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Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Aspergillus niger* strain NZYM-DB

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase EC 3.1.1.3) is produced with a genetically modified *Aspergillus niger* strain NZYM-DB by Novozymes A/S. 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 food enzyme is intended to be used in an immobilised form in the production of modified fats and oils by interesterification. Based on the estimated use levels recommended for interesterification of fats and oils and individual data from the EFSA Comprehensive European Food Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 0.75 mg TOS/kg body weight (bw) per day in European populations. 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 at the highest dose of 1,132 mg TOS/kg bw per day, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 1,500. 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 of such reactions to occur is likely to be low. Based on the data provided, the immobilisation of the food enzyme and the removal of total organic solids during fats and oils processing, the Panel concluded that the 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, lipase, *Aspergillus niger*, genetically modified microorganism

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Table of 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 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, 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 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.

Six applications have been introduced by the companies "Decernis, LLC", "Keller and Heckman LLP", the Association of Manufacturers and Formulators of Enzyme Products (AMFEP)" and "Novozymes A/S" for the authorisation of the food enzymes. Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) respectively.

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

[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.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Cyclomaltodextrin glucanotransferase from *Geobacillus stearothermophilus*, Dextranase from *Chaetomium gracile*, Subtilisin from *Bacillus licheniformis*, Mucorpepsin from *Rhizomucor miehei*, Animal rennet consisting of chymosin and pepsin from the abomasum of *Bos primigenius* (cattle), *Bubalus bubalis* (buffalo), *Capra aegagrus hircus* (goat) and *Ovis aries* (sheep), and Lipase from a genetically modified strain of *Aspergillus niger* (strain NZYM-DB) 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 food enzyme triacylglycerol lipase from the genetically modified *Aspergillus niger* strain NZYM-DB.

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 *A. niger* (strain NZYM-DB).

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 17 May 2019 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 17 May 2019, the applicant requested a clarification teleconference, which was held on 23 September 2019.

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, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) 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; Triglyceride lipase; Glycerol ester hydrolase
IUBMB No:	3.1.1.3
CAS No:	9001-62-1
EINECS No:	232-619-9

In the absence or at very low concentrations of water, the triacylglycerol lipase catalyses transesterifications of fatty acids in glycerides. It is intended to be used in an immobilised form in the production of modified fats and oils by interesterification.[4]

3.1. Source of the food enzyme

The triacylglycerol lipase is produced with a genetically modified filamentous fungus *Aspergillus niger* strain NZYM-DB, which is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany) with deposit number [redacted].[5]

[4] Technical dossier/Additional data August 2019.
[5] Technical dossier/Annex 4/Annex A4.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *A. niger* [redacted]. The first intermediate strain [redacted] is deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GMbH (DSMZ, Germany) with accession number [redacted] It was derived from the parental strain by [redacted]. Strain [redacted] was identified as *A. niger* by [redacted][6]

[redacted][7]

In *A. niger*, the following selection systems were used for development of the recipient strain:

- [redacted]

During the development of the recipient strain [redacted]

3.1.2. Characteristics of introduced sequences

The gene encoding the triacylglycerol lipase [redacted][9]

[redacted][10]

[6] Technical dossier/Annex 4/p. 7 and Annex A2.
[7] Technical dossier/Annex 4/Section 1.1.10.
[8] Technical dossier/Annex 4/Section 1.3.2.
[9] Technical dossier/Annex 4/p. 17–19.
[10] Technical dossier/Annex 4/Section 1.3.1.

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise the triacylglycerol lipase [redacted]. For this purpose, [redacted][11]

The recipient strain [redacted]

The production strain NZYM-DB contains [redacted].[12]

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* NZYM-DB differs from the recipient strain [redacted] in its capability to produce the triacylglycerol lipase enzyme [redacted][8]

The absence of antibiotic resistance genes used during the genetic modification was confirmed by Southern analysis of the production strain NZYM-DB with probes specific to [redacted] The absence of the [redacted] was also confirmed.[13]

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[14], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).[15]

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. Prior to final filtration, the food enzyme concentrate is stabilised.[16] 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.[17]

The triacylglycerol lipase is immobilised [redacted]

[11] Technical dossier/Annex 4/p. 17 and Section 1.3.1.
[12] Technical dossier/Annex 4/Section 1.3.2; and Additional data August 2019.
[13] Technical dossier/Annex 4/Annex D1; and Additional data August 2019.
[14] 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.
[15] Technical dossier/p. 12.
[16] Technical dossier/Section 3.2.1.2.5.
[17] Technical dossier/Annex 6 and Additional data August 2019.

[redacted][18]

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 [redacted] amino acids. The molecular mass, derived from the amino acid sequence, was calculated to be [redacted] kDa.[19] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches, with a single major protein band migrating between the marker proteins of 30 and 45 kDa in all batches.[20] The food enzyme was tested for α-amylase, glucoamylase and protease activities and none were detected. No other enzymatic activities were reported.[21]

The in-house determination of triacylglycerol lipase activity is based on the hydrolysis of the substrate tributyrin, forming butyric acid (reaction conditions: pH 7.0, 30°C, at least 1.5 min). The enzymatic activity is determined by titration of the released acid with sodium hydroxide. The enzyme activity is expressed in Lipase Units (CA standard) (LU(CA))/g. One LU(CA) is defined as the amount of enzyme activity which releases 1 μmol of butyric acid per minute under the given standard conditions.[22]

The free food enzyme has a temperature optimum between 50 and 70°C (pH 6.0) and a pH optimum around pH 7.0 (30°C). Thermostability was tested after a pre-incubation of the free food enzyme for 30 min at different temperatures (pH 6.0) and triacylglycerol lipase activity was stable up to 50°C. With the increasing temperature, activity was reduced, with no activity detected at 95°C.[23]

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).[24] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 10.7% and the average enzyme activity/TOS ratio was 305 LU(CA)/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4[(a)]
Triacylglycerol lipase activity	LU(CA)/g batch[(b)]	26,900	38,100	30,900	7,030
Protein	%	4.6	6.2	5.0	NA[(c)]
Ash	%	0.2	0.2	0.3	1.2
Water	%	87.8	88.0	91.3	88.0
Total organic solids (TOS)[(d)]	%	12.0	11.8	8.4	10.8
Triacylglycerol lipase activity/mg TOS	LU(CA)/mg TOS	224	323	368	65

(a): Batch used for the toxicological studies.
(b): LU(CA): Lipase Unit (CA standard) (see Section 3.3.1).
(c): NA: not analysed.
(d): TOS calculated as 100% – % water – % ash.

[18] Technical dossier/p. 53-55 and Additional data August 2019.
[19] Technical dossier/p. 31 and Annex 1.
[20] Technical dossier/p. 33.
[21] Technical dossier/p. 40.
[22] Technical dossier/Annex 3.01.
[23] Technical dossier/Annex 9.
[24] Technical dossier/p. 32 and 62, and Additional data August 2019.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 0.5 mg/kg which 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). In addition, the levels of arsenic and mercury were below the limits of detection (LoDs) of the employed methodologies. For cadmium, the highest concentration determined in the commercial batches was 0.088 mg/kg. The Panel considered this concentration as not of concern.[25,26]

The food enzyme preparation complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[27]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). [redacted] the strain unable to produce ochratoxin A and fumonisins. This was confirmed by analysis of the five batches of food enzyme in which the levels of these mycotoxins were found to be below the limits of detection.[28,29] The potential presence of other secondary metabolites 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 is sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated in three independent batches analysed in triplicate. [redacted][30] No colonies were observed.

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected [redacted][31]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian cell micronucleus test and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 4 (Table 1) has a lower specific activity (enzyme activity/TOS) compared to the three commercial food enzyme batches and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. 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, 1997) and following Good Laboratory Practice (GLP).[32] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA(pKM101) were used in the presence or absence of metabolic activation (S9-mix), applying the 'treat and plate' assay. Two separate experiments were carried out using six different concentrations of the food enzyme (156, 313, 625, 1,250, 2,500 and 5,000 μg dry matter/mL, corresponding to 140.4, 281.7, 562.5, 1,125, 2,250 and 4,500 μg TOS/mL). Toxic effects, evident as a slight reduction in the growth of the background lawn, occurred in *S.* Typhimurium TA100 and TA1535 in the absence of S9-mix at 4,500 μg TOS/mL in the first experiment, and at 2,250 and

[25] Technical dossier/p. 34 and 62, and Additional data August 2019.
[26] LoDs: Pb = 0.5 mg/kg; As = 0.1 mg/kg; Cd = 0.05 mg/kg; Hg = 0.03 mg/kg.
[27] Technical dossier/p. 34, 36 and 62; and Additional data August 2019.
[28] Technical dossier/2nd submission/p. 34 and 62, and Additional data August 2019.
[29] LoDs: Ochratoxin = 0.0003 mg/kg; Fumonisin B2 = 0.0003 mg/kg.
[30] Technical dossier/Annex 4/p. 25-26 and Annex E1.
[31] Technical dossier/Annex 4/p. 27-28 and Annex E2; and Additional data August 2019.
[32] Technical dossier/Annex 7.01.

4,500 μg TOS/mL in the second experiment. 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 did not induce gene mutations under the test conditions employed in this study.

3.4.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2010) and following GLP.[33]

Cultures of human peripheral whole blood lymphocytes were exposed to three concentrations of the food enzyme (3,000, 4,000 and 5,000 μg food enzyme/mL, corresponding to 324, 432 and 540 μg TOS/mL) following a short treatment in the presence and absence of S9-mix (3 + 21 h of recovery) and a continuous treatment without S9-mix (24 + 24 h recovery). No cytotoxicity was observed at any concentration tested and experimental condition. The frequency of binucleated cells with micronuclei (MNBN) was comparable to that of the negative controls at all concentrations tested.

The Panel concluded that the food enzyme triacylglycerol lipase did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes under the test conditions employed for 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.[34] Groups of 10 male and 10 female SPF Wistar HanTac:WH (GALAS) rats received by gavage the food enzyme in doses of 113, 374 or 1,132 mg TOS/kg bw per day. Controls received the vehicle (water).

No mortality was observed.

In high-dose males, a statistically significant increase in food consumption during weeks 7 and 9 and water consumption during days 15–18, 25–29, 39–43 and 53–57 were recorded. The Panel considered these findings as incidental since they were transient, there was no difference from controls in total accumulated food consumption, and the higher water intake was mainly driven by the animals in one cage. In addition, food and water consumptions of high-dose females were similar to those in the controls.

Among blood chemistry parameters statistically significant differences from controls included lower concentration of albumin and higher concentration of potassium in low-dose males, and higher concentration of phosphorus in low-dose females. As these findings lacked dose–response relationship and were confined to one sex they were considered by the Panel as incidental.

The spleen weight relative to brain weight was statistically significantly lower in mid-dose females as compared to that in the control group. However, absolute and relative to body weight spleen weights in this group were not statistically significantly different from the controls. As this finding had no dose–response relationship and no macroscopic and microscopic changes in spleens from this group were recorded, the finding was considered by the Panel not to be of toxicological relevance.

No other statistically significant differences from controls were observed.

Overall, the Panel identified the no-observed-adverse-effect level (NOAEL) of 1,132 mg TOS/kg bw per day, the highest dose tested.

3.4.3. 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 NZYM-DB 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, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[35]

[33] Technical dossier/Annex 7.02.
[34] Technical dossier/Annex 7.03.
[35] Technical dossier/Annex 8.

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

Respiratory allergy following occupational inhalation of lipases has been reported (Elms et al., 2003; Martel et al., 2010). Several studies have shown that adults with occupational asthma to an enzyme may be able to ingest the respiratory allergen 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 lipase have been reported in the literature.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011)[36] are used as raw materials ([redacted])[37] in 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 microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

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.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The immobilised food enzyme is used in the production of fats and oils with modified fats and oils by interesterification. The applicant estimated that up to 100 LU(CA)/g fat or oil is used,[38] corresponding to 328 mg TOS/kg fat or oil.

A flow chart depicting the manufacturing process steps of using the immobilised triacylglycerol lipase to produce modified fats/oils has been provided.[39] The feedstock is pumped through a series of fixed bed reactors containing the immobilised triacylglycerol lipase under vacuum, which removes water or alcohols formed during the reaction. In the absence of water or under water limiting conditions, triacylglycerol lipase rearranges the position of fatty acids within triacylglycerols, altering physical properties such as melting temperature or plasticity of the dietary fat. The reaction product (new glycerides with modified properties) is then filtered and deodorised to remove impurities.

AMFEP provided a reasoned explanation that due to immobilisation resulting in the physical separation of the food enzyme from the final oils, it is unlikely that any enzyme TOS would end up in the final interesterified fats (Documentation provided to EFSA no. 4). In addition, the initial washing of the resin and the down-stream purification steps applied to the fats and oils after processing, i.e. filtration and deodorisation, would also ensure the absence of TOS in the final interesterified fats.[40]

However, in the absence of experimental data[4], the extent of TOS removal or transfer of the enzyme TOS into the final fats and oil products could not be fully established.

3.5.2. Dietary exposure estimation

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 individual FoodEx categories was subsequently summed up, averaged over the total survey period 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 average 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).

[36] 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.

[37] Technical dossier/Annex 6.

[38] Technical dossier/p. 88.

[39] Additional data August 2019.

[40] Technical dossier/p. 79–86; and Additional data August 2019.

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 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 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)	0.001–0.21 (12)	0.08–0.36 (16)	0.11–0.31 (19)	0.06–0.15 (20)	0.03–0.14 (22)	0.03–0.14 (21)
Min–max 95th percentile (number of surveys)	0.002–0.53 (10)	0.26–0.75 (14)	0.28–0.61 (19)	0.20–0.34 (19)	0.10–0.33 (22)	0.08–0.35 (21)

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	
Assumption that despite the food enzyme being immobilised, all TOS is transferred into the final fat products	+
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 +: uncertainty with potential to cause overestimation of exposure. –: uncertainty with potential to cause underestimation of exposure.	+/–

TOS: total organic solids.

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.

3.6. Margin of exposure

A comparison of the NOAEL (1,132 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.001–0.36 mg/kg bw per day at the mean and from 0.002 to 0.75 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) of at least 1,509.

4. Conclusions

Based on the data provided, the Panel concluded that the immobilised food enzyme triacylglycerol lipase produced with the genetically modified *A. niger* strain NZYM-DB 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 'Lipase produced by a genetically modified strain of *Aspergillus niger* (strain NZYM-DB). April 2015. Submitted by Novozymes A/S.
2) Additional information. August 2019. Submitted by Novozymes A/S.
3) Additional information. September 2019. Submitted by Novozymes A/S.
4) Additional information on 'The transfer of enzymes into food, for fat and oil processing'. October 2017 and February 2018. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEF EFSA	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP EFSA	Panel on Food Contact Materials, Enzymes and Processing Aids
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GMbH
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FUM	fumonisin
GMO	genetically modified organism
ITS	internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
LU(CA)	Lipase unit (CA standard)
MNBN	binucleated cells with micronuclei
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–polyacrylamide 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.2021.6366#support-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 one 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, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, United Kingdom
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, 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, Greece, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain, Sweden, United Kingdom
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, United Kingdom
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, 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).