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Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Aspergillus luchuensis* strain FL100SC

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Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase EC 3.1.1.3) is produced with a genetically modified *Aspergillus luchuensis* strain FL100SC by Advanced Enzyme Technologies Ltd. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. The triacylglycerol lipase is intended to be used only in an immobilised form in the production of modified fats and oils by interesterification. Since residual amounts of total organic solids (TOS) are removed by filtration and purification steps applied during fats and oils processing for interesterification, no dietary exposure was calculated. 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) of 849 mg TOS/kg body weight (bw) per day, the highest dose tested. The similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and no match was found. The Panel considers that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided, including the immobilisation process and the absence of TOS in the final product, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: Food enzyme, triacylglycerol lipase, triacylglycerol acylhydrolase, EC 3.1.1.3, glycerol ester hydrolase, *Aspergillus luchuensis*, genetically modified microorganism

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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:

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

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 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.

Five applications have been introduced by the companies "DSM Food Specialties B.V" for the authorisation of the food enzymes Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG); "Advanced Enzyme Technologies Ltd." For the authorisation of the food enzyme Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC) and Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL100SC); "Danisco US Inc." for the authorisation of the food enzyme Glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24), and "Amano Enzyme Inc." for the authorisation of the food enzyme Catalase from *Aspergillus niger* (strain AE-CN).

Following the requirements of Article 12.1 of Commission Regulation (EU) 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] 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 Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG), Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC), Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL100SC), Glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24) and Catalase from *Aspergillus niger* (strain AE-CN) 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 triacylglycerol lipase from a genetically modified strain of *Aspergillus luchuensis* (initially indicated as *Aspergillus niger* agg.) strain FL100SC.

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 genetically modified *A. luchuensis* strain FL100SC.

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 18 May 2018, on 28 January 2019 and on 25 November 2020, and was consequently provided (see 'Documentation provided to EFSA').

Following the receipt of additional data sent by the applicant on 25 April 2019, EFSA requested a clarification teleconference on 17 June 2019, after which the applicant provided additional information on 2 July 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 guidances 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	EC 3.1.1.3
CAS No	9001-62-1
EINECS No	232-619-9

In the absence of, or at a very low concentration of water, the triacylglycerol lipase catalyses the transesterification of fatty acids in glycerides. The enzyme 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 *A. luchuensis* strain FL100SC (formerly *A. niger* agg.), which is deposited in the American Type Culture Collection

[4] Technical dossier/Additional data August 2018/p. 7.

(ATCC), with the deposit number [redacted].[5] The production strain has been identified by sequence comparison [redacted].[6]

3.1.1. Characteristics of the parental and recipient microorganisms[7]

The parental strain was initially named as *A. niger* agg. in the submitted dossier. The parental strain [redacted] was identified to belong to *Aspergillus acidus.*[8] Subsequently, the name *A. acidus* has been changed to *A. luchuensis* (Hong et al., 2014).

The recipient strain [redacted] was derived from the parental strain [redacted].

3.1.2. Characteristics of introduced sequences

The triacylglycerol lipase [redacted] used for the final transformation is synthesised from [redacted], expression in *Aspergillus* spp.

The promoter and terminator for the triacylglycerol lipase gene [redacted]. The synthetic [redacted] is cloned into [redacted]. The plasmid contains [redacted] marker gene.

[redacted] used as selection marker for *Aspergillus* spp., is from [redacted]. It was cloned in [redacted].

3.1.3. Description of the genetic modification process

The production strain was developed by [redacted].

[redacted]

Selection of transformed cells occurred [redacted]

Southern analysis showed the insertion [redacted].[9]

3.1.4. Safety aspects of the genetic modification

The recipient strain [redacted].[9]

The production strain *A. luchuensis* FL100SC differs from the recipient strain by the presence of [redacted].[9]

The absence of [redacted] of the production strain was demonstrated [redacted].[10]

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[11], 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, batch fermentation system with conventional process controls in place. After completion of the

[5] Technical dossier/Annex I-1/p. 11.
[6] Technical dossier/Annex I-1.
[7] Technical dossier/Annex M.
[8] Technical dossier/Annex I-2.
[9] Technical dossier/Annex M/p. 29.
[10] Technical dossier/Additional data April 2019/Annex A.
[11] 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.

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 ultrafiltration in which the enzyme protein is retained while most of the low molecular mass material passes the filtration membrane and is discarded.[12] 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.[13]

The food enzyme is used only in an immobilised form. Immobilisation is achieved by adsorption of the food enzyme onto the support, [redacted]. [redacted] are then washed and dried.[14]

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 produced with the genetically modified *A. luchuensis* strain FL100SC consists of a single polypeptide of [redacted]. The molecular mass of the mature protein, derived from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), was determined to be [redacted]. The SDS–PAGE analysis showed one major protein band.[15] No other enzyme activities were reported by the applicant.

The determination of enzyme activity is based on the hydrolysis of triacylglycerols to fatty acids and glycerol and is expressed in Fungal Lipase-International Standard units/g (FIP/g). One unit of enzyme activity is defined as the amount of enzyme that liberates 1 μmol fatty acids per minute from an olive oil emulsion under the standard assay conditions (temperature 37°C, incubation time 10–15 min).

The food enzyme has a temperature optimum of about 45°C (pH 7.0) and a pH optimum of about 7.5 (37°C). Thermostability was tested after a pre-incubation of the food enzyme for 120 min at different temperatures. Under the conditions of the temperature stability assay (pH 7.0), no residual activity was detected above 55°C.[16]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme were provided for three batches of a dried preparation, one of which (batch 3) was used for the toxicological testing (Table 1).[17] The mean Total Organic Solids (TOS) content of the three batches was 84.7% and the mean enzyme activity/TOS ratio was 638 FIP Standard units/mg TOS. Prior to drying, the food enzyme is stabilised with [redacted].[18]

Table 1: Composition of the dried food enzyme preparation

Parameter	Unit	Batches		
		1	2	3[(a)]
Lipase activity	FIP/g batch[(b)]	552,118	520,231	548,964
Protein	%	46.24	43.41	45.56
Ash	%	7.89	8.97	8.14
Water	%	6.63	7.45	6.95
Total Organic Solids (TOS)[(c)]	%	85.48	83.58	84.91
Lipase activity/mg TOS	FIP/mg TOS	646	622	647

(a): Batch used for the toxicological studies.
(b): FIP/g: Fungal Lipase-International Standard unit/g (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

[12] Technical dossier/Annex G and Additional data August 2018.
[13] Technical dossier/Annex G and Additional data August 2018/Annex 2.3.
[14] Technical dossier/p. 33–34.
[15] Technical dossier/p. 4/Annex B and Additional data April 2018.
[16] Technical dossier/Annex C.
[17] Technical dossier/p. 4.
[18] Additional data August 2018.

3.3.3. Purity

The lead content in two batches was below 0.25 mg/kg and in the batch used for toxicological testing, it was below 0.1 mg/kg,[19,20] which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of arsenic, cadmium and mercury were below the limits of quantification (LoQ) of the employed methodologies.[21]

The food enzyme preparation 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).[22] No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).[23]

Strains of *Aspergillus*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The presence of aflatoxins (B1, B2, G1, G2 and M1), ochratoxin A, fumonisin B1, zearalenone, deoxynivalenol, T2-toxin, HT2-toxin, ergocornine, erogocristine, ergocryptine, ergometrine, ergosine and ergotamine was examined in three food enzyme batches. The concentrations of these mycotoxins were below the respective limits of detection (LoDs) of the applied analytical methods.[24,25] 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 product was demonstrated in three independent batches analysed in triplicate. [redacted] No colonies were produced.

The absence of recombinant DNA in the food enzyme was demonstrated on three samples of the final product, each tested in triplicate. [redacted] The analysis included appropriate controls with a detection limit of 1 ng/mL enzyme product.

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided. The toxicological assays were performed with batch 3 (Table 1), which was produced according to the procedure used for commercial production and 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 performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP). Five strains of *Salmonella* Typhimurium (strains TA97a, TA98, TA100, TA102 and TA1535) were used in the presence or absence of metabolic activation (S9-mix), applying the standard plate incorporation method.[26]

Two experiments were carried out in triplicate using five different concentrations of the food enzyme (61.72, 185.18, 555.55, 1,666.67 and 5,000 μg food enzyme/plate, corresponding to 52, 157, 472, 1,415 and 4,246 μg TOS/plate). No significant toxicity was observed in any strain at any concentration level tested. Upon treatment with the food enzyme, the numbers of the revertant

[19] LoQ: Pb = 0.25 mg/kg and 0.1 mg/kg.
[20] Technical dossier/Annex D and Additional data August 2018/Annex 1.
[21] LoQ: As, Cd = 0.25 mg/kg and 0.1 mg/kg each; Hg = 0.025 mg/kg.
[22] Technical dossier/Annex A3.
[23] Technical dossier/Annex E2.
[24] Technical dossier/Annex E1 and Additional data August 2018.
[25] LoDs: aflatoxins (B1, B2, G1, G2, M1) = 1 μg/kg each; fumonisin B1 = 100 μg/kg; ochratoxin A = 1 μg/kg; T-2 toxin = 10 μg/kg; HT2-toxin = 50 μg/kg; zearalenone = 5 μg/kg; deoxynivalenol = 25 μg/kg; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine = 100 μg/kg each.
[26] Technical dossier/p. 44/Annex J.

colonies were comparable to the values observed in the vehicle control groups in all tester strains, both in the presence and absence of metabolic activation.

Therefore, the Panel concluded that the food enzyme has no mutagenic activity under the conditions employed 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, 2014) and following GLP[27] in cultured human peripheral blood lymphocytes. Two separate experiments were performed in duplicate.

Based on the result of a preliminary cytotoxicity assay, in the first experiment cells were exposed for 4 h followed by 24 h recovery to the food enzyme at 1.25, 2.5 and 5 mg/mL (corresponding to 1.1, 2.1 and 4.2 mg TOS/mL), in the presence and absence of metabolic activation (S9-mix). In the second experiment, lymphocytes were exposed to the food enzyme at 1.25, 2.5 and 5 mg/mL (corresponding to 1.1, 2.1 and 4.2 mg TOS/mL), for 4 h followed by 24 h recovery in the presence of S9-mix and for 24 h followed by 24 h recovery without S9-mix. No significant cytotoxicity was induced by treatment with the food enzyme (2.2% was the maximum reduction in mitotic index observed at 5 mg/mL, short treatment, in the presence of S9-mix).[28] The frequency of cells with structural or numerical chromosomal aberrations was similar to the values observed in the negative controls at all concentrations tested under all experimental conditions. The Panel concluded that the food enzyme did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested at up to 5 mg/mL (corresponding to 4.2 mg TOS/mL) under the experimental conditions employed.

The Panel concluded on the basis of the *in vitro* studies that there is no concern for genotoxicity of the food enzyme tested.

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

A repeated dose 90-day oral toxicity study was performed according to OECD test guideline 408 (OECD, 1998), and following GLP.[29] Groups of 10 male and 10 female Sprague–Dawley rats received the food enzyme via gavage in doses corresponding to 212, 425 or 849 mg TOS/kg body weight (bw) per day for 90 days. Two recovery groups were treated with 0 or 849 mg TOS/kg bw per day for 90 days followed by a 4-week recovery period. Controls received the vehicle (distilled water).

No mortality was observed.

Haematological examination revealed several statistically significant differences from controls. After 90 days, the treated males had a lower haemoglobin (Hb) concentration and mean corpuscular haemoglobin (MCH) at the low dose, and higher Hb concentration, haematocrit (HCT) and white blood cell (WBC) count at the mid dose. The treated females had a higher mean corpuscular volume (MCV) at the low dose, higher WBC count at the mid dose and a slightly lower platelet count at the high dose. After the recovery period, the differential count of neutrophils was slightly lower, and the differential count of lymphocytes was slightly increased in the recovery males and females relative to the recovery controls. As the findings at the end of the treatment period lacked a dose–response relationship (Hb, HCT, MCH, MCV, WBC) and the differences to controls were small (platelet count) or recorded at the end of the recovery period (differential counts of neutrophils and lymphocytes), the Panel considered these findings were not of toxicological relevance.

Clinical chemistry examination in treated rats revealed several statistically significant differences from controls. After 90 days, the treated males had a higher creatinine concentration at the mid dose, lower total protein and glucose concentrations at the high dose and lower activity of alanine aminotransferase (ALT) at all doses. The treated females had a lower alkaline phosphatase (ALP) activity at low dose, a lower total bilirubin concentration at low and high doses, an increased activity of aspartate aminotransferase (AST) at mid and high doses, increased total protein, globulin and total cholesterol concentrations at the mid dose, a higher chloride concentration at mid and high doses and a higher creatinine concentration at the high dose. After the recovery period, treated males had a higher concentration of potassium and lower concentration of chloride. After the recovery period, the globulin concentration in females was increased. As the findings at the end of treatment lacked a dose-response relationship (creatinine in males; total protein, globulin, AST, ALP, total bilirubin, total cholesterol in females) or the differences to controls were small (total protein, and ALT in males,

[27] Technical dossier/Annex J/p. 46.
[28] Technical dossier/Annex J/p. 70.
[29] Technical dossier/Annex J/p. 49.

chloride in females, glucose in males and females) or seen only at the end of the recovery period (K^+ and Cl^- in males, the globulin in females), they were considered by the Panel, not to be toxicologically relevant.

Urinalysis revealed a slightly but statistically significantly higher volume in high-dose females.

The analysis of organ weights revealed statistically significant differences in relative organ weights compared to controls: increased relative weight of adrenals in mid-dose females and decreased relative weight of ovaries in low-dose females. At the end of the recovery period on day 119, an increased relative weight of the spleen was recorded in treated females. However, in light of no corresponding gross and histopathological changes in any of these organs, the findings were considered by the Panel not of toxicological concern. Furthermore, the values of the relative epididymal weights in the mid- and high-dose males were statistically significantly lower than in the control with no apparent dose relationship. The Panel noted that the value for the main control group was higher than the value of the recovery control group. Furthermore, the decrease in the values of the absolute epididymal weights was not statistically significant. In addition, no microscopic changes were reported in the organ. In particular, the Panel noted that sloughed testicular germ cells and cell debris, which are sensitive indicators of spermatogenic disturbance in the rat, were not reported to be present in the epidydimal lumen after histological examination. This supported that the changes in epididymis weights observed at the end of the 90-day study, that is a period longer than the spermatogenic cycle, can be considered as not adverse.

No other statistically significant differences to controls were observed.

Overall, based on the data available to the Panel, a no observed adverse effect level (NOAEL) of 849 mg TOS/kg bw per day, the highest dose tested, was identified.

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. luchuensis* strain FL100SC 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 matches were found.[30]

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

Respiratory allergy following occupational inhalation of triacylglycerol lipase have been reported (Elms et al., 2003; Martel et al., 2010). However, some studies have shown that adults with occupational asthma to an enzyme used in food can commonly ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Brisman, 2002; Poulsen, 2004; Armentia et al., 2009). Adverse reactions upon ingestion of triacylglycerol lipase in individuals sensitised through the respiratory route has not been reported.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[31]) are used as raw materials ([redacted]).[32] 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 considers that under the intended condition 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 to occur is considered to be low.

[30] Technical dossier/Annex L.

[31] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[32] Technical dossier/Annex G/Appendix 2.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The immobilised triacylglycerol lipase is intended to be used in the production of modified fats and oils by interesterification. Depending on the stability of the immobilised lipase, according to the applicant, the estimated use level is between 2,800 and 28,000 FIP/kg triglycerides,[33] corresponding to 4.39–43.9 mg TOS/kg triglycerides.

A flow chart depicting the manufacturing process steps of using the immobilised lipase to produce modified fats/oils has been provided.[34] In the absence of water or under water-limiting conditions, lipase rearranges the position of fatty acids within triacylglycerols, altering physical properties, such as the melting temperature or plasticity of the dietary fat. The reaction product (new glycerides with modified properties) is then filtered and deodorised to remove impurities.

In addition to the immobilisation procedure, the initial washing of the immobilised resin and the purification steps applied to fats and oils after processing, i.e. filtration and deodorisation, would also ensure the absence of TOS in the final interesterified fats.[35] The applicant provided a laboratory study which confirmed that no leakage from the immobilised enzyme in the final food could be detected, using the total nitrogen content as an approximation of TOS.[36]

The Panel accepted the evidence provided as sufficient to conclude that the presence of residual amounts of TOS after processing is negligible.

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS during interesterification of fats and oils were considered by the Panel as sufficient to exclude this process from the exposure assessment (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated.

4. Conclusions

Based on the data provided, including the absence of TOS in the final product, the Panel concluded that the food enzyme triacylglycerol lipase produced with the genetically modified *A. luchuensis* strain FL100SC does not give rise to safety concerns under the intended conditions of use.

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

5. Documentation as provided to EFSA

1) Dossier 'Application for authorisation of Lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC)' in accordance with Regulation (EC) No 1331/2008. February 2015. Submitted by Advanced Enzyme Technologies Ltd.
2) Additional information was received from Advanced Enzyme Technologies Ltd. in August 2018.
3) Additional information was received from Advanced Enzyme Technologies Ltd. in April 2019.
4) Additional information was received from Advanced Enzyme Technologies Ltd. in July 2019.
5) Additional information was received from Advanced Enzyme Technologies Ltd. in January 2021.
6) Summary report on GMM part. December 2014. Delivered by Technical University of Denmark - DTU (Denmark).
7) Summary report on technical data. August 2016. Delivered by Hylobates Consulting and BiCT (Rome and Lodi, Italy).

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[33] Technical dossier/p. 37.
[34] Technical dossier/Figure 3.2.1.4.
[35] Technical dossier/pp. 40–42.
[36] Technical dossier/Annex N.

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Abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATCC	American Type Culture Collection
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

CFU	colony forming unit
EC	Enzyme Commission
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
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
Hb	haemoglobin
HCT	haematocrit
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	kiloDalton
LoD	limit of detection
LoQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
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
WBC	white blood cell
WHO	World Health Organization