 928. Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that this food enzyme does not give rise to safety concerns under the revised intended conditions of use.

KEYWORDS

EC 3.1.1.3, EFSA-Q-2015-00692, EFSA-Q-2023-00371, food enzyme, *Mucor circinelloides*, non-genetically modified microorganism, triacylglycerol lipase

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CONTENTS

Abstract.....	1
1. Introduction	3
1.1. Background and Terms of Reference as provided by the requestor.....	3
1.1.1. Background as provided by the European Commission.....	3
1.1.2. Terms of Reference.....	3
1.1.3. Interpretation of the Terms of Reference.....	3
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	4
2.3. Public consultation.....	4
3. Assessment.....	4
3.1. Dietary exposure.....	4
3.1.1. Revised intended use of the food enzyme	5
3.1.2. Dietary exposure estimation.....	5
3.1.3. Uncertainty analysis	6
3.2. Margin of exposure	6
4. Conclusion	6
5. Documentation as provided to EFSA	6
Abbreviations	7
Conflict of interest	7
Requestor.....	7
Question number.....	7
Copyright for non-EFSA content.....	7
Panel members.....	7
References.....	7
Appendix A.....	8
Appendix B.....	9

1 | INTRODUCTION

Article 3 of the Regulation (EC) No 1332/2008¹ provides definition for ‘food enzyme’ and ‘food enzyme preparation’.

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

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

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008² 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.

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.

Triacylglycerol lipase from a non-genetically modified strain of *Mucor javanicus* (AE-LM) is a food enzyme included in the Register of food enzymes³ to be considered for inclusion in the Union list and thus subject to a risk assessment by the European Food Safety Authority (EFSA).

On 16 December 2022, a new application has been introduced by the applicant “Amano Enzyme Inc.” for an extension of the condition of the use of the food enzyme triacylglycerol lipase from a non-genetically modified strain of *Mucor javanicus* (AE-LM).

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: triacylglycerol lipase from a non-genetically modified strain of *Mucor javanicus* (AE-LM), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.⁴

1.1.3 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of an extension of the conditions of use for the food enzyme triacylglycerol lipase from *Mucor javanicus* strain AE-LM. Following a clarification, the applicant renamed the production strain AE-LM(B) as AE-LMH.

¹Regulation (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.

²Regulation (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.

³https://food.ec.europa.eu/safety/food-improvement-agents/enzymes/eu-list-and-applications_en

⁴OJ L 354, 31.12.2008, p. 1.

The production microorganism was reclassified as *Mucor circinelloides* at the species level in the previously published scientific opinion (EFSA CEP Panel, 2023a). Therefore, EFSA continued to use the name *M. circinelloides* (instead of *M. javanicus*) also in the present opinion.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for the authorisation of the extension of use of food enzyme triacylglycerol lipase non-genetically modified *M. circinelloides* AE-LMH.

Additional information was requested from the applicant during the assessment process on 7 November 2023 and received on 21 November 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 existing guidance documents of EFSA Scientific Committee.

The current 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) and the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023b) have been followed to evaluate this application.

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002⁵ and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 4 to 25 January 2024.⁶ No comments were received.

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	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 glycerol, free fatty acids, diacylglycerols and monoacylglycerols.

All aspects concerning the safety of this food enzyme, when used in three food manufacturing processes, were evaluated in December 2022 (EFSA CEP Panel, 2023a). Following an application for use in two additional food manufacturing processes and revised use levels for those previously evaluated, EFSA revised the exposure assessment and updated the safety evaluation of this food enzyme when used in five food manufacturing processes.

3.1 | Dietary exposure

The current dietary exposure supersedes section 3.5 of the previous evaluation (EFSA CEP Panel, 2023a).

⁵Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

⁶<https://connect.efsa.europa.eu/RM/s/publicconsultation2/a01Tk0000003H4L/pc0769>

3.1.1 | Revised intended use of the food enzyme

The food enzyme is intended to be used in five food manufacturing processes at the revised recommended use levels summarised in [Table 1](#).

TABLE 1 Updated intended uses and use levels of the food enzyme.⁷

Food manufacturing process ^a	Raw material (RM)	Recommended use level (mg TOS/kg RM)	
		Current evaluation ^b	Previous evaluation ^{b,c}
Processing of cereals and other grains			
• Production of baked products	Flour	2	3–18
Processing of eggs and egg products	Eggs	8.1	3–12
Processing of dairy products			
• Production of flavouring preparations from dairy products	Various dairy materials (e.g. cheese, butter, cream)	81.3	494–2,203
• Production of cheese	Milk	8.1	
Processing of plant- and fungal-derived products			
• Production of plant-based analogues of milk and milk products	Cereals, legumes, oilseeds, nuts, etc	1,219.6	

^aThe name has been harmonised by EFSA according to the ‘Food manufacturing processes and technical data used in the exposure assessment of food enzymes’ (EFSA CEP Panel, 2023).

^bThe numbers in bold represent the maximum recommended use levels and were used for calculation.

^cThe previous evaluation is made for the food enzyme application EFSA-Q-2015-00692.

The Panel noted a substantial decrease in the use level recommended for three processes in the current assessment when compared to the previously reported levels. The applicant ascribes these changes to an optimisation of the current food manufacturing processes.⁸

The additional two uses of the food enzyme are described below.

In cheese making, the triacylglycerol lipase is added to milk together with the starter culture during coagulation.⁹ The free fatty acids released by the hydrolysis of the triacylglycerols enhance the flavour of the cheese. After coagulation, most of the food-enzyme TOS partitions into the whey and the rest remains in the curd.

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 acylglycerols.¹⁰ The hydrolysis increases the amounts of medium chain fatty acids to improve the taste. The food enzyme-TOS remains in these plant-based analogues.

Based on the thermostability evaluated previously (EFSA CEP Panel, 2023a) and the downstream processing steps applied in the food manufacturing processes, it is expected that the triacylglycerol lipase is inactivated in all the food manufacturing processes in which the food enzyme-TOS is not removed, except for plant-based beverages and cheeses, in which the triacylglycerol lipase may remain active, depending on the specific manufacturing process.

3.1.2 | Dietary exposure estimation

In accordance with the guidance document (EFSA CEP Panel, 2021), dietary exposure was calculated for the five food manufacturing processes, where the food enzyme-TOS remains in the final foods.

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, 2023b). 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 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out

⁷Additional information November 2023.

⁸Additional data November 2023.

⁹Technical dossier/Intended use(s) in food and use level(s)/Annex Flow chart and Annex 4–11- Proposed conditions of use.

¹⁰Technical dossier/Intended use(s) in food and use level(s)/Annex Flow chart/p. 5.

in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.845 mg TOS/kg bw per day in toddlers at the 95th percentile.

TABLE 2 Updated dietary exposure to the 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)	0.005–0.107 (12)	0.033–0.449 (15)	0.024–0.123 (19)	0.014–0.038 (21)	0.010–0.043 (22)	0.008–0.035 (23)
Min–max 95th percentile (number of surveys)	0.013–0.732 (11)	0.057–0.845 (14)	0.044–0.651 (19)	0.028–0.066 (20)	0.023–0.277 (22)	0.021–0.264 (22)

3.1.3 | Uncertainty analysis

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

TABLE 3 Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate.

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

Abbreviations: +, uncertainty with potential to cause overestimation of exposure; -, uncertainty with potential to cause underestimation of exposure.

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 overestimation of the exposure.

3.2 | Margin of exposure

In the previous evaluation, the Panel identified a no observed adverse effect level (NOAEL) of 784 mg TOS/kg bw per day, the highest dose tested, resulting in a margin of exposure (MOE) of at least 3240 (EFSA CEP Panel, 2023a).

A comparison of the NOAEL with the newly derived exposure estimates of 0.005–0.449 mg TOS/kg bw per day at the mean and from 0.013 to 0.845 mg TOS/kg bw per day at the 95th percentile resulted in a MOE of at least 928.

4 | CONCLUSION

Based on the data provided for the previous evaluation and the revised margin of exposure, the Panel concluded that the food enzyme triacylglycerol lipase produced with the non-genetically modified *Mucor circinelloides* strain AE-LMH does not give rise to safety concerns under the revised intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for authorisation of Triacylglycerol lipase from *Mucor javanicus* AE-LM in accordance with Regulation (EC) No 1331/2008. December 2022. Submitted by Amano Enzyme Inc.

Additional information. November 2023. Submitted by Amano Enzyme Inc.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
IUBMB	International Union of Biochemistry and Molecular Biology
MoE	margin of exposure
NOAEL	no observed adverse effect level
RM	Raw Material
TOS	total organic solids

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

QUESTION NUMBER

EFSA-Q-2023-00371

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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 ^a , Serbia ^a , 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 ^a , Serbia ^a , Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Bosnia and Herzegovina ^a , Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Montenegro ^a , Netherlands, Portugal, Romania, Serbia ^a , Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Bosnia and Herzegovina ^a , Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro ^a , Netherlands, Portugal, Romania, Serbia ^a , Slovenia, Spain, Sweden
The elderly^b	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Montenegro ^a , Netherlands, Portugal, Romania, Serbia ^a , Slovenia, Spain, Sweden

^aConsumption data from these pre-accession countries are not reported in Table 3 of this opinion; however, they are included in Appendix A for testing purpose.

^bThe 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).