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## Safety evaluation of the food enzyme triacylglycerol lipase from *Trichoderma reesei* (strain RF10625)

EFSA Panel on Food Contact Materials, Triacylglycerol lipases and Processing Aids  
(EFSA CEP Panel),

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### Abstract

The food enzyme triacylglycerol acylhydrolase (EC 3.1.1.3) is produced with a genetically modified *Trichoderma reesei* strain RF10625 by AB Enzymes. 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 baking processes and cereal-based processes. Based on the maximum use levels, dietary exposure to the food enzyme Total Organic Solids (TOS) was estimated to be up to 0.119 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 of 1,000 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, results in a margin of exposure of at least 8,400. Similarity of the amino acid sequence to those of known allergens was searched and no matches were 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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## 1. Introduction

Article 3 of the Regulation (EC) No. 1332/2008<sup>1</sup> 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<sup>2</sup> 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 Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

Four applications have been introduced by the companies "Cargill R&D Centre Europe" for the authorisation of the food enzyme Alternansucrase from *Leuconostoc citreum* (NRRL B-30894), "Intertek Scientific & Regulatory Consultancy" for the authorisation of the food enzymes Beta-galactosidase from *Bacillus circulans* (M3-1) and D-Fructose 3-Epimerase from a genetically modified strain of *Escherichia coli* (W3110-TKO), and "AB Enzymes GmbH" for the authorisation of the food enzyme Triacylglycerol lipase from a genetically modified strain of *Trichoderma reesei* (RF10625).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011<sup>3</sup> implementing Regulation (EC) No 1331/2008<sup>4</sup>, the Commission has verified that the four applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

<sup>4</sup> 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, p. 1–6.

### 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 Alternansucrase from *Leuconostoc citreum* (strain NRRL B-30894), Beta-galactosidase from *Bacillus circulans* (strain M3-1), D-Fructose 3-Epimerase from a genetically modified strain of *Escherichia coli* (W3110-TKO), and Triacylglycerol lipase from a genetically modified strain of *Trichoderma reesei* (RF10625) 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 a genetically modified *T. reesei* (strain RF10625).

## 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 *T. reesei* (strain RF10625).

Additional information was sought from the applicant during the assessment process in requests from EFSA sent on 13 July 2017 and 8 April 2019 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, 2009b) 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 the relevant existing guidance of the EFSA Scientific Committee.

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, glycerol ester hydrolase, triacylglycerol ester hydrolase
IUBMB No.:	EC 3.1.1.3
CAS No.:	9001-62-1
EINECS No.:	232-619-9

The triacylglycerol lipase catalyses, in the presence of water, the hydrolysis of the ester linkages in triacylglycerols, resulting in the generation of glycerol, free fatty acids, diacylglycerols and monoacylglycerols. The food enzyme can also convert phospholipids into lysophospholipids and glycolipids into glycomonoglycerides. It is intended to be used in baking processes and cereal-based processes.

### 3.1. Source of the food enzyme

The triacylglycerol lipase is produced with the genetically modified filamentous fungus *T. reesei* strain RF10625, which is deposited at the Westerdijk Fungal Biodiversity Institute, the Netherlands with the deposit number CBS 134213.<sup>5</sup>

<sup>5</sup> Technical dossier/Volume III/Appendix 13.

### 3.1.1. Characteristics of the parental and recipient strains

[Redacted text block]

### 3.1.2. Characteristics of introduced sequences

[Redacted text block]

### 3.1.3. Description of the genetic modification process

[Redacted text block]

### 3.1.4. Safety aspects of the genetic modification

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

[Redacted text block]

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

<sup>6</sup> Technical dossier/Volume III/Appendix 1B.

<sup>7</sup> Technical dossier/Volume III/Appendix 2A.

<sup>8</sup> Technical dossier/Volume III/Appendix 2B.

<sup>9</sup> Technical dossier/Volume III/Appendix 4B.

<sup>10</sup> Technical dossier/Additional information June 2019/Enclosure 1.

<sup>11</sup> Technical dossier/Volume III/Appendix 14.

## 3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004<sup>12</sup>, with food safety procedures based on hazard analysis and critical control points, and in accordance with current Good Manufacturing Practice.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, 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.<sup>13</sup>

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 ■ amino acids,<sup>14</sup> including a signal peptide of ■ amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be ■ kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gels showed a major protein band corresponding to an apparent molecular mass of about ■ kDa.<sup>15</sup> No other enzymatic side activities were reported.

The in-house determination of triacylglycerol lipase activity is based on hydrolysis of the substrate tributyrin, (reaction conditions: pH 7.0, 30°C, 10 min). The enzymatic activity is determined by titration of butyric acid, which is a product of the hydrolysis of tributyrin. The triacylglycerol lipase activity is expressed in ALU/g. One ALU is defined as the amount of enzyme which releases 1 μmol butyric acid in 1 min under the defined assay conditions.<sup>16</sup>

The triacylglycerol lipase has a temperature optimum around 30°C (pH 7.0) and a pH optimum around pH 7.0 to 9.0 (30°C). Thermostability was tested after a pre-incubation of the food enzyme at 85°C at different times. Under the conditions (pH 7.0) of the applied temperature stability assay, triacylglycerol lipase activity decreased by 99% after 1 min of pre-incubation.<sup>17</sup>

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four batches, three batches used for commercialisation and one batch produced for the toxicological tests (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 93.3%. The average triacylglycerol lipase activity/TOS ratio of the three food enzyme batches for commercialisation is 187.5 ALU/mg TOS.

<sup>12</sup> 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.

<sup>13</sup> Technical dossier/Annexes 12 and 13.

<sup>14</sup> Technical dossier/Annex 4C.

<sup>15</sup> Technical dossier/Annex 4A.

<sup>16</sup> Technical dossier/Annex 2.

<sup>17</sup> Technical dossier/Annex 5.



**Table 1:** Compositional data provided of the food enzyme

Parameter	Unit	Batches			
		1	2	3	4 <sup>(a)</sup>
Triacylglycerol lipase activity	ALU/g batch <sup>(b)</sup>	158,000	201,000	166,000	304,000
Protein	%	62.0	70.3	76.5	70.1
Ash	%	3.9	1.7	1.1	0.91
Water	%	4.7	4.6	4.1	4.7
Total Organic Solids (TOS) <sup>(c)</sup>	%	91.4	93.7	94.8	94.4
Triacylglycerol lipase activity/mg TOS	ALU/mg TOS	172.9	214.5	175.1	322.0

(a): Batch used for the toxicological tests.

(b): ALU/g: Lipase Units/g (see Section 3.3.1).

(c): TOS calculated as 100% – % water – % ash.

### 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 ( $\leq 5$  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 heavy metals As, Cd and Hg were below the limits of detection of the employed methodologies.<sup>18</sup>

The food enzyme 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 *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms should not exceed 30 CFU (Colony Forming Unit) per gram. No antimicrobial activity was detected in any of the batches for commercialisation (FAO/WHO, 2006).<sup>19</sup>

The presence of mycotoxins (aflatoxins B1, B2, G1 and G2, sterigmatocystin, ochratoxin A, deoxynivalenol, T2-toxin, HT-2-toxin, fumonisins B1 and B2, and zearalenone) was examined in the three food enzyme preparation batches, and were below the limits of detection (LODs) of the applied analytical methods,<sup>20</sup> except for deoxynivalenol that was detected at levels up to 105 µg/kg.<sup>19</sup> Considering the use levels, this is considered of no concern.

Strains of *Trichoderma*, in common with most filamentous fungi have the capacity to produce a range of secondary metabolites (Frisvad et al., 2017). The applicant did not provide information on other secondary metabolites produced under the conditions of fermentation which might contribute to the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.

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

### 3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme (solid form) was demonstrated in three independent batches of enzyme concentrate analysed in quadruplicate.<sup>21</sup> Five ml of concentrate was inoculated on non-selective agar plates and incubated at 30°C for 7 days.<sup>22</sup> No colonies of the production strain were produced.

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

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## 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

<sup>18</sup> LODs: As = 0.5 mg/kg; Cd = 0.05 mg/kg; Hg = 0.1 or 0.05 mg/kg.

<sup>19</sup> Technical dossier/Additional information September 2017/Att 1 and Att 2.1.

<sup>20</sup> LODs: Aflatoxins: 0.05 µg/kg; sterigmatocystin: 10 µg/kg; ochratoxin A: 0.5 µg/kg; deoxynivalenol: 40 µg/kg; T2-toxin: 20 µg/kg; HT-2-toxin: 20 µg/kg; fumonisins: 20 µg/kg; zearalenone: 50 µg/kg.

<sup>21</sup> Technical dossier/Additional information June 2019/Enclosure 2b.

<sup>22</sup> Technical dossier/Additional information June 2019/Enclosure 2a.

<sup>23</sup> Technical dossier/Volume III/Appendix 22.



been provided. The batch used in these studies (batch 4 shown in Table 1) is considered suitable as a representative 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 OECD Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP) in five strains of *Salmonella* Typhimurium (TA 98, TA 100, TA 1535, TA 1537 and TA 102) both in the presence and absence of metabolic activation (S9 mix).<sup>24</sup>

Two separate experiments were performed employing all strains and two additional confirmatory experiments were performed to verify a small increase in revertants observed in strain TA98.

Experiment 1 was carried out using the plate incorporation method. The following doses were tested: 3; 10; 33; 100; 333; 1,000; 2,500 and 5,000 µg food enzyme/plate corresponding to 2.8; 9.4; 31.1; 94.4; 314.3; 944; 2,359.5 and 4,719 µg TOS/plate. No toxic effects (determined by the observation of a reduction in the number of revertants below the indication factor of 0.5) were observed. A minor increase in the number of revertant colonies was observed in strain TA 98 in absence of metabolic activation at 5,000 µg/plate of the test substance; however, the threshold of twofold increase compared with the vehicle control was not reached and the mean value of the revertant colonies was in the range of the historical control data for the solvent.

Experiment 2 was carried out using the pre-incubation method. The following doses were tested on the basis of the results obtained in Experiment 1: 33; 100; 333; 1,000; 2,500 and 5,000 µg food enzyme/plate corresponding to 31.1; 94.4; 314.3; 944; 2359.5 and 4719 µg TOS/plate. No toxicity was detected, with the exception of strain TA 102 in presence of metabolic activation that showed a reduction in the number of revertants at 5,000 µg/plate. An increase in revertants was observed in strain TA 98 and TA 1535 without activation at 5000 µg/plate, but the mean value of the revertant colonies didn't reach the threshold of twofold increase compared with the solvent control and was in the range of the historical control data. These results were confirmed by additional experiments with strain TA 98 using the same concentrations without metabolic activation.

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* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.<sup>25</sup> The test item was tested for its ability to induce chromosomal aberrations in cultured human lymphocytes.

Two experiments were performed. In the first experiment (4 + 18 h), cultured human lymphocytes were exposed for 4 h followed by a 18 h recovery period (short term treatment) in the presence and in the absence of S9 mix at concentrations of 34.4; 60.3; 105.4; 184.5; 322.9; 565.1; 988.9; 1,730.6; 3,028.6 and 5,300 µg food enzyme/mL corresponding to 32.5; 57; 99.5; 174.1; 304.7; 533.3; 933.3; 1,633.3; 2,858.4 and 5,002 µg TOS/mL. Cytotoxicity, measured as reduction of mitotic index (MI), was detected in absence of S9 mix at the highest concentration tested 5,002 µg TOS/mL (51.7% below the solvent control), and in presence of S9 mix at 304.7 µg TOS/mL (37.5% below the solvent control) and above. Cytogenetic evaluation was performed at 1633.3; 2858.4 and 5002 µg TOS/mL without S9 mix and at 99.5; 174.1 and 304.7 µg TOS/mL with S9 mix. An increase in chromosomal aberration, was detected after exposure to 304.7 µg TOS/mL with S9 mix. This was not statistically significant, although slightly above the historical control data. Therefore, it was considered as not biologically relevant.

In the second experiment, lymphocytes were exposed continuously during 22 h (long-term treatment) to the food enzyme in the absence of S9 mix. The concentrations tested in the second experiment were 22.8; 39.9; 69.8; 122.2; 213.8; 374.1; 654.7; 1145.8; 2,005.1 and 3,508.9 µg food enzyme/mL corresponding to 21.5; 37.6; 65.9; 115.3; 201.8; 353.1; 618; 1,081.4; 1,892.4 and 3,312 µg TOS/mL. Cytotoxicity was detected starting at 1,145.8 µg food enzyme/mL (MI: 39.3% of the solvent control). Cytogenetic evaluation was performed at 353.1; 618; 1,081.4 µg TOS/mL. No increase in chromosomal aberrations with respect to the negative control were detected.

<sup>24</sup> Technical dossier/Annex 15.

<sup>25</sup> Technical dossier/Annex 16.

The test substance 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.

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

### 3.4.2. Repeated dose 90-day oral toxicity study in rats

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.<sup>26</sup> Three groups of 10 male and 10 female SPF-bred Wistar rats received by gavage the food enzyme in doses corresponding to 50; 200 and 1,000 mg TOS/kg body weight (bw) per day. Controls received the vehicle (distilled water). Doses were selected on the basis of a dose range finding study performed not following GLP<sup>26</sup> and submitted by the applicant.

No mortality was observed.

High-dose females had a statistically significantly higher value for fore limb grip strength in comparison to controls. As it was an isolated finding, it was not considered toxicologically relevant.

A statistically significantly higher relative feed intake was recorded on a single occasion (days 85–92) in all treated male groups as compared to that in the control group. The difference originated from a lower control value compared to a previous period, while the values of the treated males were comparable to those in a previous period. Therefore, this finding was considered of no toxicologic significance.

Statistically significant differences from controls in haematological parameters included for treated males reduced haemoglobin distribution width and increased prothrombin time at the high dose, decreased relative lymphocyte count and increased relative neutrophil count at the mid dose, and decreased relative lymphocyte count and increased eosinophil count at the low dose. For females, statistically significant differences from controls included increased absolute eosinophil count, and prothrombin time at the high dose, reduction of a total white blood cell count and of relative and absolute lymphocyte counts, and increase in relative eosinophil count at the mid dose.

Clinical chemistry examination revealed several statistically significant differences from controls such as higher concentrations of glucose, chloride and potassium, lower concentrations of calcium and total bilirubin in high-dose males, and higher concentration of chloride in mid-dose males. High- and mid-dose females had a statistically significantly higher sodium concentration.

All differences in haematology and clinical chemistry parameters were unrelated to dose and inconsistent between sexes. Therefore, they were considered by the Panel as of no toxicological relevance.

No other statistically significant differences from controls were observed.

Based on the results of this study, the Panel identified a no-observed-adverse-effect-level (NOAEL) of 1,000 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 *T.reesei* strain RF10625 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.<sup>27</sup>

No information is 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 lipase have been reported in the literature.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011) are used as raw materials ( ) in the media fed

<sup>26</sup> Technical dossier/Annex 17.

<sup>27</sup> Technical dossier/Volume II/Annex 18.

to the production strain. However, during the fermentation process, these products will be degraded and utilised by the strain for cell growth, cell maintenance and production of enzyme protein. In addition, the fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that 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 triacylglycerol lipase produced with the genetically modified *T. reesei* strain RF10625 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 food enzyme is intended to be used in baking processes and cereal-based processes at a recommended use level of up to 10 mg TOS/kg flour.<sup>28</sup>

In baking processes, the triacylglycerol lipase is added to the raw materials during the preparation of the dough. It is used to facilitate the handling of the dough, improve the dough structure and behaviour, as well as to reduce batter viscosity, thus contributing to an improved and consistent baking process.

In cereal-based processes, the triacylglycerol lipase is added to the raw materials during the preparation of the dough to improve the dough processability and to reduce oil uptake during frying. It is used to improve the strength and stability of the dough, thus facilitating its handling.

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

#### 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<sup>29</sup> 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 one 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).

<sup>28</sup> Technical dossier/Additional information September 2017.

<sup>29</sup> <http://www.efsa.europa.eu/en/food-consumption/comprehensive-database>

**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)	0.008–0.035 (10)	0.029–0.074 (14)	0.035–0.065 (19)	0.018–0.040 (18)	0.012–0.027 (19)	0.011–0.025 (18)
Min–max 95th percentile (number of surveys)	0.033–0.119 (8)	0.067–0.105 (12)	0.059–0.118 (19)	0.034–0.078 (17)	0.025–0.047 (19)	0.021–0.043 (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
<b>Model input data</b>	
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	+/-
<b>Model assumptions and factors</b>	
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.

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 (1,000 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.008–0.074 mg TOS/kg bw per day at the mean and from 0.021–0.119 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 8,403.

## 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 *T. reesei* strain RF10625 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) Application for authorisation of a triacylglycerol lipase from a genetically modified strain of *Trichoderma reesei* in accordance with Regulation (EC) No 1331/2008. 2015. Submitted by AB Enzymes.
- 2) Additional information. September 2017. Submitted by AB Enzymes.
- 3) Additional information. June 2019. Submitted by AB Enzymes.
- 4) Summary report on GMM part for triacylglycerol lipase produced by *Trichoderma reesei* strain RF10625, EFSA-Q-2016-00212. Delivered by the Technical University of Denmark (Kongens Lyngby, Denmark).

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## Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practices
GMM	genetically modified microorganism
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
ITS	internal transcribed spacer
LOD	limit of detection
MI	mitotic index
MOE	margin of exposure
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid
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/full/10.2903/j.efsa.2019.5837>).

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: The contribution of FoodEx categories to the dietary exposure to the food enzyme–TOS



## 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 <sup>(a)</sup>	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 <sup>(a)</sup>	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).