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Safety evaluation of the food enzyme cellulase from the non-genetically modified *Penicillium funiculosum* strain DP-Lzc35

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Abstract

The food enzyme cellulase (4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase; EC 3.2.1.4) is produced with the non-genetically modified *Penicillium funiculosum* strain Lzc35 by Danisco US Inc. The cellulase is intended to be used in distilled alcohol production, baking and brewing processes. Since residual amounts of total organic solids (TOS) are removed by distillation, dietary exposure was only calculated for baking and brewing processes. Based on the proposed maximum use levels, dietary exposure to the food enzyme–TOS was estimated to be up to 0.416 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 84 mg TOS/kg bw per day, the highest dose tested, which when compared with the estimated dietary exposure, resulted in a margin of exposure of at least 200. Similarity of the amino acid sequence of the food enzyme to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood 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.

Keywords: food enzyme, cellulase, β-1-4-glucanase, EC 3.2.1.4, *Penicillium funiculosum*

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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 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.

Five applications have been introduced by the companies 'Danisco US Inc.' for the authorisation of the food enzyme Cellulase from *Penicillium funiculosum* (strain DP-Lzc35), 'Advanced Enzyme Technologies Ltd.' for the authorisation of the food enzyme Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL108SC), 'Avances Bioquimicos Alimentacion, S.L.' for the authorisation of the food enzyme Catalase from porcine livers and 'Nagase (Europa) GmbH' for authorisation of the food enzymes 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and Urase from *Lactobacillus fermentum* (strain 48/72).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011 implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall 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.

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 Cellulase from *Penicillium funiculosum* (strain DP-Lzc35), Triacylglycerol lipase from a genetically modified strain of *Aspergillus niger agg* (strain FL108SC), Catalase from porcine livers, 1,4-alpha-glucan branching enzyme from *Geobacillus stearothermophilus* (strain TRBE14) and Urase from *Lactobacillus fermentum* (strain 48/72) 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 request to carry out the safety assessment of the food enzyme Cellulase from *Penicillium funiculosum* (strain DP-Lzc35).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier supporting the application for authorisation of the food enzyme cellulase from a non-genetically modified *P. funiculosum* (strain DP-Lzc35).

Additional information was requested from the applicant during the assessment process on 10 December 2018 and 15 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) and following the relevant existing guidance's of EFSA Scientific Committees.

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

3. Assessment

IUBMB nomenclature:	Cellulase
Systematic name:	1,4-(1,3;1,4)-β-D-glucan-4-glucanohydrolase
Synonyms:	carboxymethyl cellulase; β-1-4-glucanase
IUBMB No:	EC 3.2.1.4
CAS No:	9012-54-8
EINECS No:	232-734-4.

Cellulase catalyses the hydrolysis of 1-4-β-glycosidic linkages in cellulose and other β-glucans resulting in the generation of shorter β-D-glucan chains. It is intended to be used in baking and brewing processes, and distilled alcohol production.

3.1. Source of the food enzyme

The cellulase production strain *Penicillium funiculosum* Lzc35 is deposited at the Westerdijk Fungal Biodiversity Institute (CBS, The Netherlands) with deposit number [redacted].[3] The strain is not genetically modified.

The production strain was derived from the wild type strain *P. funiculosum*, which is deposited in the International Mycological Institute strain collection as [redacted].[4] The production strain was optimised for enzyme production during multiple rounds of classical mutagenesis.

The strain has been taxonomically identified as belonging to the *P. funiculosum* group by 18S rRNA and ITS analysis.[5]

[3] Technical dossier/Additional information September 2019/Annex AC.
[4] Technical dossier/1st submission/Annex J.
[5] Technical dossier/1st submission/Annex J; Additional information September 2019/Annex AD/Annex AE/Annex AF.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[6], with food safety procedures based on HACCP (Hazard Analysis and Critical Control Points), and in accordance with current Good Manufacturing Practice (GMP).[7]

The production strain is grown as a pure culture using a typical industrial medium in a contained, 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.[8] 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 cellulase is a single polypeptide chain of [redacted] amino acids.[10] The molecular mass, derived from the amino acid sequence, was calculated to be [redacted] kDa.[11] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all batches. The gel showed a single major protein band corresponding to an apparent molecular mass of about number [redacted] kDa. The protein profile also included bands of lower staining intensity.[12] The presence of endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase activities have been reported by the applicant.[13]

The in-house determination of cellulase activity is based on the hydrolysis of carboxymethylcellulose. The products of this reaction (β-1,4-glucan oligosaccharides) are determined colorimetrically by measuring the increase in reducing groups using a 3,5-dinitrosalicylic acid reagent (reaction conditions: pH 5.0 and 50°C). One unit of cellulase activity is defined as the amount of enzyme which produces 1 micromole glucose equivalents per minute under the conditions described for the assay.[14]

The food enzyme has a temperature optimum of about 60°C (pH 5.0) and a pH optimum of about 5.0 (50°C). To assess the temperature stability of the enzyme, an azurine-coloured, cross-linked barley-β-glucan was used as substrate. Coloured oligomers are released by cellulase activity, and the absorbance at 590 nm was determined to quantitate the remaining activity. Under the conditions (pH 5) of the applied temperature stability assay, the cellulase activity decreased rapidly at 65°C, showing no residual activity after 40 min.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for six commercial batches and three additional batches used for toxicological tests (Table 1).[16] The average total organic solids (TOS) content of the six commercial food enzyme batches is 32.1% and the average enzyme activity/TOS ratio is 45.9 U/mg TOS.

[6] 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.
[7] Technical dossier/1st submission/Annex M.
[8] Technical dossier/1st submission/pg. 43; Additional information September 2019/Annex AA/Annex X/Annex Y.
[9] Technical dossier/1st submission/Annex N/Annex O; Additional information September 2019/Annex Z.
[10] Technical dossier/1st submission/Annex H.
[11] Technical dossier/1st submission/Annex H; Additional information September 2019.
[12] Technical dossier/1st submission/pg 30.
[13] Technical dossier/1st submission/pg. 33; Additional information September 2020.
[14] Technical dossier/1st submission/Annex D.
[15] Technical dossier/1st submission/pg. 34–35/Annex I.
[16] Technical dossier/1st submission/Annex F; Additional information September 2019/Annex X; Additional information September 2020/Annex AG/Annex AH/Annex AM.

Table 1: Compositional data for six commercial batches of the food enzyme and three additional batches used for the toxicological tests

Parameter	Unit	Batch				
		Mean[a]	Minimum–Maximum	1[b]	2[c]	3[d]
Cellulase activity	CMC DNS U/g batch[e]	14,700	13,230–16,712	14,383	2,041	3,823
Protein	%	21.63	19.97–24.76	12.61	NA[f]	NA
Ash	%	0.21	0.07–0.34	0.53	NA	0.40
Water	%	67.74	58.58–70.90	70.24	NA	91.23
Total organic solids (TOS)[g]	%	32.05	27.61–33.88	29.23	5.74	8.37
Cellulase Units/mg TOS	CMC DNS U/mg TOS	45.90	45.66–51.17	49.20	35.60	45.70

(a): Mean values of six commercial batches.
(b): Batch used for Ames test.
(c): Batch used for chromosomal aberration assay.
(d): Batch used for the repeated dose 90-day oral toxicity study in rats.
(e): CMC DNS: Carboxymethylcellulose 3,5-dinitrosalicylic acid (see Section 3.3.1).
(f): NA: not analysed.
(g): TOS calculated as 100% − % water − % ash.

3.3.3. Purity

The lead content in the six commercial batches and in the batch used for genotoxicity testing was below 5 mg/kg which complies[17] with the specification for lead (≤ 5 mg/kg)[18] as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[19] No antimicrobial activity was detected in any of these batches (FAO/WHO 2006).[18]

Strains of *Penicillium*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites. The potential presence of the aflatoxins, ochratoxin, zearalenone, fumonisin and sterigmatocystin[20] in the food enzyme was examined and all values were below the limits of detection (LODs) of the applied analytical methods.[21] The possible presence of other secondary metabolites is addressed by the toxicological examination of the food enzyme–TOS.

3.3.4. Viable cells of the production strain

The absence of the production strain in the food enzyme was demonstrated in eighteen independent batches. Two agar plates were inoculated with 0.5 mL of food enzyme each and incubated at 25°C for five days. No colonies were produced.[22]

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 batches 1, 2 and 3 (Table 1) used in these studies have similar or lower chemical purity, and thus are considered suitable as test items.[23]

[17] Technical dossier/Additional information September 2019/Annex X/Annex Y; Additional information September 2020/Annex AM.
[18] Technical dossier: pg. 32 and Annex F.
[19] Technical dossier: p. 41 and Annex G.
[20] LOD: aflatoxin = 0.5 μg/kg, ochratoxin = 2 μg/kg; zearalenone = 25 μg/kg; fumonisin = 100 μg/kg; sterigmatocystin = 100 μg/kg.
[21] Technical dossier/1st submission/Annex L/Additional information September 2019/Annex X/Annex Y; Additional information September 2020/Annex AI.
[22] Additional information September 2020/Annex AJ/Annex AK/Annex AL.
[23] Technical dossier/1st submission/Annex T (CoA); Additional information September 2019/Annex X; Additional information September 2020/Annex AG/Annex AH/Annex AM.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in four strains of *Salmonella* Typhimurium (TA 1535, TA 1537, TA 98 and TA 100) and *E. coli* WP2uvrA, in the presence or absence of metabolic activation (S9-mix) applying the treat and plate method.[24] A preliminary toxicity test was conducted in *S.* Typhimurium TA100 at a range of concentrations from 5 to 5,000 μg TOS/plate. No cytotoxicity or increase of revertants was observed at any concentration tested. Two separate experiments in triplicate were carried out using five different concentrations of the food enzyme: 50, 158, 500, 1,581 and 5,000 μg TOS/plate. No increase in revertant colony numbers above the control values was observed in any tested strains at any concentrations of the food enzyme.

Therefore, the Panel concluded that the food enzyme did not induce gene mutations under the conditions employed.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1983) and following GLP in cultured human peripheral blood lymphocytes.[25] Two experiments were performed in duplicate. The concentrations used in the chromosome aberration assay were set based on the results of a dose range finding test. The highest concentrations applied (2.93 U/mL without and 5.86 U/mL with S9-mix) reduced the mitotic index to 54% and 67% of the vehicle control, respectively. In the first experiment, lymphocyte cultures were exposed to the food enzyme for 3 h + 15 h recovery in the presence of S9-mix at the concentrations 1.46, 2.93 and 5.86 U/mL (corresponding to 41.06, 82.4 and 164.8 μg TOS/mL), and for 18 h continuous treatment without S9-mix at 0.18, 1.46, and 2.93 (corresponding to 5.01, 41.06 and 82.4 TOS/mL). In the second experiment without S9-mix, lymphocyte cultures were exposed continuously for 18 h at 1.46, 2.93 and 5.86 U/mL (corresponding to 41.06, 82.4 and 164.8 μg TOS/mL), and for 32 h at 2.93 U/mL (82.4 μg TOS/mL) with S9-mix the cultures were treated for 3 h + 15 h recovery at 1.46, 2.93 and 5.86 U/mL (corresponding to 41.06, 82.4 and 164.8 μg TOS/mL) and for 3 h + 29 h recovery at 5.86 U/mL (164.8 μg TOS/mL). No statistically significant increase in the frequency of structural chromosomal aberrations was observed after treatment with the food enzyme at any concentrations analysed.

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

Overall, the Panel considered that the genotoxicity studies provided were sufficient for the evaluation of the enzyme cellulase and concluded that they did not indicate DNA reactivity at the gene or chromosome level.

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

A repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1981) and following GLP.[26] Groups of 10 male and 10 female SPF Sprague–Dawley [Crl: CD®BR strain] rats were given by gavage the food enzyme at doses of 50, 250 and 1,000 mg/kg body weight (bw) per day, corresponding to 4, 21 and 84 mg TOS/kg bw per day. Controls received the vehicle (distilled water).

No mortality was observed.

At necropsy, the relative brain weight in high-dose group females was slightly but statistically significantly increased (about 8%). As no microscopic brain changes were observed, and the value was within the relevant historical control ranges, this finding was considered by the Panel of no toxicological relevance.

No other statistically significant effects were observed.

The Panel identified a no observed adverse effect level (NOAEL) of 84 mg TOS/kg bw per day, the highest dose tested.

[24] Technical dossier/Additional information September 2020/Annex AM.
[25] Technical dossier/1st submission/pg. 61/Annex S.
[26] Technical dossier/1st submission/Annex U.

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 this cellulase produced with the *P. funiculosum* (strain DP-Lzc35) 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.[27]

No information is available on oral and respiratory sensitisation or elicitation reactions of this cellulase.

Respiratory allergic reactions following occupational inhalation of cellulase 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). Information on adverse reactions upon ingestion of cellulase 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[28]) are used as raw materials ([redacted]). In addition, [redacted] and [redacted], known allergens, are also present in the media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial/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.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed as in the case of distilled alcohol production. However, the food enzyme remains in the baked products and in the beer.

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 can be excluded for distilled alcohol production. The risk cannot be excluded for baking and brewing processes, but the likelihood of such reactions to occur 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 three food manufacturing processes at the recommended use levels summarised in Table 2.

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant[29]

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Baking processes[(b)]	Flour	Up to 35 mg TOS/kg flour
Brewing processes	Cereals	Up to 28.3 mg TOS/kg cereals
Distilled alcohol production	Cereals	Up to 18.4 mg TOS/kg cereals

TOS: total organic solids.

(a): The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' – not yet published at the time of adoption of this opinion.

(b): Additional information September 2019.

[27] Technical dossier/1st submission/pg. 65/Annex V.

[28] 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.

[29] Technical dossier/pg. 54–58.

In baking processes, the food enzyme is added to the raw materials during the preparation of the dough. It is used to hydrolyse β-glucans, so contributing to the reduction of dough viscosity.

In brewing processes, the food enzyme is added during the mashing step or the fermentation step, where it aids the degradation of cereal β-D-glucans. The hydrolysis decreases viscosity, thereby improving yield and consistency of the wort. The degradation also promotes the release of molecules such as proteins, pectins, colour or flavours.

The food enzyme remains in the dough and wort. Based on data provided on thermostability (see Section 3.3.1), it is expected that the cellulase is inactivated during the baking and brewing processes.

In distilled alcohol production, the food enzyme is added during the slurry mixing step, in the liquefaction step and if needed in the pre-saccharification or fermentation step. This is to improve the saccharification and fermentation processes.

Concerning distilled alcohol production, technical information and experimental data provided on the removal of food enzyme–TOS was considered by the Panel as sufficient to exclude these processes from the exposure assessment (Annex B in EFSA CEF Panel, 2016).

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by distillation (by > 99%), foods derived through this process, i.e. distilled alcohols, were excluded from the estimation.

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

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Section 3.5.1/Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from individual FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the average and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 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 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 40 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 23 European countries (Appendix B).

Table 3: Summary of estimated dietary exposure to food enzyme–TOS in six population groups

Population group	Estimated exposure (mg/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)	0.007–0.100 (12)	0.074–0.215 (16)	0.085–0.206 (19)	0.047–0.131 (20)	0.040–0.109 (22)	0.038–0.073 (21)
Min–max 95th percentile (number of surveys)	0.038–0.416 (10)	0.185–0.367 (14)	0.165–0.382 (19)	0.103–0.267 (19)	0.088–0.252 (22)	0.076–0.134 (21)

TOS: total organic solids.

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

Table 4: 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	+/–
Exclusion of other processes from the exposure estimate: –distilled alcohol production	–

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

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

The exclusion of one food manufacturing processes (distilled alcohol production) from the exposure assessment was based on > 99% of TOS removal during these processes and is not expected to have an impact on the overall estimate derived.

3.6. Margin of exposure

A comparison of the NOAEL (84 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.007–0.215 mg TOS/kg bw per day at the mean and from 0.038 to 0.416 mg TOS/kg bw per day at the 95th percentile, resulted in a margin of exposure (MOE) of at least 202.

4. Conclusions

Based on the data provided, the removal of TOS during distilled alcohol production, and the derived margin of exposure for baking and brewing processes, the Panel concluded that the food enzyme cellulase produced with the non-genetically modified *P. funiculosum* strain DP-Lzc35 does not give rise to safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Dossier 'Cellulase from *Penicillium funiculosum* (DP-Lzc35)'. March 2015. Submitted by Danisco US Inc.
2) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.
3) Additional information. September 2019. Submitted by DuPont Nutrition and Biosciences.
4) Additional information. September 2020. Submitted by DuPont Nutrition and Biosciences.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
GLP	Good Laboratory Practice
GM	genetically modified
GMP	Good Manufacturing Practice
ITS	internal transcribed spacer
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
LOD	limit of detection
MOE	margin of exposure
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Cooperation and Development
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://doi.org/10.2903/j.efsa.2021.6365).

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 food enzyme–TOS dietary exposure.

Appendix B – Population groups considered for the exposure assessment

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

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