Adopted: 18 September 2024

DOI: 10.2903/j.efsa.2024.9035

efsa JOURNAL

SCIENTIFIC OPINION

Safety evaluation of the food enzyme triacylglycerol lipase from the non-genetically modified *Aspergillus tubingensis* strain NL151

EFSA Panel on Food Enzymes (FEZ) | Holger Zorn | José Manuel Barat Baviera | Claudia Bolognesi | Francesco Catania | Gabriele Gadermaier | Ralf Greiner | Baltasar Mayo | Alicja Mortensen | Yrjö Henrik Roos | Marize L. M. Solano | Monika Sramkova | Henk Van Loveren | Laurence Vernis | Andrew Chesson | Lieve Herman | Magdalena Andryszkiewicz | Daniele Cavanna | Ana Gomes | Natália Kovalkovičová | Sandra Rainieri | Giulio Di Piazza | Rita Ferreira de Sousa | Yi Liu

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) is produced with the non-genetically modified *Aspergillus tubingensis* strain NL151 by Shin Nihon Chemical Co., Ltd. The food enzyme was free from viable cells of the production organism. It is intended to be used in six food manufacturing processes. Dietary exposure was estimated to be up to 0.278 mg total organic solids (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 1669 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 6004. A search for homology of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that, the risk of allergic reactions upon dietary exposure cannot be excluded, but the likelihood is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns, under the intended conditions of use.

KEYWORDS

Aspergillus niger, *Aspergillus tubingensis*, EC 3.1.1.3, food enzyme, non-genetically modified microorganism; EFSA-Q-2016-00654, triacylglycerol acylhydrolase, 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[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 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 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.

Five applications have been introduced by the applicants "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzymes Triacylglycerol lipase from *Aspergillus niger* (strain NL 151), Aspergillopepsin I from *Aspergillus niger* (strain AP 233) and Pectinase from *Rhizopus oryzae* (strain MC3-3-9), "Alpha Ingredients S.r.l." for the authorisation of the food enzyme Transglutaminase from *Streptomyces mobaerensis* (strain DSM40587) and "Laboratorios Arroyo S.S." for chymosin and pepsin from stomachs of calves and cows.

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

1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the following food enzymes Triacylglycerol lipase from *Aspergillus niger* (strain NL 151), Aspergillopepsin I from *Aspergillus niger* (strain AP 233), Pectinase from *Rhizopus oryzae* (strain MC3-3-9), Transglutaminase from *Streptomyces mobaerensis* (strain DSM40587) and chymosin and pepsin from stomachs of calves and cows in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

[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.

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 the non-genetically modified *A. niger* strain NL 151.

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

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. niger* strain NL 151.

Additional information was requested from the applicant during the assessment process on 16 November 2021, 29 February 2024 and received on 26 September 2023, 22 March 2024 (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 16 November 2021, the applicant requested a clarification teleconference on 21 September 2022, after which the applicant provided additional data on 26 September 2023.

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 the 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. Additional information was requested 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
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. 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 application is intended to be used in six food manufacturing processes: processing of cereals and other grains for the production of (1) baked products, (2) brewed products, (3) non-wine vinegars; (4) processing of fats and oils for the production of modified fats and oils by interesterification and processing of dairy products for the production of (5) processed cheese and (6) flavouring preparations.

3.1 | Source of the food enzyme[5]

The food enzyme triacylglycerol lipase is produced with the non-genetically modified filamentous fungus *A. tubingensis* (formerly *A. niger*) strain NL 151, which is deposited in [redacted] with the deposit number [redacted].[6]

[4]Technical dossier/p. 20, 67.
[5]Technical dossier/p. 31–37; Technical dossier/Annex I; Technical dossier/Additional data, 26 September 2023/Annex; Attachment 1; Attachment 2.
[6]Technical dossier/Additional data, 26 September 2023/Annex; Attachment 2.

The production strain NL 151 was [redacted]. It was identified as *A. tubingensis* [redacted][7]

3.2 | Production of the food enzyme[8]

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 under solid state fermentation with conventional process controls in place. After completion of the fermentation the enzyme is extracted with water and the biomass and other solids are removed from the fermentation broth by centrifugation followed by microfiltration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[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[13]

The triacylglycerol lipase is a single polypeptide chain of [redacted] amino acids.[14] The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[15] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected mass of the enzyme.[16]

No other enzyme activities were reported.[17]

The applicant's in-house determination of triacylglycerol lipase activity[18] is based on the hydrolysis of olive oil (reaction conditions: [redacted]) and determined by measuring the release of fatty acids by titration. The triacylglycerol lipase activity is expressed in Units (U)/g. One unit is defined as the amount of enzyme which releases 1 µmol of fatty acid from olive oil per minute under the condition of the assay.[19]

The food enzyme has a temperature optimum around 37°C (pH 6.0) and a pH optimum around pH 4.0 (37°C).[20] Thermostability was tested after a pre-incubation of the food enzyme for 15 min at different temperatures (pH 6.0). Triacylglycerol lipase activity was stable up to 55°C. With the increasing temperature activity was reduced, with no activity detected after incubation at 70°C for 20 min.[21]

3.3.2 | Chemical parameters[22]

Data on the chemical parameters of the food enzyme were provided for three batches intended for commercialisation and two batches produced for the toxicological tests (Table 1).[23] The mean total organic solids (TOS) of the three food enzyme batches intended for commercialisation was 16.7% and the mean enzyme activity/TOS ratio was 129.2 U/mg TOS.

[7]Technical dossier/Additional data, 26 September 2023/Annex; Attachment 1.
[8]Technical dossier/p. 37–40, 42–43; Technical dossier/Annex III; Technical dossier/Additional data, 26 September 2023/Annex; Attachment 3; Technical dossier/Additional data, 26 September 2023/Annex/Response to EFSA Question 5.
[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/p. 38; Technical dossier/Additional data, 26 September 2023/Annex; Attachment 3.
[11]Technical dossier/p. 38–39.
[12]Technical dossier/p. 37–38; Technical dossier/Annex III.1-III.17; Technical dossier/Additional data, 26 September 2023/Annex/Response to EFSA Question 5.
[13]Technical dossier/p. 20–31; Technical dossier/Annex II.
[14]Technical dossier/p. 21; Technical dossier/Annex VII.1.
[15]Technical dossier/Additional data, 26 September 2023/Annex/Response to EFSA Question 4.
[16]Technical dossier/p. 20–22.
[17]Technical dossier/p. 29.
[18]Technical dossier/p. 24; Technical dossier/Annex II.1.
[19]Technical dossier/p. 24; Technical dossier/Annex II.1.
[20]Technical dossier/p. 27–28; Technical dossier/Annex II.1.
[21]Technical dossier/p. 27; Technical dossier/Annex II.1.
[22]Technical dossier/p. 40–42, 56; Technical dossier/Annex II; Annex IV; Annex V; Technical dossier/Additional data, 26 September 2023/Attachment 4.
[23]Technical dossier/p. 41, 56; Technical dossier/Annex IV.1; Annex V.1.

TABLE 1 Composition of the food enzyme.[24]

Parameters	Unit	Batches				
		1	2	3	4[a]	5[b]
Triacylglycerol lipase activity	U/g[c]	20,100	25,100	19,400	20,700	6350
Protein	%	11.0	11.8	11.3	12.4	NA[d]
Ash	%	0.4	0.4	0.5	0.4	0.2
Water	%	82.9	82.9	82.9	82.9	94.5
Total organic solids (TOS)[e]	%	16.7	16.7	16.6	16.7	5.3
Activity/TOS ratio	U/mg TOS	120.4	150.3	116.9	124.0	119.8

[a]Batch used for Ames test, an in vitro mammalian chromosomal aberration test, an in vivo mammalian erythrocyte micronucleus test and a repeated dose 90-day oral toxicity study.

[b]Batch used for in vitro mammalian cell micronucleus test.

[c]U: Unit (see Section 3.3.1).

[d]NA: not analysed.

[e]TOS calculated as 100% – % water – % ash.

3.3.3 | Purity[25]

The lead content in the three commercial batches and in two batches used for toxicological studies was below 5 mg/kg[26] which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the arsenic concentration was below the limits of detection of the employed method.[27,28]

The food enzyme complies with the microbiological criteria for total coliforms, *Escherichia coli* and *Salmonella*, as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[29] No antimicrobial activity was detected in any of the tested batches.[30]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins (B1, B2, G1, G2), ochratoxin A, sterigmatocystin, T-2 toxin and zearalenone was examined in the three commercial food enzyme batches. All were below the limit of detection (LoD) of the applied methods.[31,32] 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[33]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted]. No colonies were produced. A positive control was included.[34]

3.4 | Toxicological data[35]

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

Batches 4 and 5 (Table 1) used in these studies had similar activity/TOS values as the batches intended for commercialisation and were considered suitable as a test items.

[24]Technical dossier/p. 40–42, 56; Technical dossier/Annex II; Annex IV; Annex V; Technical dossier/Additional data, 26 September 2023/Attachment 4.

[25]Technical dossier/p. 41–42; Technical dossier/Annex IV; Annex V; Technical dossier/Additional data, 26 September 2023/Attachment 4.

[26]Technical dossier/p. 41; Technical dossier/Annex IV.1; Technical dossier/Additional data, 26 September 2023/Attachment 4.

[27]Technical dossier/p. 41; Technical dossier/Annex IV.1: LoDs: Pb = 5 mg/kg; As = 3 mg/kg.

[28]Technical dossier/p. 41; Technical dossier/Annex IV.1; Technical dossier/Additional data, 26 September 2023/Attachment 4.

[29]Technical dossier/p. 41, 56; Technical dossier/Annex IV.1; Technical dossier/Additional data, 26 September 2023/Attachment 4.

[30]Technical dossier/p. 41; Technical dossier/Annex IV.1; Annex IV.2.

[31]Technical dossier/p. 42; Technical dossier/Annex IV.3.

[32]Technical dossier/Annex IV.3: LoDs: aflatoxins (B1, B2, G1, G2) = 0.5 μg/kg each; ochratoxin A = 0.5 μg/kg; sterigmatocystin = 20 μg/kg; T-2 toxin = 0.1 mg/kg; zearalenone = 50 μg/kg.

[33]Technical dossier/Additional data, 26 September 2023/Annex.

[34]Technical dossier/Additional data, 26 September 2023/Annex.

[35]Technical dossier/p. 51–57; Technical dossier/Annex VI.

3.4.1 | Genotoxicity

3.4.1.1 | *In vitro assays*

3.4.1.1.1 | *Bacterial reverse mutation test*

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[36]

Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvrA* were used with or without metabolic activation (S9-mix).

A growth inhibition experiment was carried out applying the pre-incubation method, using six concentrations of the food enzyme ranging from 0.0207 and 2070 U/plate, corresponding to 0.167 and 16,694 μg TOS/plate. Growth stimulation, as indicated by the thickening of the background bacterial lawn, was observed at 2070 U/plate in all strains in the presence and absence of S9-mix. Upon treatment with the food enzyme, a two-fold increase in the number of revertant colonies was observed at the highest concentration of 2070 U/plate in *S*. Typhimurium strains TA98 and TA1537 without S9-mix and in *S*. Typhimurium strains TA100, TA1535, TA98 and TA1537 with S9-mix.

A dose-finding experiment was carried out applying the pre-incubation method, using six concentrations of the food enzyme ranging from 8.52 to 2070 U/plate, corresponding to 69, 206, 619, 1855, 5565 and 16,694 μg TOS/plate. Growth stimulation, as indicated by the thickening of the background bacterial lawn, was observed at 2070 U/plate in all strains in the presence and absence of S9-mix. Upon treatment with the food enzyme, a two-fold increase in the number of revertant colonies was observed at concentration of 2070 U/plate in *S*. Typhimurium strains TA100, TA1535 both with and without S9-mix, at concentrations of 690 U/plate and 2070 U/plate in *S*. Typhimurium strain T98 without S9-mix and at concentration of 2070 U/plate in *S*. Typhimurium strain T98 without S9-mix.

Based on these results, the main test was carried out applying the pre-incubation method, using six concentrations of the food enzyme from 64.7 to 2070 U/plate (corresponding to 522, 1040, 2089, 4177, 8387 and 16,694 μg TOS/plate) in *S*. Typhimurium TA1537 and *E. coli* WP2*uvrA*. Growth stimulation, as indicated by the thickening of the background bacterial lawn, was observed at ≤ 518 U/plate in *E. coli* WP2*uvrA* and at ≤ 1040 U/plate in *S*. Typhimurium TA1537, in the presence and absence of S9-mix. 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. For the remaining strains, to avoid possible false positives due to free histidine, the 'treat and wash' method was applied, using six concentrations of the food enzyme from 8.52 to 2070 U/plate, corresponding to 69, 206, 619, 1855, 5565 and 16,694 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.

A confirmatory experiment was carried out applying the 'treat and wash' method, using six concentrations of the food enzyme from 64.7 to 2070 U/plate (corresponding to 522, 1040, 2089, 4177, 8387 and 16,694 μg TOS/plate) in *S*. Typhimurium strains TA100; TA1535 and TA98. 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.

3.4.1.1.2 | *In vitro mammalian chromosomal aberration test*

The in vitro mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[37] An experiment was performed with duplicate cultures of Chinese hamster lung fibroblast cells (CHL/IU). The cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

A preliminary test to establish cytotoxicity was carried out in a short-term treatment (6 h exposure and 18 h recovery period) with S9-mix and in a continuous treatment (24 h exposure without recovery period) without S9-mix at concentrations ranging from 0.0207 and 2070 U/mL, corresponding to 0.167 and 16,694 μg TOS/mL. A reduction in mitotic index was observed at the highest concentration tested (5.8% vs. 9.6% in the controls in the short-term treatment with S9-mix and 1.6% vs. 7.2% in the controls in the continuous treatment without S9-mix).

A dose-finding study was performed at concentrations ranging from 64.7 to 2070 U/mL (corresponding to 522 and 16,694 μg TOS/mL), and no inhibition of cell growth by 50% or more was observed.

Based on these results, the cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 518, 1040 and 2070 U/mL (corresponding to 4177, 8387 and 16,694 μg TOS/mL) in a short-term treatment (6 hours exposure and 18 hours recovery period) either with and without S9-mix, and in a long-term treatment (24 h exposure without recovery period) without S9-mix. In the short-term treatment, cytotoxicity (measured as inhibition of cell

[36]Technical dossier/Annex VI.1.

[37]Technical dossier/Annex VI.2.

growth) of 44.8% was observed at the highest concentration tested without S9-mix. In the long-term treatment without S9-mix, cytotoxicity of 46.3% was observed at the highest concentration tested. The frequency of structural chromosomal aberrations was statistically significantly different to the negative controls at concentrations of 8387 and 16,694 μg TOS/mL tested in the short-term and long-term treatment without S9-mix and at concentration of 16,694 μg TOS/mL tested in the short-term treatment with S9-mix. Statistically non-significant increase in the frequency of numerical chromosomal aberrations was reported at concentrations of 8387 and 16,694 μg TOS/mL tested in the short-term treatment without S9-mix.

The Panel concluded that food enzyme triacylglycerol lipase did induce an increase in the frequency of structural chromosome aberrations under the test conditions applied in this study.

3.4.1.1.3 | *In vitro mammalian cell micronucleus test*[38]

The in vitro mammalian cell micronucleus test was carried out according to the OECD Test Guideline 487 (OECD, 2016) and following GLP.[39] An experiment was performed with duplicate cultures of human peripheral whole blood lymphocytes. The cell cultures were treated with the food enzyme with or without metabolic activation (S9-mix).

In a range-finding test no cytotoxicity above 50% was seen at any concentration tested up to 5000 μg TOS/mL in the short-term treatment with and without S9-mix. However, cytotoxicity was seen at concentrations from 1080 μg TOS/mL upwards in the long-term treatment without S9-mix.

Based on these results, in the main experiment, cells were exposed to the food enzyme and scored for the frequency of bi-nucleated cells with micronuclei (MNBN) at concentrations of 1000, 2000 and 5000 μg TOS/mL in the short-term treatment (3 h exposure and 21 h recovery period) either with or without S9-mix and at concentrations of 500, 650 and 1000 μg TOS/mL in the long-term treatment (24 h exposure and 24 h recovery period) without S9-mix.

Cytotoxicity of 57%, based on replication index, was reported at the highest concentration tested in the long-term treatment. The frequency of MNBN was not statistically significantly different to the negative controls at all concentrations tested.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce an increase in the frequency of MNBNs under the test conditions applied in this study.

3.4.1.2 | *In vivo assay*

3.4.1.2.1 | *In vivo mammalian erythrocyte micronucleus test*

The in vivo mammalian erythrocyte micronucleus test in rats was carried out according to the OECD Test Guideline 474 (OECD, 1997c) and following GLP.[40]

Five Sprague–Dawley Crl:CD(SD) [SPF] rats (males) per group were treated with a single oral administration of the food enzyme at doses of 5180, 104,000 and 207,000 U/kg bw (corresponding to 417.7, 838.7 and 1669 mg TOS/kg bw per day) by gavage for 2 consecutive days (24 h interval). Bone marrow was sampled 24 h after the final dosing.

No statistically significant increases in the frequency of micronucleated cells in the treated animals and no statistically significant difference in the ratio of immature erythrocytes to total number of erythrocytes were observed in comparison to the controls.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce an increase in the frequency of micronucleated immature erythrocytes in the rat bone marrow under the test conditions applied in this study, however, the study was considered inconclusive because no evidence of bone marrow exposure was provided.

Conclusions on Genotoxicity

Based on the negative results obtained with the Ames test and with the in vitro mammalian cell micronucleus test in human peripheral lymphocytes, the Panel concluded that there was no concern for genotoxicity of the food enzyme triacylglycerol lipase. The Panel considered that the positive results reported in the in vitro chromosomal aberration test with transformed rodent cell line were overruled by those obtained with the primary human cell culture.

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, 1998).[41]

Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received by gavage the food enzyme in doses of 2070, 20,700 or 207,000 U/kg body weight (bw) per day, corresponding to 16.7; 166.9 or 1669 mg TOS/kg bw per day. The

[38]Technical dossier/Additional data, 26 September 2023/Annex; Attachment 4.

[39]Technical dossier/Additional data, 26 September 2023/Annex; Attachment 4.

[40]Technical dossier/Annex VI.3.

[41]Technical dossier/Annex VI.4.

doses were established in a repeated dose 14-day oral toxicity study in rats.[42] Controls received the vehicle (water for injection).

No mortality was observed.

Haematological investigation showed a statistically significant decrease in mean cell haemoglobin concentration (MCHC) in low- and high-dose males (–2% and –1%, respectively), an increase in monocyte (Mono) ratio in mid-dose males (+45%), an increase in platelet (PLT) count in low- and high-dose females (+16% and +13%, respectively), an increase in lymphocyte (Lymph) ratio (+7%) and a decrease in neutrophil (Neu) ratio (–30%) in high-dose females. 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 (MCHC, Mono ratio, PLT), there were no changes in other relevant parameters (in white blood cell count) and the changes were within the historical control values (all parameters except for Mono ratio for which the background data were not provided).

Clinical chemistry investigation revealed a statistically significant decrease in glucose concentration in low-dose females (–10%) and an increase in γ-glutamyl transpeptidase activity in low- and mid-dose females (+50% and +50%, respectively). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (both parameters), there was no dose–response relationship (both parameters) and the change was small (glucose).

The urinalysis revealed a statistically significant decrease in the potassium concentration (–25%) and the total potassium excretion (–19%) in high-dose males and a decrease in the total sodium excretion in high-dose females (–33%). 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 (in absence of any changes in serum electrolytes, in absolute and relative kidney weights, in absence of any gross and histological findings in the organ), and the changes were within the historical control values.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1669 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity[43]

The allergenicity assessment considered only the food enzyme and not additives, preservatives, carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the food enzyme triacylglycerol lipase produced with the *A. tubingensis* strain NL 151 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.[44]

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

Respiratory allergy following occupational inhalation of triacylglycerol lipase has been reported (Elms et al., 2003; Martel et al., 2010). Brant et al. (2004) reported occupational asthma in two patients in the detergent industry, caused by cellulase and lipase from *Aspergillus oryzae*. In addition, there were case reports of allergies due to inhalation with a digestive enzyme drug containing α-amylase and lipase derived from porcine pancreas (Shin et al., 2008). However, several studies have shown that adults with occupational asthma caused by an enzyme are usually able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Armentia et al., 2009; Brisman, 2002; Cullinan et al., 1997; Poulsen, 2004). In addition, no allergic reactions upon dietary exposure to any lipase have been reported in the literature.

Aspergillus species are known to cause respiratory allergy (Shen & Han, 1998). Oral allergic reactions to *Aspergillus* do occur (Xing et al., 2022) but are rare.

[redacted], a product that may cause allergies (listed in the Regulation (EU) No 1169/2011[45]) is used as raw material. 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 mostly 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. The Panel, however, considered that residual amounts of potentially allergenic proteins could still be present in the food enzyme.

Overall, the Panel considered that a risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

[42]Technical dossier/Annex VI.5.

[43]Technical dossier/p. 57–58; Technical dossier/Annex VII; Technical dossier/Additional data, 22 March 2024.

[44]Technical dossier/p. 57–58; Technical dossier/Annex VII; Technical dossier/Additional data, 22 March 2024/Attachment 1.

[45]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.

3.5 | Dietary exposure

3.5.1 | Intended uses of the food enzyme

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

TABLE 2 Intended uses and recommended use levels of the food enzyme as provided by the applicant.[46]

Food manufacturing process[a]	Raw material (RM)	Maximum recommended use level (mg TOS/kg RM)[b]
Processing of dairy products		
• Production of processed cheese[c]	Cheese	**11.6**
• Production of flavouring preparations from dairy products	Milk	**11.6**
Processing of cereals and other grains		
• Production of baked products	Flour (wheat or rye)	**7.8**
• Production of brewed products	Rice	**23.3**
• Production of non-wine vinegar	Rice	**23.3**
Processing of fats and oils		
• Production of modified fats and oils by interesterification	Soy, corn, canola, palm oil	**155**

[a]The 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).

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

[c]This food manufacturing process is not included in the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023). For calculation, see Appendix C.

In the production of processed cheese, the food enzyme is added to cheese during ripening to release fatty acids and consequently intensify the flavour.[47] The food enzyme–TOS remain in the processed cheeses.

In the production of flavouring preparations from dairy products, the food enzyme is added to cheese slurry to produce enzyme-modified cheese or to butterfat slurry to produce enzyme-modified dairy preparations.[48] The hydrolysis of milk fat by triacylglycerol lipase releases free fatty acids, intensifying the flavour in these products, which are incorporated into a variety of foods (e.g. snacks, beverages) to impart flavours. The food enzyme–TOS remain in these flavouring preparations.

In the production of baked products, the food enzyme is added to flour during dough making.[49] The triacylglycerol lipase hydrolyses fats and oils in flour, which improves gas retention and the dough structure. The food enzyme–TOS remain in the baked products.

In brewing processes for the production of rice wine and in the production of non-wine vinegars, the food enzyme is added to rice during steeping.[50] The triacylglycerol lipase releases unsaturated fatty acids from rice triglycerides, facilitating flavour development in rice wines or vinegars. The food enzyme–TOS remain in the final foods.

In the production of modified fats and oils by interesterification, at low-water content, triacylglycerol lipases catalyse the exchange of fatty acids at the sn1- and sn3-position of the triglycerides, modifying the properties of the resulting oils or fats (e.g. melting point and nutritional properties).[51] Interesterified fats can be incorporated into many foods as ingredients, e.g. as alternatives to hydrogenated fats in food formulation, such as spreads, bakery products or cocoa butter substitutes (Berry et al., 2019). Despite the request from EFSA, the applicant did not provide analytical data to establish the extent of possible removal of the food enzyme–TOS from the modified fats.[52] In the absence of analytical data, the Panel decided to proceed with the dietary exposure assessment by considering that all 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 processes, it is expected that this triacylglycerol lipase is inactivated in all the food manufacturing processes listed in Table 2, with the exception of cheese, in which it may remain in its active form, depending on the processing conditions.

[46]Technical dossier/Additional data, 26 September 2023/Table 2; Technical dossier/Additional data, 22 March 2024/Response to EFSA Question 2.

[47]Technical dossier/Additional data, 26 September 2023/Attachment 5/p. 4.

[48]Technical dossier/Additional data, 26 September 2023/Attachment 5/p. 5.

[49]Technical dossier/Additional data, 26 September 2023/Attachment 5/p. 1.

[50]Technical dossier/Additional data, 26 September 2023/Attachment 5/p. 2, 6.

[51]Technical dossier/Additional data, 26 September 2023/Attachment 5/p. 3.

[52]Technical dossier/Additional data, 26 September 2023/Response to EFSA Question 10.

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) together with the information provided in Appendix C.

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 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 in 26 European countries (Appendix B). The highest dietary exposure was estimated to be 0.278 mg TOS/kg bw per day in children 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)	0–0.084 (12)	0.016–0.128 (15)	0.040–0.127 (19)	0.024–0.065 (21)	0.019–0.058 (22)	0.017–0.050 (23)
Min–max 95th percentile (number of surveys)	0–0.228 (11)	0.075–0.275 (14)	0.103–0.278 (19)	0.067–0.151 (20)	0.059–0.160 (22)	0.043–0.122 (22)

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	+
For estimating exposure to processed cheese, the food groups selected included only processed cheese defined by Codex[53]	–
Exposure to food enzyme–TOS always calculated based on the recommended maximum use level	+
To estimate the exposure from the production of brewed products, rice wine is the only product indicated by the applicant, but the calculation included also the FoodEx categories related to beers	+
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.

[53]Process(ed) cheese and spreadable process(ed) cheese are covered by codex stan A-8(b)-1978 CXSa08be.doc(dairyconsultant.co.uk).

3.6 | Margin of exposure

A comparison of the NOAEL (1669 mg TOS/kg bw per day) identified from the 90-day rat study with the derived exposure estimates of 0–0.128 mg TOS/kg bw per day at the mean and from 0 to 0.278 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 6004.

4 | CONCLUSIONS

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 *A. tubingensis* strain NL 151 does not give rise to safety concerns under the intended conditions of use.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Application for the authorisation of triacylglycerol lipase from *Aspergillus niger* strain NL 151 as a food enzyme in the European Union. 9 March 2015. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. 26 September 2023. Submitted by Shin Nihon Chemical Co., Ltd.

Additional information. 22 March 2024. Submitted by Shin Nihon Chemical Co., Ltd.

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
CHL/IU	Chinese hamster lung-derived fibroblasts
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organisation of the United Nations
FEZ	EFSA Panel on Food Enzymes
FoodEx	a standardised food classification and description system
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MCHC	mean cell haemoglobin concentration
MNBN	bi-nucleated cells with micronuclei
Mono	monocyte
Neu	neutrophil
[redacted]	[redacted]
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
NA	not analysed
OECD	Organisation for Economic Co-operation and Development
RM	raw material
SPF	Specific pathogen free
TOS	total organic solids
WHO	World Health Organization

ACKNOWLEDGEMENTS

The Panel wishes to thank the following for the support provided to this scientific output: Erik Boinowitz.

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-2016-00654

COPYRIGHT FOR NON-EFSA CONTENT

PANEL MEMBERS

José Manuel Barat Baviera, Claudia Bolognesi, Francesco Catania, Gabriele Gadermaier, Ralf Greiner, Baltasar Mayo, Alicja Mortensen, Yrjö Henrik Roos, Marize de Lourdes Marzo Solano, Monika Sramkova, Henk Van Loveren, Laurence Vernis, and Holger Zorn.

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.

REFERENCES

Armentia, A., Dias-Perales, A., Castrodeza, J., Dueñas-Laita, A., Palacin, A., & 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.

Berry, S. E., Bruce, J. H., Steenson, S., Stanner, S., Buttriss, J. L., Spiro, A., Gibson, P. S., Bowler, I., Dionisi, F., Farrell, L., Glass, A., Lovegrove, J. A., Nicholas, J., Peacock, E., Porter, S., Mensink, R. P., & Hall, W. L. (2019). Interesterified fats: What are they and why are they used? A briefing report from the roundtable on Interesterified fats in foods. *Nutrition Bulletin*, *44*, 363–380.

Brant, A., Hole, A., Cannon, J., Helm, J., Swales, C., Welch, J., Newman Taylor, A., & Cullinan, P. (2004). Occupational asthma caused by cellulase and lipase in the detergent industry. *Occupational and Environmental Medicine*, *61*, 793–795.

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

Cullinan, P., Cook, A., Jones, M., Cannon, J., Fitzgerald, B., & Taylor, A. J. (1997). Clinical responses to ingested fungal alpha-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy*, *52*(3), 346–349. https://doi.org/10.1111/j.1398-9995.1997.tb01003.x

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

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

EFSA (European Food Safety Authority). (2011). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). (2009). Guidance of EFSA prepared by the Scientific Panel of Food Contact Materials, Enzymes, Flavourings and Processing Aids on the submission of a dossier on food enzymes. *EFSA Journal*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2009.1305

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*, *17*(6), 5741. 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, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., 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., … Chesson, A. (2021). Scientific Guidance for the submission of dossiers on food enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambre, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Riviere, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Apergi, K., … Chesson, A. (2023). Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, *21*(7), 8094. https://doi.org/10.2903/j.efsa.2023.8094

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*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

Elms, J., Fishwick, D., Walker, J., Rawbone, R., Jeffrey, P., Griffin, P., Gibson, M., & Curran, A. D. (2003). Prevalence of sensitisation to cellulase and xylanase in bakery workers. *Occupational and Environmental Medicine*, *60*(10), 802–804. https://doi.org/10.1136/oem.60.10.802

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. https://www.fao.org/3/a-a0675e.pdf

Frisvad, J. C., Møller, L. L. H., Larsen TO, Kumar, R., & Arnau, J. (2018). Safety of the fungal workhorses of industrial biotechnology: Update on the mycotoxin and secondary metabolite potential of *Aspergillus niger, Aspergillus oryzae,* and *Trichoderma reesei*. *Applied Microbiology and Biotechnology*, *102*, 9481–9515. https://doi.org/10.1007/s00253-018-9354-1

[redacted]

Martel, C., Nielsen, G. D., Mari, A., Licht, T. R., & Poulsen, L. K. (2010). Bibliographic review on the potential of microorganisms, microbial products and enzymes to induce respiratory sensitization. *EFSA Supporting Publication*, *7*(9), EN-75. 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. https://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. 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). (1997c). OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 474: Mammalian erythrocyte micronucleus test. 21 July 1997. 10 pp. https://www.oecd.org/chemicalsafety/risk-assessment/1948442.pdf

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. https://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

OECD (Organisation for Economic Co-Operation and Development). (2016). OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 487: *In vitro* mammalian cell micronucleus test. 29 July 2016. 29 pp. https://doi.org/10.1787/9789264264861-en

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

Shen, H. D., & Han, S. H. (1998). Characterization of allergens of *Penicillium* and *Aspergillus* species. *Journal of Microbiology, Immunology and Infection*, *31*, 141–145.

Shin, S. Y., Hur, G. Y., Ye, Y. M., & Park, H. S. (2008). A case of occupational rhinitis caused by porcine pancreatic extract developing into occupational asthma. *Journal of Korean Medical Science*, *23*(2), 347–349.

Xing, H., Wang, J., Sun, Y., & Wang, H. (2022). Recent advances in the allergic cross-reactivity between fungi and foods. *Journal of Immunology Research*, 7583400. https://doi.org/10.1155/2022/7583400

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: EFSA FEZ Panel (EFSA Panel on Food Enzymes), Zorn, H., Barat Baviera, J. M., Bolognesi, C., Catania, F., Gadermaier, G., Greiner, R., Mayo, B., Mortensen, A., Roos, Y. H., Solano, M. L. M., Sramkova, M., Van Loveren, H., Vernis, L., Chesson, A., Herman, L., Andryszkiewicz, M., Cavanna, D., Gomes, A., … Liu, Y. (2024). Safety evaluation of the food enzyme triacylglycerol lipase from the non-genetically modified *Aspergillus tubingensis* strain NL151. *EFSA Journal*, *22*(10), e9035. https://doi.org/10.2903/j.efsa.2024.9035

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, the 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, the 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*, the 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*, the 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*, the 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).

APPENDIX C

FoodEx1 categories and technical conversion factors considered for the exposure estimation to the processed cheeses

FoodEx_code	FoodEx_name	FoodEX hierarchical level	Tf1	Tf2	Tf3
A.08.08.003	Cheese, processed, sliceable	4	1	1.00	1.00
A.08.08.004	Cheese, processed spreadable	4	1	1.00	1.00
A.08.08.005	Cheese, processed, with condiments	4	1	0.94	1.00
A.08.08.006	Cheese, processed, with ham	4	1	0.74	1.00
A.08.08.007	Cheese, processed, with mushrooms	4	1	0.44	1.00
A.08.08.008	Cheese, processed, with pepper herbs	4	1	0.94	1.00
A.08.08.009	Cheese, processed, with walnuts	4	1	0.94	1.00
A.08.08.010	Cheese, processed, low fat	4	1	1.00	1.00
A.08.08.011	Cheese, processed cheese, plain	4	1	1.00	1.00
A.08.08.120	Cheese, Mozzarella	4	1	1.00	0.08*

* Only mozzarella-like cheese toppings are considered.