Adopted: 13 March 2024

DOI: 10.2903/j.efsa.2024.8724

SCIENTIFIC OPINION

efsa JOURNAL

Safety evaluation of the food enzyme phosphodiesterase I from the non-genetically modified *Leptographium procerum* strain FDA

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) | Claude Lambré | José Manuel Barat Baviera | Claudia Bolognesi | Pier Sandro Cocconcelli | Riccardo Crebelli | David Michael Gott | Konrad Grob | Evgenia Lampi | Marcel Mengelers | Alicja Mortensen | Gilles Rivière | Inger-Lise Steffensen | Christina Tlustos | Henk Van Loveren | Laurence Vernis | Holger Zorn | Yrjö Roos | Magdalena Andryszkiewicz | Daniele Cavanna | Ana Criado | Simone Lunardi | Elsa Nielsen | Karin Nørby | Yi Liu | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme phosphodiesterase I (oligonucleotide 5′-nucleotidohydrolase; EC 3.1.4.1) is produced with the non-genetically modified *Leptographium procerum* strain FDA by DSM Food Specialties B.V. The food enzyme is free from viable cells of the production organism. It is intended to be used in the processing of yeast and yeast products. Dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.171 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 1000 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 5848. 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 the 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

5′-exonuclease, EC 3.1.4.1, food enzyme, *Leptographium procerum*, non-genetically modified microorganism, oligonucleotide 5′-nucleotidohydrolase, phosphodiesterase I

CONTENTS

1 | INTRODUCTION

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

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

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

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

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

All food enzymes currently on the EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008[1] on food enzymes.

Two applications have been introduced by the company DSM Food Specialties B.V for the authorisation of the food enzymes glucose oxidase from a genetically modified strain of *Aspergillus niger* (strain ZGL), and phosphodiesterase I from *Leptographium procerum* (strain FDA).

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 two applications fall within the scope of the food enzyme Regulation and contains 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 glucose oxidase from a genetically modified strain of *Aspergillus niger* (strain ZGL), and phosphodiesterase I from *Leptographium procerum* (strain FDA) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2 | Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission's request to carry out the safety assessment of the food enzyme phosphodiesterase I from the non-genetically modified *Leptographium procerum* strain FDA.

[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.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme phosphodiesterase I from a non-genetically modified *Leptographium procerum* strain FDA.

Additional information was requested from the applicant during the assessment process on 31 October 2014, 17 June 2015 and 27 April 2023 and received on 30 April 2015, 26 August 2015 and 26 October 2023, respectively (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, 2009a) 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, 2009b) has 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

IUBMB nomenclature	Phosphodiesterase I
Systematic name	Oligonucleotide 5′-nucleotidohydrolase
Synonyms	5′-Exonuclease;5′-phosphodiesterase;5′-nucleotide phosphodiesterase
IUBMB No	3.1.4.1
CAS No	9025-82-5
EINECS No	232-806-5

Phosphodiesterases I catalyse the sequential removal of 5′-nucleotides from the 3′-hydroxy termini of 3′-hydroxy-terminated oligonucleotides. The enzyme under assessment is intended to be used in the processing of yeast and yeast products.

3.1 | Source of the food enzyme

The phosphodiesterase I is produced with the non-genetically modified filamentous fungus *Leptographium procerum* strain FDA, which is deposited at the Westerdijk Fungal Biodiversity Institute (the Netherlands) with the deposit number [redacted].[4] The production strain was identified as *L. procerum* [redacted].

3.2 | Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004,[6] with food safety procedures based on Hazard Analysis and Critical Control Points, and in accordance with current good manufacturing practice.[7]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, 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 filtration. 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. The food enzyme is further purified by ion-exchange

[4]Technical dossier/Additional data October 2023/Annex 1.

[5]Technical dossier/Additional data October 2023/Annex 2.

[6]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.

[7]Technical dossier/p. 42 and Annex 9.

chromatography.[8] 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.[9]

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 phosphodiesterase I is a single polypeptide chain of [redacted] amino acids.[10] The molecular mass of the mature protein, calculated from the amino acid sequence, is around [redacted] kDa.[11] The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches.[12] The gels showed a single major protein band corresponding to an apparent molecular mass of about [redacted] kDa. No other enzymatic activities were reported.[13]

The in-house determination of phosphodiesterase I activity is based on the hydrolysis of guanosine 2′- and 3′-monophosphate (reaction conditions: pH 5.6, 65°C, 20 min). The enzymatic activity is determined by measuring the release of phosphate, which forms a yellow-coloured complex with a molybdate/vanadate reagent which is measured spectrophotometrically at 405 nm. The enzyme activity is expressed in 5′-phosphodiesterase units (5′FDU)/mL. One 5′FDU is defined as the amount of enzyme that liberates 1 µmol phosphate per minute under the conditions of the assay.[14]

The food enzyme has a temperature optimum around 65°C (pH 5.6) and a pH optimum around pH 7.5 (65°C). Thermostability was tested after pre-incubation of the food enzyme at different temperatures and for different time periods. The enzyme activity decreased when incubated at 65°C for 2 min, showing no residual activity above 80°C.[15]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch produced for the toxicological tests (Table 1).[16] The mean total organic solids (TOS) of the batches for commercialisation was 17.3% and the mean enzyme activity/TOS ratio was 41.1 5′FDU/mg TOS.

TABLE 1 Composition of the food enzyme.

		Batches			
Parameters	**Unit**	**1**	**2**	**3**	**4[a]**
Phosphodiesterase I activity	5′FDU/g[b]	7390	6410	7485	4465[d]
Protein	%	2.8	2.0	2.6	1.4[e]
Ash	%	0.31	0.28	0.5	0.48[d]
Water	%	81.9	82.2	83	89.9[d]
Total organic solids (TOS)[c]	%	17.8	17.5	16.5	9.6[d]
Activity/TOS ratio	5′FDU/mg TOS	41.5	36.6	45.3	46.6[d]

[a]Batch used for Ames test, chromosomal aberration test and repeated 90-day oral toxicity study.
[b]5′FDU: 5′ phosphodiesterase units (see Section 3.3.1).
[c]TOS calculated as 100% – % water – % ash.
[d]Value determined under GLP conditions.
[e]Value determined under non-GLP conditions.

[8]Technical dossier/pp. 42–47 and Annex 10.
[9]Technical dossier/Annex 11.
[10]Technical dossier/Additional data April 2015/Annex 6.
[11]Technical dossier/Additional data April 2015.
[12]Technical dossier/p. 34.
[13]Technical dossier/p. 35; Additional data April 2015/Annex 3; Additional data October 2023/Answer 6.
[14]Technical dossier/p. 34 and Annex 2.
[15]Technical dossier/pp. 35–36; Additional data April 2015.
[16]Technical dossier/p. 32/Annex 2; Additional data April 2015/Annexes 3 and 7; Additional data August 2016.

3.3.3 | Purity

The lead content in the three commercial batches was below 5 mg/kg, which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[17]

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). No antimicrobial activity was detected in any of the tested batches.

Strains of *Leptographium*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The applicant did not provide information on the potential secondary metabolites produced under the conditions of fermentation that might be present in the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

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

3.3.4 | Viable cells of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted].[18]

3.4 | Toxicological data

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, has been provided.

The batch 4 (Table 1) used in these studies has a similar activity/TOS value as the batches intended for commercialisation and was considered suitable as a test item.

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).[19]

Two independent experiments were carried out with four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA, either with or without metabolic activation (S9-mix), applying the pre-incubation method and triplicate plating. In the first experiment, eight concentrations of the food enzyme were tested: 3, 10, 33, 100, 333, 1000, 3330 and 5000 µg TOS/plate. In the second experiment, all strains were tested at five concentrations: 100, 333, 1000, 3330 and 5000 µg TOS/plate.

No cytotoxicity or precipitation was observed in any strain or at any concentration of the food enzyme tested, with or without S9-mix. In the first experiment, no increase of revertant colony numbers was reported in any strain, at any concentration tested with or without S9-mix. In the second experiment with S9-mix, a concentration-dependent increase in revertant counts was seen for TA98, TA100 and TA1535 (mutation factor of 2, 2.4 and 3.8, respectively) compared to vehicle controls. These increases were due to the comparison with the low value of the concurrent controls at the lower limit of the historical control data and were not observed in the first experiment. The Panel considered these increases as not biologically relevant. For all remaining results, no biologically relevant increases in the number of revertant colonies above the control values were seen in the first or the second experiment, with or without S9-mix.

The Panel concluded that the food enzyme phosphodiesterase I 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.[20]

Two separate experiments were performed with duplicate cultures of human peripheral whole blood lymphocytes. In the range-finding test, the cell cultures were treated with the food enzyme either with or without metabolic activation (S9-mix).

[17]Technical dossier/Additional data April 2015/Annexes 3 and 7.

[18]Technical dossier/Additional data October 2023/Annex 3.

[19]Technical dossier/Annex 12.

[20]Technical dossier/Annex 13.

No cytotoxicity above 50% was seen at any concentration tested up to 5000 µg TOS/mL in a short-term treatment (3-h exposure with 21-h recovery period) with or without S9-mix. In long-term treatments (24-h or 48-h exposure without recovery), cytotoxicity of 50% was seen above 3330 or 1000 µg TOS/mL, respectively, without S9-mix and no cytotoxicity was seen up to 5000 µg TOS/mL in a short-term treatment with long-term recovery (3-h exposure with 45-h recovery period) with S9-mix.

In the first experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations after short-term treatment at concentrations of 1000, 3330 and 5000 µg TOS/mL, either with or without S9-mix. In the second experiment, cells were exposed to the food enzyme and scored for chromosomal aberrations at concentrations of 300, 4000 and 5000 µg TOS/mL after 24-h exposure without S9-mix, at 100, 2000 and 4000 µg TOS/mL after 48-h exposure without S9-mix and at 1000, 3330 and 5000 µg TOS/mL, after 3-h exposure and 45-h recovery with S9-mix.

A cytotoxicity of 43% and 56% was observed at the highest concentration tested after 24- and 48-h treatment, respectively. The frequency of chromosomal aberrations was not statistically significantly different when compared to the negative controls at any of the concentrations tested with or without S9-mix, in any of the experiments. All results were within the historical control range.

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

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

The repeated dose 90-day oral toxicity study was performed following GLP[21] and in accordance with the OECD Test Guideline 408 (OECD, 1998) with the following deviation: Urea was not determined in the clinical chemistry investigation. The Panel considered that this deviation is minor and did not have an impact on the evaluation of the study.

Groups of 10 male and 10 female Wistar (HsdCpd:Wu) rats received by gavage the food enzyme in doses of 100, 300 or 1000 mg TOS/kg bw per day. Controls received the vehicle ('Milli-Q' water).

No mortality was observed.

Body weight gain was statistically significantly increased on days 85–90 of administration in mid-dose males (+285%) and on days 22–29 in low- and mid-dose females (+63%, +64%). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically, there was no dose–response relationship and the changes were without a statistically significant effect on the final body weight and the final body weight gain.

In the functional observations, a statistically significant higher ambulatory time was observed in interval 1 (+30%), 3 (+48%) and total (+31%) in low-dose males and a higher motor activity score in interval 1 in low- and mid-dose females (+26%, +20%) and in interval 3 in high-dose females (+25%); and a lower ambulatory time in interval 1 in low-dose females (–21%), a lower horizontal counts in interval 1 in low- and mid-dose females (–33%, –26%) and a lower ambulatory counts in interval 1 in low- and mid-dose females (–38%, –31%). The Panel considered the changes as not toxicologically relevant, as they were only recorded sporadically (all parameters), they were only observed in one sex (motor activity, horizontal counts, ambulatory counts), there was no consistency between the changes in males and females (ambulatory time) and there was no dose–response relationship (all parameters except for motor activity in interval 3 in females).

Haematological investigations revealed a statistically significant decrease in the haemoglobin concentration (Hb) in low- and high-dose males (–4%, –4%) and in all treated female groups (–4%, –3%, –4%), in the haematocrit (Hct) in high-dose females (–3%), in the mean corpuscular haemoglobin concentration (MCHC) in low- and mid-dose females (–3%, –3%) and an increased prothrombin time (PT) in mid-dose females (+4%). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex (Hct, MCHC, PT), there was no dose–response relationship (Hb, MCHC, PT), the changes were small (all parameters), there were no changes in other relevant parameters (for Hb, Hct, MCHC – in the red blood cell count; for PT – in the platelet count and in the other blood coagulation parameter investigated, i.e. the activated partial thromboplastin time) and the changes were within the historical control values.

Clinical chemistry investigations revealed a statistically significant decrease in albumin (+5%) and in the albumin/globulin ratio (+13%) in high-dose males. The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex, the changes were small and the changes were within the historical control values.

Statistically significant changes in organ weights included a decrease in the relative thymus weight in low- and high-dose males (–20%, both). The Panel considered the changes as not toxicologically relevant, as they were only observed in one sex, there was no dose–response relationship and there were no histopathological changes in thymus.

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

The Panel identified a no observed adverse effect level (NOAEL) of 1000 mg TOS/kg bw per day, the highest dose tested.

3.4.3 | Allergenicity

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

[21]Technical dossier/Annex 14.

The potential allergenicity of the phosphodiesterase I produced with the *Leptographium procerum* strain FDA 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.[22]

No information was available on oral and respiratory sensitisation or elicitation reactions of this phosphodiesterase I. No allergic reactions upon dietary exposure to phosphodiesterases have been reported.

Soybean flour or meal, products that may cause allergies or intolerances (listed in the Regulation (EU) No 1169/2011[23]), are used as raw materials. However, during the fermentation process, these will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that no potentially allergenic residues from these sources are present in the food enzyme.

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

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in the processing of yeast and yeast products 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.[24]

Food manufacturing process[a]	Raw material (RM)	Recommended use level (mg TOS/kg RM)[b]
Processing of yeast and yeast products	Autolysed yeast, whole yeast cell, yeast extracts	978–**2934**
	Yeast cell walls	194–1467

[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.

In yeast processing, the food enzyme is added to different yeast components (whole yeast cells, autolysed yeast, yeast extracts or yeast cell walls)[25] to hydrolyse oligonucleotides and produce 5′-guanosine monophosphate (5′-GMP) and other 5′-nucleotides. 5′-GMP modifies the taste characteristics in yeast extracts. These are used (in paste or powder form) as an ingredient in a wide range of foods, such as soups and savoury sauces.[26] The food enzyme-TOS remains in yeast extracts and the final foods.

Based on data provided on thermostability (see Section 3.3.1), it is expected that the food enzyme is inactivated during the processing of yeast and yeast products.

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).

[22]Technical dossier p. 14, pp. 60–61/Additional data April 2015/Annex 6.

[23]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.

[24]Technical dossier/Additional information October 2023-Part I/Answer 12.

[25]Technical dossier/p. 52.

[26]Technical dossier/p. 70.

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 0.171 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)	0.001–0.026 (12)	0.006–0.073 (15)	0.005–0.059 (19)	0.003–0.026 (21)	0.002–0.015 (22)	0.001–0.027 (23)
Min–max 95th percentile (number of surveys)	0.001–0.117 (11)	0.016–0.093 (14)	0.016–0.171 (19)	0.010–0.076 (20)	0.006–0.052 (22)	0.004–0.070 (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	
The higher use level given for the process 'processing of yeast and yeast products' was used in the calculation.	+
Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level	+
Selection of broad FoodEx categories for the exposure assessment	+
Use of recipe fractions in disaggregation 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.

The conservative approach applied to estimate the exposure to the food enzyme-TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to an overestimation of the exposure.

3.6 | Margin of exposure

A comparison of the NOAEL (1000 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0.001–0.073 mg TOS/kg bw per day at the mean and from 0.001–0.171 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure of at least 5848.

4 | CONCLUSIONS

Based on the data provided and the derived margin of exposure, the Panel concluded that the food enzyme phosphodiesterase I produced with the non-genetically modified *Leptographium procerum* strain FDA does not give rise to safety concerns under the intended conditions of use.

DOCUMENTATION AS PROVIDED TO EFSA

Technical dossier "Application for authorisation of phosphodiesterase I from *Leptographium procerum* in accordance with Regulation (EC) No 1331/2008". 26 November 2013. Submitted by DSM Food Specialties B.V.

Additional information. 30th April 2015. Submitted by DSM Food Specialties B.V.

Additional information. 26th August 2015. Submitted by DSM Food Specialties B.V.

Additional information. 26th October 2023. Submitted by DSM Food Specialties B.V.

Summary report on genotoxicity and subchronic toxicity study. May 2014. Delivered by Forschungs- und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Freiburg, Germany.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	good laboratory practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
MOE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
TOS	total organic solids
WHO	World Health Organization

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-2013-01006

PANEL MEMBERS

José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

COPYRIGHT FOR NON-EFSA CONTENT

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

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). (2009a). Guidance of EFSA prepared by the scientific panel of food contact material, enzymes, flavourings and processing aids on the submission of a dossier on food enzymes. *EFSA Journal*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority). (2009b). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal*, *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 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), 1–31. 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 micro-organisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

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

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). (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

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 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., Roos, Y., Andryszkiewicz, M., … Chesson, A. (2024). Safety evaluation of the food enzyme phosphodiesterase I from the non-genetically modified *Leptographium procerum* strain FDA. *EFSA Journal*, *22*(4), e8724. https://doi.org/10.2903/j.efsa.2024.8724

APPENDIX A

Dietary exposure estimates to the food enzyme–TOS in details

Appendix A can be found in the online version of this output (in the 'Supporting information' section). The file contains two sheets, corresponding to two tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

APPENDIX B

Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[a]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

[a]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).