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Safety evaluation of the food enzyme triacylglycerol lipase from *Aspergillus niger* (strain LFS)

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (EFSA CEP Panel), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüschweiler, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Klaus-Dieter Jany*, Sirpa Kärenlampi*, André Penninks*, Jaime Aguilera, Margarita Aguileria-Gómez, Davide Arcella, Christine Horn, Natália Kovalkovičová, Yi Liu, Joaquim Manuel Maia and Andrew Chesson

Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is produced with a genetically modified *Aspergillus niger* strain LFS by DSM Food Specialties B.V.. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and recombinant DNA. The triacylglycerol lipase food enzyme is intended to be used in baking processes. Based on the maximum use levels, dietary exposure to the food enzyme-total organic solids (TOS) was estimated to be up to 0.020 mg TOS/kg body weight (bw) per day. The toxicity studies were carried out with an asparaginase from *A. niger* (strain ASP). The Panel considered this enzyme as a suitable substitute to be used in the toxicological studies, because they derive from the same recipient strain, the location of the inserts are comparable, no partial inserts were present and the production methods are essentially the same. Genotoxicity tests did not raise 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 (NOAEL) at the highest dose of 1,038 and 1,194 mg TOS/kg bw per day (for males and females, respectively) that, compared with the estimated dietary exposure, results in a sufficiently high margin of exposure (MoE) (of at least 51,900). Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood to occur is considered to be 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.

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Keywords: food enzyme, triacylglycerol lipase, triacylglycerol acylhydrolase, EC 3.1.1.3, lipase, *Aspergillus niger*, genetically modified microorganism

Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

* Member of the former Working Group on "Enzymes" of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Beat Johannes Brüschweiler, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Alicja Mortensen, Gilles Rivière, Vittorio Silano, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn

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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 micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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:

i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
ii) there is a reasonable technological need;
iii) 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 on food enzymes.

Two applications have been introduced by the companies 'DSM Food Specialties B.V" and "Amano Enzyme inc" for the authorisation of the food enzymes Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* (strain LFS) and AMP deaminase from *Aspergillus melleus* (strain AE-DN).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls 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 triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* (strain LFS) and AMP deaminase from *Aspergillus melleus* (strain AE-DN) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 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, p. 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 a genetically modified *Aspergillus niger* (strain LFS).

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 genetically modified *Aspergillus niger* (strain LFS).

Additional information was requested from the applicant during the assessment process on 19 May 2015, 22 May 2017, 13 July 2017, 23 February 2018 and 25 June 2018, and was consequently provided (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) as well as in the EFSA 'Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011) and following relevant guidelines of the EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature: Triacylglycerol lipase
Systematic name: Triacylglycerol acylhydrolase
Synonyms: Lipase
IUBMB No.: EC 3.1.1.3
CAS No.: 9001-62-1
EINECS No.: 232-619-9

The triacylglycerol lipase catalyses the hydrolysis of triacylglycerols into fatty acids, and mono- and diacylglycerols. The triacylglycerol lipase preferably hydrolyses ester bonds at positions one and three of the triacylglycerol molecule. However, triacylglycerol lipase hydrolyses also ester bonds of other lipids, including phospholipids and glycolipids. It is intended to be used in baking processes.

3.1. Source of the food enzyme

The triacylglycerol lipase is produced with a genetically modified filamentous fungus *A. niger* strain LFS. According to the CEF Guidance, the certificate of deposit of the strain in a public validated culture collection should be provided. [4] The Panel noted that this would not allow a verification of the strain independently of the company.

[5]

3.1.1. Characteristics of the parental and recipient microorganisms

[4] Technical dossier/Annex II-17.
[5] Technical dossier/Annex II-2.

[6]

3.1.2. Characteristics of introduced sequences

3.1.3. Description of the genetic modification process

[7]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *A. niger* LFS differs from the parental strain

Genotypic stability of the production strain was confirmed

[6] Technical dossier/Additional information June 2015/Annex-3.
[7] Technical dossier/Additional information November 2018.

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004[8], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

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 leaving a supernatant containing the food enzyme. 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 weight material passes the filtration membrane and is discarded. 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.

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 is a single polypeptide chain of 346 amino acids, including a pre-pro sequence of 30 amino acids and a sequence of 42 amino acids at the C-terminus, which are cleaved off during secretion of the enzyme. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be 28.7 kDa. The homogeneity was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. Gels presented for three independent food enzyme batches were comparable, showing the target protein migrating at about 41 kDa in all batches.[9] No enzymatic side activities were reported.

The in-house determination of triacylglycerol lipase activity is based on hydrolysis of the substrate *p*-nitrophenyl-palmitate, resulting in the release of *p*-nitrophenol (reaction conditions: pH 8.5, 37°C, 5 min). The enzyme activity is expressed in DSM Lipase Units (DLU)/g. One DLU is defined as the amount of enzyme that liberates 1 μmol *p*-nitrophenol per minute under the standard conditions of the assay.[10]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature maximum around 25°C (pH 8.5) and no distinct pH maximum in the pH range of 6–9. No thermostability studies were provided, but considering the temperature profile data no enzyme activity was detectable at temperatures higher than 50°C.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 12.1% (range 9.3–14.9%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 100.2 DLU/mg TOS.

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[8] 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.

[9] Technical dossier/Additional information August 2017.

[10] Technical dossier/Annex I-2.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches		
		1	2	3
Triacylglycerol lipase activity	DLU/g batch [(a)]	8,120	11,700	17,500
Protein	%	5.9	8.1	9.8
Ash	%	0.58	0.54	0.55
Water	%	90.1	87.3	84.6
Total organic solids (TOS)[(b)]	%	9.3	12.2	14.9
Triacylglycerol lipase activity/mg TOS	DLU/mg TOS	87.3	95.9	117.4

(a): DLU: DSM Lipase Units (see Section 3.3.1).
(b): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The food enzyme preparation (includes 80% flour as formulation agent) complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[11,12]

The food enzyme preparation complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 colony forming unit (CFU) per gram.[12] No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[12]

Strains of *Aspergillus* in common with most filamentous fungi have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The applicant did not provide information on the secondary metabolites produced under the conditions of fermentation which might contribute to the food enzyme–TOS. The absence of ochratoxin A and fumonisins was demonstrated. The possible presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme–TOS.[13,14]

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain [15]

The absence of recombinant DNA in the enzyme product was demonstrated [16]

3.4. Toxicological data

3.4.1. Choice of test item

No toxicological studies were provided for the triacylglycerol lipase food enzyme produced with the *A. niger* strain LFS. Instead, the applicant argued that the assessment of this triacylglycerol lipase produced by *A. niger* strain LFS could be based on toxicological data from another food enzyme – an asparaginase produced with the *A. niger* strain ASP, previously submitted to EFSA (Question No EFSA-Q-2013-00895) following the EFSA guidance (EFSA CEF Panel, 2009).

The focus of the toxicological studies of food enzymes is the assessment of non-protein components of TOS. Only rarely is the enzyme protein itself considered a potential hazard.

[11] Limit of detection (LOD): Pb = 0.006 mg/L; Technical dossier/Annex I-4 and Additional information August 2017.
[12] Technical dossier/Annex I-3.
[13] Technical dossier/Annex I-4.
[14] Limit of quantification (LOQ): Ochratoxin A = 0.1 μg/kg; Fumonisins (B_1, B_2 and B_3) = 10 μg/kg each toxin.
[15] Technical dossier/Annex II-13 and Additional information June 2015.
[16] Technical dossier/Annex II-16 and Additional information October 2014.

The production strain of the asparaginase was developed from the same recipient strain as that for the triacylglycerol lipase under assessment (*A. niger* LFS) Therefore, the genetic differences between *A. niger* LFS and *A. niger* ASP are not expected to result in a different toxigenic potential.

The batch of asparaginase food enzyme from the *Aspergillus niger* strain ASP, used for toxicological studies, was produced according to a standard procedure similar to the one described in Section 3.2 of this opinion.[17] According to the data provided by the applicant, the raw materials used and the steps involved in the manufacturing of the asparaginase and triacylglycerol lipase food enzymes from *A. niger* strains (ASP and LFS, respectively) are essentially the same in both processes, and the temperature and pH conditions used during fermentation are similar. To produce the final non-formulated ASP batch used for toxicological testing, an additional spray-drying step was performed. The spray-dried batch compared to the mean of ASP commercial batches resulted in a similar specific activity of 38.5 vs 38.1 Asparaginase Units (ASPU)/mg TOS and a higher level of inorganic constituents.

Taking the microbiological and technical data into account, the Panel considered the asparaginase as a suitable substitute for the triacylglycerol lipase in the toxicological studies.

3.4.2. Genotoxicity

3.4.2.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was made according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[18] Four strains of *Salmonella* Typhimurium (TA1535, TA100, TA1537 and TA98) and *E. coli* WP2*uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the 'plate incorporation assay'. One experiment in triplicate was performed using five different concentrations of the food enzyme (62, 185, 556, 1,667 and 5,000 μg/plate corresponding to ca. 56, 166, 499, 1,495 and 4,484 μg TOS/plate). No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

The Panel concluded that the food enzyme asparaginase did not induce gene mutations under the test conditions employed in this study.

3.4.2.2. *In vitro* mammalian chromosomal aberration test

An *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP in human peripheral blood lymphocytes with and without metabolic activation (S9-mix).[19]

In a first experiment, the cultures were exposed at concentrations of 2,000, 3,000 and 5,000 μg of food enzyme/mL (corresponding to ca. 1,794, 2,690 and 4,484 μg TOS/mL) applying a 4 + 24 h treatment in the presence and absence of S9-mix, and a continuous treatment 24 + 24 h treatment without S9-mix. In a second experiment, 3,000, 4,000 and 5,000 μg of food enzyme/mL (corresponding to ca. 2,690, 3,587 and 4,484 μg TOS/mL) were tested in a pulse treatment 4 + 24 h with the S9-mix. A slight cytotoxicity was observed after the pulse treatment with and without metabolic activation. The test substance was clearly cytotoxic at the highest concentration analysed after a continuous treatment (mitotic index was reduced to the 46% of that of the concurrent controls). The enzyme preparation did not induce a significant increase in structural or numerical chromosome aberrations in cultured human blood lymphocytes, in either of the two independently repeated experiments.

[17] Technical dossier/Additional information September 2017.
[18] Technical dossier/Annex I-16.
[19] Technical dossier/Annex I-17.

The Panel concluded that the food enzyme did not induce chromosome aberrations in cultured human blood lymphocytes, under the test conditions employed for this study.

3.4.3. 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.[20] Groups of 20 male and 20 female Wistar rats received 0.2%, 0.6% or 1.8% of the food enzyme in the diet in doses corresponding to 117, 351 and 1,038 mg TOS/kg body weight (bw) per day for males, and 135, 405 and 1,194 mg TOS/kg bw per day for females. Controls received the same diet with no enzyme added.

No mortality was observed.

Among haematology parameters the absolute number and the percentage of monocytes were statistically significantly increased in high-dose males on day 8 of the study, but not on day 44 or at termination. Therefore, the Panel considered these findings as incidental. The number (in all male dose groups) and percentage (in low- and high-dose males) of basophiles were statistically significantly decreased at termination. As the differences to controls were minor, the decreases were not dose-related and other related effects were absent, these findings were not considered to be of toxicological significance.

Clinical chemistry examination revealed a statistically significantly increased blood urea concentration in low- and mid-dose females on day 44 (mmol/L: 6.2 $\pm$ 0.2 and 7.2 $\pm$ 0.7 vs 5.6 $\pm$ 0.1 in the control group). As these findings were not observed at termination, the differences to controls were minor and not dose related they were considered as incidental and not of toxicological significance.

In urinalysis, the microscopy of the sediment revealed a slight but statistically significant increase in triple phosphate crystals in high-dose males, which were not considered of toxicological importance.

The relative weights of testes (8.38 g/kg bw) and epididymides (3.49 g/kg bw) in the low-dose group were statistically significantly lower than in controls (9.18 g/kg bw and 3.69 g/kg bw, respectively). As these findings were not present at higher doses and were not associated with any microscopically changes in these organs, they were considered as incidental and not of toxicological relevance.

No other significant effects were observed.

The Panel identified a no observed adverse effect level (NOAEL) at the highest dose tested equal to 1,038 and 1,194 mg TOS/kg bw per day for males and females, respectively.

3.4.4. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient, which may be used in the final formulation.

The potential allergenicity of the triacylglycerol lipase produced with the genetically modified *A. niger* strain LFS was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[21]

No information was available on oral sensitisation or elicitation reactions of this triacylglycerol lipase. Several studies have shown that adults with occupational asthma may be able to ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). In addition, no allergic reactions upon dietary exposure to any triacylglycerol lipase have been reported in the literature. Therefore, it can be concluded that the likelihood of an allergic reaction upon oral ingestion of this triacylglycerol lipase, produced with the genetically modified *A. niger* strain LFS, in individuals respiratory sensitised to triacylglycerol lipase, cannot be excluded, but the likelihood of such a reaction to occur is considered to be low.

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

[20] Technical dossier/Annex I-18.
[21] Technical dossier/Annex I-19.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes at a recommended use level of up to 1.64 mg TOS/kg flour.

The food enzyme is used to facilitate handling of the dough, to improve its structure and behaviour during baking as well as to reduce batter viscosity. The food enzyme is added during the preparation of the dough at the beginning of this process.

The food enzyme remains in the dough. Based on the biochemical data provided (see Section 3.3.1), it is expected that the triacylglycerol lipase would be inactivated during baking processes.

3.5.2. Dietary exposure estimation

Chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for bodyweight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed average 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 2: Summary of 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)	0.000–0.005 (10)	0.003–0.010 (14)	0.004–0.009 (19)	0.002–0.006 (18)	0.002–0.004 (19)	0.002–0.003 (18)
Min–max 95th percentile (number of surveys)	0.002–0.020 (8)	0.009–0.017 (12)	0.008–0.018 (19)	0.005–0.012 (17)	0.004–0.007 (19)	0.003–0.006 (18)

TOS: total organic solids.

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 3.

Table 3: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact
Model input data	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Possible national differences in categorisation and classification of food	+/–
Model assumptions and factors	
FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS	+
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	+/–

TOS: total organic solids.
+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

The conservative approach applied to the exposure estimate to food enzyme–TOS, in particular assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure to this specific enzyme under the intended condition of use.

3.6. Margin of exposure

A comparison of the NOAEL (1,038 mg TOS/kg bw per day for males and 1,194 mg TOS/kg bw per day for females) from the 90-day study with the derived exposure estimates of 0.000–0.010 mg TOS/kg bw per day at the mean and from 0.002–0.020 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MoE) of at least 51,900.

The Panel considered the MoE sufficient to accommodate any potential additional uncertainties resulting from read-across from toxicological studies performed on asparaginase, produced using the same recipient strain, as a substitute.

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 genetically modified *A. niger* strain LFS does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considers the food enzyme free from viable cells of the production organism and recombinant DNA.

Documentation provided to EFSA

1) Technical dossier "Triacylglycerol lipase from *Aspergillus niger* (strain LFS)". November 2014. Submitted by DSM Food Specialities B.V.
2) Additional information received, June 2015. DSM Food Specialities B.V.
3) Additional information received, August 2017. DSM Food Specialities B.V.
4) Additional information received, September 2017. DSM Food Specialities B.V.
5) Additional information received, April 2018. DSM Food Specialities B.V.
6) Additional information received, November 2018. DSM Food Specialities B.V.
7) Summary report on technical data and dietary exposure related to triacylglycerol lipase from a genetically modified strain of *Aspergillus niger* (strain LFS) by DSM. May 2015. Delivered by Hylobates Consulting and BiCT.
8) Summary report on GMM part for Triacylglycerol lipase from *Aspergillus niger* (strain LFS), EFSA-Q-2014-00325. December 2014. Delivered by Technical University of Denmark (Lyngby, Denmark).

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Abbreviations

ASPU	Asparaginase Unit
bp	base pair
bw	body weight
CAS	Chemical Abstracts Service
CBS	Centraalbureau voor Schimmelcultures
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DLU	DSM Lipase Unit
EC	European Commission and Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Addictives
LOD	limit of detection
LOQ	limit of quantification
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–poly acrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5630).

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 one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(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).