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SCIENTIFIC OPINION

Revised dietary exposure assessment of the food enzyme triacylglycerol lipase from the genetically modified *Aspergillus oryzae* strain NZYM-LH

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is produced with the genetically modified *Aspergillus oryzae* strain NZYM-LH by Novozymes A/S. A safety evaluation of this food enzyme was made previously, in which EFSA concluded that, under the intended conditions of use, this food enzyme did not give rise to safety concerns. Due to the implementation of a new methodology to estimate the dietary exposure to food enzymes in 2016, the European Commission requested EFSA to revise the exposure assessment of this food enzyme by using this new methodology. In this assessment, EFSA realigned the intended uses of this food enzyme to two food manufacturing processes and recalculated the dietary exposure. Dietary exposure to the food enzyme-TOS was calculated to be up to 0.284 mg TOS/kg body weight (bw) per day in European populations. When combined with the no observed adverse effect level previously reported (1080 mg TOS/kg bw per day, the highest dose tested), the Panel derived a margin of exposure of at least 3803. Based on the revised exposure estimate, the margin of exposure calculated thereof and the previous evaluation, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

Aspergillus oryzae, EC 3.1.1.3, EFSA-Q-2012-01009, EFSA-Q-2024-00608, food enzyme, genetically modified microorganism, triacylglycerol lipase

CONTENTS

1 | INTRODUCTION

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

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

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

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

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

Regulation (EC) No 1332/2008[1] lays down the rules on food enzymes used in foods with a view to ensuring the effective functioning of the internal market whilst ensuring a high level of protection of human health.

Food enzymes shall be subject to safety evaluation by the European Food Safety Authority (EFSA) and approval via a Union list. The inclusion of a food enzyme in the Union list is considered by the Commission on the basis of the opinion from EFSA, taking into account also other general criteria such as technological need, consumer aspects and, where relevant, other legitimate factors. For every food enzyme included in the positive list intended uses in food and specifications, including the criteria on purity and the origin of the food enzyme, shall be laid down.

The establishment of the Union list will take place in a single step after the Authority has issued an opinion on each food enzyme for which an application complying with the validity criteria laid down in accordance with Article 9(1) of Regulation (EC) No 1331/2008[2] had been submitted in accordance with Article 17(2) of Regulation (EC) No 1332/2008.

Pursuant to Article 9 of Regulation (EC) No 1331/2008 the Commission adopted implementing measures that are laid down in Regulation (EU) No 234/2011[3] as regards the content, drafting and presentation of applications submitted under each sectoral food law, arrangements for checking the validity of applications and the type of information that should be included in the opinion of EFSA.

Article 5 of Regulation (EU) No 234/2011 requires applicants to take into account the latest guidance documents adopted or endorsed by the Authority available at the time of the submission of the application for the safety evaluation of a food enzyme.

In 2009, EFSA published the first guidance on the Submission of a Dossier on Food Enzymes.[4]

Based on experience gained in assessing the submitted dossiers, EFSA, on its own initiative, has updated the approach and methodology to assess the dietary exposure on food enzymes, resulting in the adoption of the statement on the exposure assessment of food enzymes by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (EFSA CEF Panel, 2016). In 2021, the Statement was incorporated into the revised 'Scientific Guidance for the submission of dossiers on Food Enzymes'.[5]

[1]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3]Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, p. 15.

[4]The EFSA Journal (2009) 1305, 1-26.

[5]https://doi.org/10.2903/j.efsa.2021.6851.

As this approach has been applied since 2016 to the evaluation of all food enzymes, the outdated methodology, based on the 2009 EFSA guidance, had been used only in five opinions adopted before that time (i.e. EFSA-Q-2012-00897, EFSA-Q-2012-01009, EFSA-Q-2013-00197, EFSA-Q-2013-00198 and EFSA-Q-2014-00499).

In order to ensure a consistent approach, the Commission considers that it is desirable to update the exposure assessment in the five opinions on food enzymes adopted before 2016 by following the current exposure approach defined in Chapter 5 of the 'Scientific Guidance for the submission of dossiers on Food Enzymes' and its implementing document 'Food manufacturing processes and technical data used in the enzyme exposure assessment' published in 2023.[6]

The new rules on transparency introduced by Regulation (EU) 2019/1381 of the European Parliament and of the Council on the transparency and sustainability of the EU risk assessment in the food chain shall not apply to those applications, as they were submitted before Regulation (EU) 2019/1381 entered into application.

1.1.2 | Terms of Reference

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission requests EFSA to update the opinions on food enzymes adopted before 2016, following the exposure approach defined in the 'Scientific Guidance for the submission of dossiers on Food Enzymes' published in 2021 and its implementing document published in 2023.

1.1.3 | Interpretation of the Terms of Reference

The present scientific opinion addresses one of the five requests from the European Commission to revise the dietary exposure and update the safety evaluation for EFSA-Q-2012-01009.

2 | DATA AND METHODOLOGIES

2.1 | Data

The data used to update the dietary exposure evaluation were those provided by the applicant under the food enzyme application EFSA-Q-2012-01009.

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 '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, 2023) have been followed for the evaluation.

3 | ASSESSMENT

IUBMB nomenclature	Triacylglycerol lipase
Systematic name	Triacylglycerol acylhydrolase
Synonyms	Lipase; triglyceride lipase
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 were evaluated in June 2014 (EFSA CEF Panel, 2014). Following a request from the European Commission, EFSA realigned the intended uses to two food manufacturing processes according to the current classification (EFSA CEP Panel, 2023), recalculated the dietary exposure using the approach described in the 'Scientific Guidance for the submission of dossiers on Food Enzymes' (EFSA CEP Panel, 2021) and revised the margin of exposure.

[6] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.8094.

3.1 | Dietary exposure

The current dietary exposure supersedes section 3.6, 3.7 and 4 of the previous evaluation (EFSA CEF Panel, 2014).

3.1.1 | Intended use of the food enzyme

The intended uses reported in the current evaluation correspond to those in the previous evaluation, except that they have been reclassified according to the current guidance document (EFSA CEP Panel, 2023). The food enzyme is intended to be used in two food manufacturing processes at the use levels summarised in Table 1.

TABLE 1 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[7]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[b,c]
Processing of cereals and other grains		
• Production of baked products	Flour	**20.4**
• Production of cereal-based products other than baked	Flour	**20.4**

[a]The names reported in the EFSA-Q-2012-01009 have been harmonised by EFSA in accordance with the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

[b]The levels were the same as those reported in the scientific opinion of EFSA-Q-2012-01009.

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

The food enzyme-TOS are not removed in the two food manufacturing processes.

3.1.2 | Dietary exposure estimation

Chronic exposure to the food enzyme-TOS was calculated using the FEIM webtool[8] 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 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. They ranged from 0.008 to 0.158 mg TOS/kg bw per day at the mean and from 0.022 to 0.284 mg TOS/kg bw per day at the 95th percentile, with the highest dietary exposure being 0.284 mg TOS/kg bw per day in toddlers at the 95th percentile. Detailed mean and 95th percentile exposure to the food enzyme-TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 48 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 26 European countries (Appendix B).

TABLE 2 Updated dietary exposure to the 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)	0.020–0.108 (12)	0.054–0.158 (15)	0.027–0.133 (19)	0.008–0.081 (21)	0.025–0.048 (22)	0.023–0.050 (23)
Min–max 95th percentile (number of surveys)	0.058–0.273 (11)	0.129–0.284 (14)	0.065–0.250 (19)	0.022–0.144 (20)	0.050–0.109 (22)	0.046–0.093 (22)

[7]Technical dossier/p. 73.

[8]Version 1.1.1-1.

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 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	+/–

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 an overestimation of the exposure.

3.2 | Margin of exposure

In the previous evaluation, the Panel identified a no observed adverse effect level (NOAEL) of 1080 mg TOS/kg bw per day (the highest dose tested) and derived a margin of exposure of at least 3186 (EFSA CEF Panel, 2014).

In the present opinion, a comparison of the NOAEL with the newly derived exposure estimates resulted in a margin of exposure of at least 3803.

4 | CONCLUSION

Based on the revised exposure estimation, the margin of exposure calculated thereof and the previous evaluation, the Panel concluded that the food enzyme triacylglycerol lipase produced with the genetically modified *Aspergillus oryzae* strain NZYM-LH does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier "Lipase from a genetically modified strain of Aspergillus oryzae (strain NZYM-LH)". November 2012. Submitted by Novozymes A/S.

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
EC European Commission
EINECS European Inventory of Existing Commercial Chemical Substances
EU European Union
IUBMB International Union of Biochemistry and Molecular Biology
NOAEL no observed adverse effect level
RM raw material
TOS total organic solids

REQUESTOR

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 2 of this opinion, however, they are included in Appendix A 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).