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# Safety evaluation of the food enzyme triacylglycerol lipase from the genetically modified *Ogataea polymorpha* strain DP-Jzk33

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## Abstract

The food enzyme triacylglycerol lipase (triacylglycerol acylhydrolase EC 3.1.1.3) is produced with the genetically modified *Ogataea polymorpha* strain DP-Jzk33 by Danisco US Inc. 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. It is intended to be used in baking and cereal-based processes. Based on the maximum use levels recommended for baking and cereal-based processes 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 0.520 mg TOS/kg body weight (bw) per day. 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 669 mg TOS/kg bw per day, the highest dose tested. Comparison with the estimated dietary exposure results in a margin of exposure of at least 1,287. A search was made of the similarity of the amino acid sequence of the lipase to those of known allergens 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 Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.



**Keywords:** food enzyme, triacylglycerol lipase, EC 3.1.1.3, *Ogataea polymorpha*, *Hansenula polymorpha*, genetically modified microorganism

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# 1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions 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 microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

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

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

The 'Guidance on submission of a dossier on a food enzyme for 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 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 has been introduced by the companies "Meiji Seika Pharma Co., Ltd." for the authorisation of the food enzyme Cellulase from *Talaromyces cellulolyticus/Talaromyces pinophilus* (strain *Acremonium cellulolyticus*); "Danisco US Inc." for the authorisation of the food enzymes Aspergillopepsin I from a genetically modified strain from *Trichoderma reesei* (strain DP-NZq40) and Triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33); "Neova Technologies Inc." for the authorisation of the food enzyme Trypsin and Chymotrypsin from porcine pancreatic glands, and "Novozymes A/S." for the authorisation of the food enzyme Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[2], the Commission has verified that the five 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, p. 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, p. 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 assessment on the food enzymes Cellulase from *Talaromyces cellulolyticus/Talaromyces pinophilus* (strain *Acremonium cellulolyticus*), Aspergillopepsin I from a genetically modified strain from *Trichoderma reesei* (strain DP-NZq40), Triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (strain DP-Jzk33), Trypsin and Chymotrypsin from porcine pancreatic glands and Peptidase from a strain of *Aspergillus oryzae* (strain NZYM-EX) 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 *Ogataea polymorpha* (formerly known as *Hansenula polymorpha*; strain DP-Jzk33).

# 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 the genetically modified *O. polymorpha* strain DP-Jzk33.

Additional information was sought from the applicant during the assessment process on 19 January 2018, 11 September 2019 and 2 October 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 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance of 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; triglyceride lipase |
| IUBMB No: | EC 3.1.1.3 |
| CAS No: | 9001-62-1 |
| EINECS No.: | 232-619-9. |

Triacylglycerol lipases catalyse, 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. In the absence of water, or at a very low concentration of water, interesterification, i.e. the exchange of free fatty acids in positions 1 and 3 between two or more triacylglycerols, may occur. It is intended to be used in baking and cereal-based processes.

## 3.1. Source of the food enzyme

The triacylglycerol lipase is produced with the genetically modified methylotrophic yeast *O. polymorpha* (previously known as *Hansenula polymorpha*) (synonyms: *Pichia angusta, Torulopsis methanothermos*) strain DP-Jzk33 ([redacted]), which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS), the Netherlands, with the deposit number [redacted].[4]

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

The production strain DP-Jzk33 was characterised as *O. polymorpha* [redacted].[5]

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

The parental strain is *O. polymorpha* isolate [redacted]. Its genome has been sequenced ([redacted]).

The recipient strain [redacted] was derived from the parental strain by [redacted].[6]

### 3.1.2. Characteristics of the introduced sequences

[redacted][7]

### 3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise triacylglycerol lipase [redacted][8]

### 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 *O. polymorpha* DP-Jzk33 [redacted]

The genetic stability of the production strain was demonstrated [redacted].

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

## 3.2. Production of the food enzyme

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

[5] Technical dossier/1st submission/Annex G.
[6] Technical dossier/1st submission/Annex Z.
[7] Technical dossier/2nd submission/Annex W.
[8] Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.

The production strain is grown as a pure culture using a typical industrial medium in a submerged batch or fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[9]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

## 3.3. Characteristics of the food enzyme

### 3.3.1. Properties of the food enzyme

The triacylglycerol lipase is a single polypeptide chain of [redacted] amino acids, including the signal sequence.[10] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa. The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Gel showed a major protein band at [redacted] kDa. No other enzymatic side activities were reported.

The in-house determination of triacylglycerol lipase activity is based on hydrolysis of the substrate of lecithin (reaction conditions: pH 7.0, temperature 37°C, reaction time 10 min). The enzyme activity is expressed in activity units/g (TIPU/g). One TIPU unit is defined as the amount of enzyme which liberates 1 mol free fatty acid per minute under the conditions of the assay.[11]

The food enzyme has a temperature optimum around 40°C (pH 7.0) and a pH optimum around 7.0 – 8.0 (temperature 37°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 7.0). The enzyme activity decreased rapidly above 45°C, showing no residual activity when incubated at 50°C or above.[12]

### 3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for five food enzyme batches, three batches to be used for commercialisation[13] and two batches produced for the toxicological tests (Table 1). The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 8.7%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation was 141.3 TIPU/mg TOS.

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

| Parameter | Unit | Batch | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4(a) | 5(b) |
| Triacylglycerol lipase activity | TIPU/g batch(c) | 10,993 | 11,326 | 12,380 | 5,800 | 13,587 |
| Protein | % | 4.6 | 6.2 | 2.8 | 0.4 | 3.5 |
| Ash | % | 1.1 | 0.4 | 0.9 | 0.2 | NA |
| Water | % | 90.7 | 88.2 | 92.7 | 93.1 | NA |
| Total organic solids (TOS)(d) | % | 8.2 | 11.4 | 6.4 | 6.7 | 8.0 |
| Activity/mg TOS | TIPU/mg TOS | 134.1 | 99.4 | 193.4 | 86.6 | 169.8 |

NA: not analysed.
(a): Batch used for the repeated dose 90-day oral toxicity study.
(b): Batch used for the *in vitro* chromosomal aberration and bacterial reverse mutation tests.
(c): TIPU: activity units (see Section 3.3.1).
(d): TOS calculated as 100% - % water - % ash (batches 1-5).

[9] Technical dossier/Additional data August 2019/Annex AG_SI.
[10] Technical dossier/1st submission/Annex I.
[11] Technical dossier/1st submission/Annex D.
[12] Technical dossier/1st submission/Annex J.
[13] Technical dossier/Additional data August 2019/Annex AE_SI.

### 3.3.3. Purity

The lead content in the three commercial batches and in the batch used for toxicological studies was below 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).[14]

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 colony forming units (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[14]

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

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

The absence of the production strain in the food enzyme was demonstrated in nine independent batches. For each batch, [redacted]. The results were negative.[15]

A test for recombinant DNA in the food enzyme was made by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected [redacted].[16]

## 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. Batches 4 and 5 (Table 1) were used for the toxicological assays (batch 4 used for the repeated dose 90-day oral toxicity study; batch 5 for the *in vitro* chromosomal aberration test and for the bacterial reverse mutation test). Batch 4 has the lowest specific activity (enzyme activity/mg TOS), which indicates that it is cruder than the three batches for commercialisation and its use for toxicological testing was considered suitable. The specific activity of batch 5 is comparable to those of the commercial batches.

### 3.4.1. Genotoxicity

#### 3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 471 of Chemicals (OECD, 1997) and following Good Laboratory Practice (GLP).[17] Four strains of *Salmonella* Typhimurum (TA97, TA98, TA100, TA1535) and *E. coli* WP2uvrA were used in the presence or absence of metabolic activation, applying the plate incorporation method. Two experiments were carried out in triplicate using five different concentrations of the dehydrated food enzyme containing 99.1% of dry matter (10, 100, 500, 2,000 and 5,000 μg of food enzyme/plate, corresponding to 9.7, 97, 486, 1,945 and 4,862 μg TOS/plate). No cytotoxicity was observed at any concentration of the test substance. Upon treatment with the food enzyme there was no significant increase in revertant colony numbers above the control values in any strain with or without S9-mix.

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

#### 3.4.1.2. *In vitro* mammalian chromosomal aberration test

An *in vitro* mammalian chromosomal aberration test was carried out according to the OECD Test Guideline 473 (OECD, 2016) and following GLP[18] in human peripheral blood lymphocytes.

A dose-finding study was performed at concentrations ranging from 0.5 to 5,000 μg/mL of food enzyme. Cytotoxicity, measured as reduction in mitotic index (MI) relative to the vehicle control, was

[14] LOD: Pb = 0.05 mg/kg; Additional data August 2019.
[15] Technical dossier/Additional data August 2019/Annex AI_SI.
[16] Technical dossier/Additional data August 2019/Annex AJ_SI.
[17] Technical dossier/1st submission/Annex Q.
[18] Technical dossier/Additional data August 2019/Annex AL_SI.

observed in the absence of metabolic activation at 5,000 μg/mL (53%) for the 4-hour exposure and at 500 μg/mL (74%) for the 20-hour exposure. A 62% reduction of MI was observed at 5 μg/mL for the 4-hour exposure in the presence of S9-mix. Based on these results, the concentrations selected for evaluation of chromosomal aberrations were 250, 500 and 1,500 μg/mL (corresponding to 19.88, 39.75 and 119.25 μg TOS/mL) and 1, 2 and 3 μg/mL (corresponding to 0.08, 0.16 and 0.24 TOS/mL) applying a short-term treatment (4 hours followed by 16 hours recovery) in the absence and in the presence of S9-mix, respectively, and 50, 150 and 175 μg/mL (corresponding to 3.97, 11.92 and 13.91 μg TOS/mL) applying a continuous treatment (20 hours) in the absence of S9-mix. No significant increases in structural or numerical aberrations (polyploid or endoreduplicated cells) were observed at any dose in treatment groups with or without S9-mix.

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

### 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.[19] Groups of 10 SPF Hsd:Sprague Dawley™:SD™ rats of both sexes received the food enzyme by gavage for 13 weeks, at dose levels of 5,800, 17,400 and 58,000 units (TIPU) of food enzyme/kg body weight (bw) per day corresponding to 67, 201 and 669 mg TOS/kg bw per day, respectively. Controls received the vehicle (water).

The mean food intake (period 1–90 days) of low-dose females and the mean water intake (period 1–90 days) in mid- and high-dose females were statistically significantly lower than in the controls. The differences to controls were small and did not affect terminal body weight. Therefore, these differences from the controls were considered by the Panel not to be toxicologically significant.

Clinical chemistry examination revealed a statistically significant and dose-dependent increase in the serum bilirubin levels in males and females from the mid- and high-dose groups. The Panel noted that the increase in bilirubin concentration was not associated with any microscopical changes in the liver. Therefore, it was considered by the Panel as not toxicologically significant.

No other significant differences from the controls were observed. The Panel identified the no observed adverse effect level (NOAEL) of 669 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 allergenicity of triacylglycerol lipase produced with the genetically modified *O. polymorpha* strain DP-Jzk33 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 criterion, no match was found.

No information was available on oral and respiratory sensitisation or elicitation reactions of this triacylglycerol lipase.

Respiratory allergy following occupational inhalation of triacylglycerol lipase has 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). Information on adverse reactions upon ingestion of triacylglycerol lipase in individuals sensitised through the respiratory route has not been reported.

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 to occur is considered to be low.

[19] Technical dossier/1st submission/Annex T and Additional data December 2019/Annexes AN_SI, AO_SI and AP_SI.

## 3.5. Dietary exposure

### 3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking and cereal-based processes at a recommended use level of up to 43.7 mg TOS/kg flour.[20]

In baking processes, the triacylglycerol lipase is added to the raw materials during the preparation of the dough. It is used to facilitate handling of the dough, to improve its 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 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 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 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).

**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** | 3–11Months | 12–35 months | 3–9 years | 10–17 years | 18–64 years | ≥ 65 years |
| **Min–max mean (number of surveys)** | 0.033–0.152 (10) | 0.128–0.324 (14) | 0.154–0.282 (19) | 0.081–0.175 (18) | 0.053–0.117 (19) | 0.048–0.110 (18) |
| **Min–max 95th percentile (number of surveys)** | 0.145–0.520 (8) | 0.294–0.460 (12) | 0.259–0.514 (19) | 0.147–0.341 (17) | 0.111–0.204 (19) | 0.091–0.188 (18) |

### 3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 3.

[20] Technical dossier/Additional data August 2019.

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

| Sources of uncertainties | Direction of impact |
|---|---|
| **Model input data** | |
| Consumption data: different methodologies/representativeness/underreporting/ misreporting/no portion size standard | +/– |
| Use of data from food consumption surveys of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile) | + |
| Possible national differences in categorisation and classification of food | +/- |
| **Model assumptions and factors** | |
| FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS | + |
| Exposure to food enzyme–TOS was always calculated based on the recommended maximum use level | + |
| Selection of broad FoodEx categories for the exposure assessment | + |
| Use of recipe fractions in disaggregation FoodEx categories | +/- |
| Use of technical factors in the exposure model | +/- |

+: 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 (669 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates in six human population groups of 0.033–0.324 mg TOS/kg bw per day at the mean and from 0.091-0.520 mg TOS/kg bw per day at the 95th percentile, resulted in margins of exposure (MOE) above 1287, indicating that there is no safety concern.

## 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 *O. polymorpha* strain DP-Jzk33 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 its recombinant DNA.

## Documentation provided to EFSA

1) Dossier "Application for authorisation of triacylglycerol lipase from a genetically modified strain of *Hansenula polymorpha* (DP-Jzk33) in accordance with Regulation (EC) No 1331/2008", June 2015. Submitted by Danisco US Inc.
2) Additional information was received from Danisco US Inc in August 2019.
3) Additional information was received from Danisco US Inc in December 2019.
4) Summary report on GMM part. July 2017. Delivered by contractor (DTU, Kongens Lyngby, Denmark).

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## Abbreviations

| | |
|---|---|
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CBS | Westerdijk Fungal Biodiversity Institute, the Netherlands |
| CEF | EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids |
| CEP | EFSA Panel on Food Contact Materials, Enzymes and Processing Aids |
| CFU | colony forming units |
| FAO | Food and Agriculture Organization of the United Nations |
| GLP | Good Laboratory Practice |
| GMO | genetically modified organisms |
| GMP | Good Manufacturing Practice |
| HACCP | Hazard Analysis and Critical Control Points |

| | |
|---|---|
| IUBMB | International Union of Biochemistry and Molecular Biology |
| MI | mitotic index |
| MOE | margins of exposure |
| NOAE | no observed adverse effect level |
| OECD | Organisation for Economic Cooperation and Development |
| PCR | polymerase chain reaction |
| SDS–PAGE | sodium dodecyl sulfate–polyacrylamide gel electrophoresis |
| TIPU | triacylglycerol lipase activity units |
| 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.2020.6048#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, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom |
| Toddlers | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom |
| Children[(a)] | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom |
| Adolescents | From 10 years up to and including 17 years of age | Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom |
| Adults | From 18 years up to and including 64 years of age | Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom |
| The elderly[(a)] | From 65 years of age and older | Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom |

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