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Safety evaluation of the food enzyme triacylglycerol lipase from non-genetically modified *Limtongozyma cylindracea* strain MS-5-OF

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is produced with the non-genetically modified yeast *Limtongozyma cylindracea* strain MS-5-OF by Meito Sangyo Co., Ltd. The food enzyme is free from viable cells of the production organism. It is intended to be used in five food manufacturing processes: brewing processes, baking processes, milk processing for cheese production, production of free fatty acids by hydrolysis and production of flavouring preparations from dairy products. Dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 1.033 mg TOS/kg body weight (bw) per day in European populations. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 2,084 mg TOS/kg bw per day, the highest dose tested, which, when compared with the estimated dietary exposure, results in a margin of exposure of at least 2,017. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no match was found. The Panel considered that a risk of allergic reactions by 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: food enzyme, triacylglycerol lipase, triacylglycerol acylhydrolase, EC 3.1.1.3, *Limtongozyma cylindracea* (*Candida cylindracea*), non-genetically modified yeast

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1. Introduction

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

'Food enzyme' means a product obtained from plants, animals or 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.

Four applications have been introduced by the companies 'Puratos NC sa.', 'Novozymes A/S', 'Meito Sangyo Co., Ltd' and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) for the authorisation of the food enzymes inulinase from a genetically modified strain of *Aspergillus oryzae* (strain MUCL 44346), trypsin from porcine pancreatic glands, triacylglycerol lipase from *Candida cylindracea*, and cellulase, glucanase and hemicellulose covering xylanase and mannanase from *Aspergillus niger*, respectively.

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 four 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 food enzymes inulinase from a genetically modified strain of *Aspergillus oryzae*

[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. 15–24.

(strain MUCL 44346), trypsin from porcine pancreatic glands, triacylglycerol lipase from *Candida cylindracea*, and cellulase, glucanase and hemicellulose covering xylanase and mannanase from *Aspergillus niger* in accordance with Article 17.3 of Regulation (EC) No 1332/2008[1] on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme triacylglycerol lipase from *Candida cylindracea*.

Recent data identified the production microorganism as *Limtongozyma cylindracea* (basionym: *C. cylindracea*) strain MS-5-OF (Section 3.1).[4] Therefore, this name will be used in this opinion instead of *C. cylindracea*.

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 *C. cylindracea*.

Additional information was requested from the applicant during the assessment process on 10 October 2022 and received on 11 April 2023 (see 'Documentation provided to EFSA').

2.2. Methodologies

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

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

3. Assessment[5]

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

In the presence of water, triacylglycerol lipases catalyse the hydrolysis of the ester linkages in triacylglycerols, resulting in the generation of glycerol, free fatty acids, diacylglycerols and monoacylglycerols. The food enzyme under application is intended to be used in five food manufacturing processes: brewing processes, baking processes, milk processing for cheese production, production of free fatty acids by hydrolysis and production of flavouring preparations from dairy products.

3.1. Source of the food enzyme[6]

The enzyme is produced with the non-genetically modified yeast *L. cylindracea* (basionym: *C. cylindracea*) strain MS-5-OF, which is deposited at [redacted] with the deposit number [redacted].[7]

[4] Technical dossier/Additional data, 11 April 2023.
[5] Technical dossier/p. 4; Technical dossier/Extension_intended uses UPDATED.
[6] Technical dossier/p. 8, 40; Technical dossier/Additional data, 11 April 2023.
[7] Technical dossier/Additional data, 11 April 2023/Attachment A.

The production strain was identified as *L. cylindracea* by [redacted].[8]

The parent strain of the production strain was originally isolated [redacted]. The production strain, strain MS-5-OF, was obtained [redacted].[4]

The species *L. cylindracea* is included in the list of organisms for which the qualified presumption of safety (QPS) approach may be applied, when used for production purposes only (EFSA, 2007; EFSA BIOHAZ Panel, 2022). The qualification 'for production purpose only' requires the demonstration of the absence of viable cells of the production organism in the final product.

3.2. Production of the food enzyme[9]

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current Good Manufacturing Practice.[11]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch or fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by centrifugation. The supernatant containing the enzyme is then precipitated by addition of [redacted]. The precipitate containing the enzyme is recovered by centrifugation and dried under vacuum to remove [redacted]. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[12]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The triacylglycerol lipase activity expressed by *L. cylindracea* derives from five isoenzymes Lip1, Lip2, Lip3, Lip4 and Lip5. Each are single polypeptide chains of [redacted] or [redacted] amino acids[13] with molecular masses of the mature proteins, calculated from their amino acid sequences, ranging from [redacted] to [redacted] kDa.[14] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed a single major protein band corresponding to an apparent molecular mass of about [redacted] kDa[15], consistent with the expected mass of the enzyme. No other enzymatic activities were reported.[16]

The in-house determination of triacylglycerol lipase activity is based on hydrolysis of olive oil (reaction conditions: pH 7.0, 37°C, 30 min). The enzymatic activity is determined by the addition of sodium hydroxide and back-titrating the excess with acid as a measure of the free fatty acids released from the olive oil emulsion. One unit (U) of activity is defined as the quantity of enzyme that will liberate 1 μmol of fatty acids per minute under the conditions of the assay.[17]

The food enzyme has a temperature optimum between 40°C and 50°C (pH 7.0) and a pH optimum between pH 6 and 8 (37°C).[18] Thermostability was measured by pre-incubation of the food enzyme for 30 min at various temperatures. Enzyme activity was lost at 70°C.[19]

[8] Technical dossier/Additional data, 11 April 2023/Attachment B.
[9] Technical dossier/p. 8–9, 46–54; Technical dossier/Additional data, 11 April 2023/Attachment D; Attachment H.
[10] 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.
[11] Technical dossier/p. 8, 44, 46; Technical dossier/Additional data, 11 April 2023/Attachment D.
[12] Technical dossier/p. 8, 46; Technical dossier/Annex 5; Technical dossier/Additional data, 11 April 2023.
[13] Technical dossier/p. 7, 37; Technical dossier/Additional data, 11 April 2023/Attachment E.
[14] Technical dossier/p. 37; Technical dossier/Additional data, 11 April 2023.
[15] Technical dossier/p. 35–36.
[16] Technical dossier/p. 7, 38.
[17] Technical dossier/p. 7, 38; Technical dossier/Annex 2.
[18] Technical dossier/p. 7–8, 39–40; Technical dossier/Annex 2.
[19] Technical dossier/p. 39–40.

3.3.2. Chemical parameters[20]

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[21] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 62.5% and the mean enzyme activity/TOS ratio was 739.3 U/mg TOS.

Table 1: Composition of the food enzyme[20]

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Triacylglycerol lipase activity	U/g[(b)]	500,000	450,000	435,000	430,000
Protein	%	24.8	24.8	23.6	25.5
Ash	%	33.5	31.9	33.3	31.7
Water	%	4.4	4.7	4.8	4.0
Total organic solids (TOS)[(c)]	%	62.1	63.4	61.9	64.3
Activity/TOS	U/mg TOS	805	710	703	669

(a): Batch used for the toxicological studies.
(b): U: activity unit (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity[22]

The lead content in the three commercial batches and in the batch used for toxicological studies was below 5 mg/kg[23], which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme 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).[24] No antimicrobial activity was detected in any of the tested batches.[24]

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

3.3.4. Viable cells[25]

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. For each analysis, [redacted]. No colonies were produced. Positive controls were included for each of the samples.

3.4. Toxicological data[26]

Although the production strain is now considered to meet the requirements of the QPS approach to safety assessment, this possibility was not available to the applicant at the time of application. As required at that time, a battery of toxicological tests, including a bacterial reverse mutation test (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, were provided and are assessed below. The batch 4 (Table 1) used in these studies had lower activity/TOS value than the commercial batches and was consequently considered suitable as a test item.

[20] Technical dossier/Additional data, 11 April 2023/Attachment F; Attachment G.
[21] Technical dossier/p. 34–35, 75; Technical dossier/Annex 1; Annex 2; Annex 3; Annex 6; Annex 7; Annex 8.
[22] Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 11 April 2023/Attachment F; Attachment G.
[23] Technical dossier/Annex 1; Annex 3; Technical dossier/Additional data, 11 April 2023/Limit of detection (LoD) for lead = 5 μg/g.
[24] Technical dossier/p. 7, 36–37; Technical dossier/Annex 1; Annex 3.
[25] Technical dossier/Additional data, 11 April 2023/Attachment C; Attachment F; Attachment G.
[26] Technical dossier/p. 71–76.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation test (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[27]

Four strains of *Salmonella Typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used with or without metabolic activation (S9-mix), applying the pre-incubation method. Based on the results of a dose-finding test, two experiments were carried out in triplicate, using five concentrations ranging from 313 to 5,000 μg/plate, corresponding to 201, 402, 804, 1,608 and 3,215 μg TOS/plate.

No cytotoxicity was observed in any strain tested, with or without 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.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce gene mutations under the test conditions applied in this study.

3.4.1.2. *In vitro* mammalian 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.[28]

An experiment was performed with duplicate cultures of Chinese hamster lung-derived fibroblasts (CHL/IU). In a cell-growth inhibition test, the cells were exposed to the food enzyme at concentrations of 39 to 5,000 μg/mL (corresponding to 25 to 3,215 μg TOS/mL) in the short-term treatment (6 h exposure and 18 h recovery period) with or without metabolic activation (S9-mix) and in the long-term treatment (24 h exposure without recovery) without S9-mix.

No cytotoxicity above 50% was seen at any concentration tested. In the main experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 1,250, 2,500 and 5,000 μg/mL (corresponding to 804, 1,608 and 3,215 μg TOS/mL) in the short-term treatment either with or without S9-mix and at concentrations of 625, 1,250, 2,500 and 5,000 μg/mL (corresponding to 402, 804, 1,608 and 3,215 μg TOS/mL) in the long-term treatment.

Cytotoxicity (56% relative cell counts) was seen only at the highest concentration tested in the long-term treatment. The frequency of structural and numerical chromosomal aberrations was not statistically significantly different to the negative controls at any concentrations tested.

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

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[29] Groups of 10 male and 10 female Sprague–Dawley (Crl:CD(SD)) rats received 1.0, 2.5 and 5.0% of the food enzyme in the diet, equal to 408, 1,027 and 2,084 mg TOS/kg bw per day in males, and 477, 1,211 and 2,379 mg TOS/kg bw per day in females, for 91 days. Controls received the powdered basal diet CR-LPF.

No mortality was observed.

In clinical observations, a statistically significant decrease in rearing count in mid-dose males (−33%) was observed in week 5. The Panel considered the change as not toxicologically relevant, as it was only recorded sporadically, it was only observed in one sex and there was not dose–response relationship.

In the functional observations, a statistically significant increase in motor activity in mid- and high-dose males (+84%, +94%, respectively) at the 50–60 min interval (week 12), an increase in the total value (0–60 min) in high-dose males (+26%) and an increase in motor activity in mid-dose females (+97%) at the 50–60 min interval (week 12) was observed. The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically (motor activity in females) and were within the historical control values (motor activity in males).

[27] Technical dossier/Annex 6.
[28] Technical dossier/Annex 7.
[29] Technical dossier/Annex 8; Technical dossier/Additional data, 11 April 2023/Attachment I.

Haematological investigations revealed a statistically significant decrease in percentage of basophiles in mid- and high-dose females (−33% and −33%, respectively), a decrease in percentage of lymphocytes in low-dose females (−12%), an increase in the percentage and the count of neutrophils in low-dose females (+66% and +69%, respectively), an increase in platelet count in high-dose males (+10%) and in prothrombin time in high-dose males (+11%). 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 (percentage of basophiles, percentage of lymphocytes, percentage and count of neutrophils), the changes were small (all changes in population of leukocytes and in platelet count), there were no changes in other relevant parameters (total number of leukocytes, other coagulation parameters like activated thromboplastin time and fibrinogen) and the changes were within the historical control values.[30]

Clinical chemistry investigations revealed a statistically significant decrease in calcium in high-dose males (−3%) and a statistically significant increase in glucose in low-dose females (+15%). 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 (glucose) and the changes were within the historical control values.[30]

Urinalysis revealed a statistically significant increase in the excretion of potassium into urine in high-dose males (+60%) and high-dose females (+50%). The Panel considered the change as not toxicologically relevant, as there was no change in other relevant parameters and the change was within the historical control values (the excretion of potassium into urine).[30]

Statistically significant changes in organ weights included a decrease in absolute (−19%) and relative (−17%) weight of the adrenals in mid-dose males. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex and there was no dose–response relationship, there were no histopathological changes in adrenals in high-dose males (mid-dose not examined) and the changes were within the historical control values.[30]

Microscopic examination revealed no more than mild corticomedullary mineralization in the kidneys in low-, mid- and high-dose females (3/10, 6/10, 10/10 vs 0/10 in the control). The Panel considered this finding as a histological manifestation of nephrocalcinosis, which occurs with dietary imbalance of calcium to phosphorus (Ca:P) ratio in the laboratory rat, particularly in females. The test material contained 6.07% P and 1.45% Ca. The Ca:P ratio in the experimental diets decreased with the increase in the dose level of the test item. This, according to the Panel, explained a dose-dependent increase in the incidence of the corticomedullary mineralization in the treated female groups. Consequently, the Panel considered that this finding arose as a result of a progressive imbalance of Ca:P in the diet and not as an adverse effect of the test item.

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

The Panel identified a no observed adverse effect level (NOAEL) of 2,084 mg TOS/kg bw per day in males and of 2,379 mg TOS/kg bw per day in females, the highest doses tested.

3.4.3. Allergenicity[4]

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

The potential allergenicity of the food enzyme triacylglycerol lipase from the non-genetically modified *L. cylindracea* strain MS-5-OF 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.

No information was available on oral sensitisation or elicitation reactions of this triacylglycerol lipase.

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 may be able to ingest these respiratory allergens without

[30] Technical dossier/Additional data, 11 April 2023/Attachment I.

acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any lipase have been reported in the literature.

[redacted], a product that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[31]), is used as a raw material. In addition, [redacted], known sources of allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the yeast biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues from these sources are not expected to be present in the food enzyme.

The Panel considered that, under the intended conditions of use, the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme[32]

The food enzyme is intended to be used in five 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[33]

Food manufacturing process[(a)]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[(b)]
Brewing processes	Cereals	0.2–**40**
Baking processes	Flour	0.1–**10**
Milk processing for cheese production	Milk	0.005–**0.5**
Production of free fatty acids by hydrolysis	Edible fats and oils	0.1–**1,500**
Production of flavouring preparations from dairy products	Milk	5–**800**
	Cheese, butter, etc.	0.5–200

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

In brewing processes for the production of Japanese sake, the food enzyme is added to rice during steeping or liquefaction before the fermentation step.[34] The triacylglycerol lipase releases unsaturated fatty acids from rice triglycerides, facilitating the flavour development in sake.[35] The food enzyme–TOS remains in the sake.

In baking processes, the food enzyme is added to flour during dough making. The triacylglycerol lipase hydrolyses fats and oils in flour, which improves gas retention and improves the dough structure.[36] The food enzyme–TOS remains in the bakery products.

In cheese production, the food enzyme is added to milk before the coagulation step.[37] The hydrolysis releases fatty acids from the milk fat, thereby intensifying flavour in the curd, consequently shortening the ripening time of the cheese. The food enzyme–TOS remains in the cheese and the whey.

[31] 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.
[32] Technical dossier/p. 11, 56–65, 102; Technical dossier/Extension_intended uses UPDATED; Technical dossier/Additional data, 11 April 2023.
[33] Technical dossier/p. 65; Technical dossier/Extension_intended uses UPDATED.
[34] Technical dossier/p. 89.
[35] Technical dossier/p. 88.
[36] Technical dossier/p. 97.
[37] Technical dossier/p. 92.

For the production of free fatty acids, the food enzyme is added after the degumming step. The hydrolysis of triglycerides results in the production of oils rich in free polyunsaturated fatty acids.[38] The subsequent steps such as molecular distillation, decolorization and deodorization are expected to remove the food enzyme–TOS from the final purified oils. However, the applicant did not support this assumption with data.[39] In line with the guidance document (EFSA CEP Panel, 2021), EFSA considered a scenario of 100% carry-over of the food enzyme–TOS into the final foods, when performing the estimation using the set of input data compiled in Appendix C.

In the production of flavouring preparations from dairy products, the food enzyme is added to the cheese to produce enzyme-modified cheese (EMC)[40] or to milk or butter to produce enzyme-modified milk or dairy preparations.[41] The hydrolysis of milk fat by triacylglycerol lipase releases free fatty acids, intensifying flavour in these products, which are incorporated into a variety of foods (e.g. snacks, beverages) to impart flavours.[42] The food enzyme–TOS remains in these flavouring preparations.

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 shown in Table 2.

3.5.2. Dietary exposure estimation

Chronic exposure to the food enzyme–TOS was calculated by combining the maximum recommended use level with individual consumption data (EFSA CEP Panel, 2021). The estimation involved selection of relevant food categories and application of technical conversion factors (EFSA CEP Panel, 2023). Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 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 43 dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B). The highest dietary exposure was estimated to be 1.033 mg TOS/kg bw per day in infants at the 95th percentile.

Table 3: Summary of the estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.022–0.260 (12)	0.025–0.140 (15)	0.022–0.057 (19)	0.007–0.034 (21)	0.019–0.061 (22)	0.014–0.055 (23)
Min–max 95th percentile (number of surveys)	0.086–1.033 (11)	0.064–0.479 (14)	0.011–0.113 (19)	0.036–0.068 (20)	0.049–0.211 (22)	0.033–0.197 (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.

[38] Technical dossier/p. 94.
[39] Technical dossier/Additional data, 11 April 2023/Response 6.2.
[40] Technical dossier/p. 90.
[41] Technical dossier/p. 95.
[42] Technical dossier/Additional data, 11 April 2023/Responses 6.3, 6.4 and 6.5.

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

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

+: Uncertainty with potential to cause overestimation of exposure.
–: Uncertainty with potential to cause underestimation of exposure.
FoodEx: a standardised food classification and description system.

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.

3.6. Margin of exposure

A comparison of the NOAEL (2,084 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.007–0.26 mg TOS/kg bw per day at the mean and from 0.011–1.033 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 2,017.

4. Conclusions

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme triacylglycerol lipase from the non-genetically modified *L. cylindracea* strain MS-5-OF does not give rise to safety concerns under the intended conditions of use.

5. Documentation as provided to EFSA

Technical dossier Application for authorisation of triacylglycerol lipase from *Candida cylindracea* in accordance with Regulation (EC) No 1331/2008. 6 February 2015. Submitted by Meito Sangyo Co., Ltd.

Additional information. 11 April 2023. Submitted by Meito Sangyo Co., Ltd.

Summary report on technical data and dietary exposure related to triacylglycerol lipase from *Candida cylindracea* by Meito Sangyo Co. Ltd. 5 July 2016. Delivered by Hylobates Consulting and BiCT (Roma, Italy).

Summary report on genotoxicity and subchronic toxicity study related to triacylglycerol lipase produced with a strain of *Candida cylindracea* by Meito Sangyo Co., Ltd. 2016. Delivered by FoBIG (Germany).

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[redacted]

Abbreviations

AMFEP	the Association of Manufacturers and Formulators of Enzyme Products
[redacted]	[redacted]
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 BIOHAZ Panel	EFSA Panel on Biological Hazards
EFSA GMO Panel	EFSA Panel on Genetically Modified Organisms
EINECS	European Inventory of Existing Commercial Chemical Substances
EMC	enzyme-modified cheese
f1, f2, f3	technical factors
FFA	free fatty acids
FAO	Food and Agricultural Organization of the United Nations
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
[redacted]	[redacted]
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kilodalton
LoD	limit of detection
MoE	margin of exposure
[redacted]	[redacted]
NOAEL	no observed adverse effect level
non-GM	non-genetically modified
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety
[redacted]	[redacted]
RM	raw material
TOS	total organic solids
U	Unit
WHO	World Health Organization

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[(a)]	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*, 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 – FoodEx categories and technical factors used to estimate the dietary exposure via the 'Production of free fatty acids by hydrolysis'

FoodEx1 hierarchical code	FoodEx matrix description	FoodEx1 hierarchical level	f1 (converting fats/oils to free fatty acids [FFA])	f2 (average fraction of FFA in respective FoodEx category)	f3 (percentage of FoodEx category containing FFA)
A.01.06.003.001	Cereal bar with fruits	4	2.5	0.005	0.001
A.01.06.003.003	Cereal bar with chocolate	4	2.5	0.005	0.001
A.11.04	Vegetable oil (unspecified)	3	2.5	0.02	0.001
A.17.01	Infant formulae (unspecified)	4	2.5	0.002	1
A.17.01.001	Infant formula, milk-based	3	2.5	0.002	1
A.17.01.002	Infant formula, hypoallergenic	3	2.5	0.002	1
A.17.01.003	Infant formula, soya-based	3	2.5	0.002	1
A.17.01.004	Infant formula, milk and soya-based	3	2.5	0.002	1
A.17.01.005	Infant formula, based on protein hydrolysates	3	2.5	0.002	1
A.17.02	Follow-on formulae (unspecified)	4	2.5	0.002	1
A.17.02.001	Follow-on formula, milk-based	3	2.5	0.002	1
A.17.02.002	Follow-on formula, hypoallergenic	3	2.5	0.002	1
A.17.02.003	Follow-on formula, soya-based	3	2.5	0.002	1
A.17.02.004	Follow-on formula, milk and soya-based	3	2.5	0.002	1
A.17.02.005	Follow-on formula, based on protein hydrolysates	3	2.5	0.002	1
A.18.02.004	Supplements containing special fatty acids	3	2.5	0.8	1

f1, f2, f3: technical factors; FFA: free fatty acids; FoodEx: a standardised food classification and description system.