SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 19 September 2019

doi: 10.2903/j.efsa.2019.5839

Safety evaluation of the food enzyme cellulase from *Trichoderma reesei* (strain DP-Nzc36)

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Abstract

The food enzyme cellulase (4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase; EC 3.2.1.4) is produced with the genetically modified *Trichoderma reesei* strain DP-Nzc36 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. The food enzyme is intended to be used in distilled alcohol production, starch processing for the production of glucose syrups and brewing processes. Since residual amounts of the food enzyme are removed by distillation and starch processing, no dietary exposure was calculated for these food processes. Based on the maximum use levels recommended for brewing 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 0.131 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 at least 97.6 mg TOS/kg bw per day which, compared to the estimated dietary exposure, results in a margin of exposure of at least 745. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure can be excluded in distilled alcohol production and is considered to be low when the enzyme is used in starch processing and brewing processes. 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, 4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase, EC 3.2.1.4, endo-1,4-β-D-glucanase, *Trichoderma reesei*, genetically modified microorganism

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Question number: EFSA-Q-2015-00454

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Note: The full opinion will be published in accordance with Article 12 of Regulation (EC) No 1331/2008 once decision on confidentiality will be received from the European Commission.

Suggested citation: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Silano V, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mortensen A, Riviere G, Steffensen I-L, Tlustos C, van Loveren H, Vernis L, Zorn H, Herman L, Marcon F, Gomes A, Kovalkovičová N, Liu Y, Maia J and Chesson A, 2019. Scientific Opinion on the safety evaluation of the food enzyme cellulase from *Trichoderma reesei* (strain DP-Nzc36). EFSA Journal 2019;17(10):5839, 15 pp. https://doi.org/10.2903/j.efsa.2019.5839

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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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 microorganisms 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:

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 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 CEF Panel, 2009) 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 have been introduced by the companies "Amano Enzyme Inc." for the authorisation of the food enzymes Pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL) and Rhizopuspepsin from *Rhizopus niveus* (strain AE-N), "Caglificio Clerici S.p.A." for the authorisation of the food enzyme Rennet paste fromabomasum of goat (*Capra aegagrus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), and "Danisco US Inc." for the authorisation of the food enzyme Cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36).

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008[3], 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, 11.03.2011, 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 Pullulanase from *Klebsiella pneumoniae* (strain AE-PUL), Pullulanase from *Pullulanibacillus naganoensis* (strain AE-PL), Rhizopuspepsin from *Rhizopus niveus* (strain AE-N), Rennet paste fromabomasum of goat (*Capra aegagrus hircus*), sheep (*Ovis aries*) and cattle (*Bos primigenius*), and Cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36) in accordance with the 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 cellulase from a genetically modified *Trichoderma reesei* (strain DP-Nzc36).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme cellulase from a genetically modified *Trichoderma reesei* strain DP-Nzc36.

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 25 October 2018 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, 2009) 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 EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with 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: 4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
Synonyms: endo-1,4-β-D-glucanase
IUBMB No: EC 3.2.1.4
CAS No: 9012-54-8.

The cellulase catalyses the hydrolysis of 1,4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans, resulting in the generation of mono, di-, tri-, tetra- and oligosaccharides composed of glucose residues. It is intended to be used in brewing processes, distilled alcohol production and starch processing for the production of glucose syrups.

3.1. Source of the food enzyme

The cellulase is produced with the genetically modified filamentous fungus *T. reesei* strain DP-Nzc36 [redacted], which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [redacted].[4]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is [redacted] *T. reesei* [redacted]. The American Type Culture Collection (ATCC) designation for *T. reesei* strain [redacted].[5]

[4] Technical dossier/Additional data June 2019/Annex AI_SI.
[5] Technical dossier/2nd submission/Annex X updated.

The recipient strain *T. reesei* was developed from strain by The recipient strain was characterised as *T. reesei* on the basis of .[6]

3.1.2. Characteristics of the introduced sequences

[7]

.[5]

3.1.3. Description of the genetic modification process

[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 *T. reesei* DP-Nzc36 The genetic stability of the production strain was demonstrated .[10]

[9]

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

The production strain is grown as a pure culture using a typical industrial medium in a submerged fermentation system with conventional process controls in place. After completion of the fermentation,

[6] Technical dossier/Additional data June 2019/Annex AJ_SI.
[7] Technical dossier/1st submission/Annex V.
[8] Technical dossier/2nd submission/Annex AC.
[9] Technical dossier/1st submission/Annex Y and Additional data June 2019.
[10] 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 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.

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.[11] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[12] The protein pattern of the food enzyme was investigated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Gels showed a major protein band at 44 kDa and some minor protein bands of higher and lower molecular mass.[13] No other enzymatic side activities were reported.

The in-house determination of cellulase activity is based on hydrolysis of the substrate carboxymethyl cellulose (CMC) (reaction conditions: pH 4.8, temperature 50°C, reaction time 10 min). The enzymatic activity is determined by measuring the release of reducing carbohydrates, which react with 3,5 dinitrosalicylic acid (DNS) producing a colour. The cellulase activity is quantified relative to an enzyme standard and expressed in carboxymethyl cellulose units/g (CMCU/g). One unit of cellulase activity corresponds to the amount of enzyme required to generate 1 μmol of glucose reducing sugar equivalents per minute under the conditions of the assay.[14]

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum around 65°C (pH 4.5) and a pH optimum around 4.5–5.5 (temperature 50°C). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.5). The enzyme activity decreased rapidly above 55°C, showing no residual activity when incubated at 75°C or above.[15]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for four food enzyme batches, three batches to be used for commercialisation and one batch produced for the toxicological tests (Table 1).[16] The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 15.1%. The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 73.8 CMCU/mg TOS.

Table 1: Compositional data of the food enzyme

Parameter	Unit	Batch			
		1	2	3	4[(a)]
Cellulase activity	CMCU/g batch[(b)]	11,098	11,552	10,833	15,345
Protein	%	9.43	9.46	9.01	12.5
Ash	%	0.04	0.04	0.04	0.04
Water	%	84.2	85.1	85.4	84.1
Total Organic Solids (TOS)[(c)]	%	15.8	14.9	14.6	15.9
Activity/mg TOS	CMCU/mg TOS	70.1	77.3	74.0	96.5

(a): Batch used for the toxicological studies.
(b): CMCU: Carboxymethyl cellulose units (see Section 3.1.3).
(c): TOS calculated as 100% - % water - % ash.

[11] Technical dossier/1st submission/Annex I.
[12] Technical dossier/Additional data June 2019.
[13] Technical dossier/Additional data June 2019/Annex AF_SI.
[14] Technical dossier/1st submission/Annex E.
[15] Technical dossier/1st submission/Annex E.1 and Additional data June 2019/Annex AH_SI.
[16] Technical dossier/Additional data June 2019/Annexes AD_SI and 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).[17]

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).[18]

The presence of mycotoxin T2 toxin was examined in three food enzyme batches. This mycotoxin was found not to be present at detectable levels in the food enzyme.[19]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The applicant did not provide information on the secondary metabolites, other than the mycotoxin indicated above, 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 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 batches, tested in quadruplicate. [redacted].[20]

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].[21]

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 batch 4 (Table 1) used in these studies has a slightly higher activity/mg TOS than the three batches for commercialisation However, this value was still comparable to those of the commercial batches, and thus, batch 4 was considered suitable for toxicological testing.

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, 1997a) and following Good Laboratory Practice (GLP).[22] Four strains of Salmonella Typhimurium (TA1535, TA1537, TA98 and TA100) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation (S9 mix), applying the direct plate incorporation method (experiment 1) and the pre-incubation test (experiment 2). The experiments were carried out in triplicate using five different concentrations of the food enzyme (50, 150, 500, 1500 and 5,000 μg total protein/plate, corresponding to 64, 191, 636, 1,908 and 6,360 μg TOS/plate). No cytotoxicity was observed at any concentration level 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.

[17] LOD: Pb = 0.05 mg/kg; Additional data June 2019.
[18] Technical dossier/2nd submission/Annex H updated and Additional data June 2019/Annexes AD_SI and AE_SI.
[19] LOD: T2 Toxin = 10 μg/kg; Technical dossier/2nd submission/Annex H and Additional data June 2019/Annex AD_SI.
[20] Technical dossier/2nd submission/Annex H updated and Additional data June 2019/Annex AL_SI.
[21] Technical dossier/Additional data June 2019/Annex AM_SI.
[22] Technical dossier/1st submission/Annex R.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosome aberration test was carried out according to the OECD Test Guideline 473 (OECD, 1997b) and following GLP.[23] Cultured human peripheral blood lymphocytes were treated with the food enzyme, vehicle control or appropriate positive controls. Two experiments were performed in duplicate. Based on the results obtained in a preliminary toxicity test, the cultures were exposed to the food enzyme at 156, 312, 625, 1,250, 2,500, 5,000 μg total protein/mL (corresponding to 199, 398, 795, 1,590, 3,180 and 6,360 μg TOS/mL, based on total protein of 12.5% according to study protocol), applying a short-term treatment (4 + 20 h of recovery) in the presence and absence of S9-mix, and a continuous treatment (24 + 0 h of recovery) in the absence of S9-mix. No precipitation or significant changes in pH and osmolarity were detected. Cytotoxicity, measured as mitotic inhibition, did not exceed 58% of concurrent negative control values. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in negative controls and within the range of the laboratory historical solvent control data.

The Panel concluded that 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 rodents

A repeated dose 90-day oral toxicity study was performed according to OECD Test Guideline 408 (OECD, 1998), and following GLP.[24] Groups of 10 male and 10 female Wistar Han™: RccHan: WIST rats received the food enzyme by gavage for 13 weeks, at dose levels of 20, 40 and 80 mg total protein/kg body weight (bw) per day corresponding to 24.4, 48.8 and 97.6 mg TOS/kg bw per day, respectively. Controls received the vehicle (0.9% saline).

No mortality was observed.

Concerning organ weights, the only statistically significant difference to controls was slightly but statistically significantly increased in absolute and relative adrenal weights of high-dose males. As this finding was not accompanied with histopathological changes in the adrenal glands, there was no clear dose–response relationship and it was not seen in high-dose females, the Panel did not consider this as an adverse effect.

Histopathological examinations revealed minimal centrilobular hepatocytes hypertrophy of the liver in 3 out of 10 high-dose males. The Panel noted that this finding correlated with a slight and not statistically significant increase in absolute and relative liver weights in this group as compared to the controls. The Panel further noted that hepatocyte enlargement is a common histologic finding in rodent liver which, in the absence of associated degenerative or inflammatory changes, is considered as an adaptive response. This finding was not recorded at lower doses in males and was absent in females. Therefore, the Panel considered this finding as not adverse.

No other statistically significant differences were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 97.6 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 cellulase produced with the genetically modified *T. reesei* strain DP-Nzc36 was assessed by comping 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no match was found.

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

Respiratory allergy following occupational inhalation of cellulase 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 cellulase in individuals sensitised through the

[23] Technical dossier/1st submission/Annex S.
[24] Technical dossier/1st submission/Annex T.

respiratory route has not been reported. Therefore, it can be concluded that an allergic reaction upon oral ingestion of cellulase produced with the genetically modified *T. reesei* strain DP-Nzc36, in individuals respiratory sensitised to cellulase cannot be excluded, but the likelihood of such reaction to occur is considered to be low.

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 is the case for distilled alcohol production.

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 starch processing 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.[25]

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

Food manufacturing process[(a)]	Raw material	Recommended dosage of the food enzyme
Brewing processes	Cereals	Up to 28.5 mg TOS/kg cereals
Distilled alcohol production	Cereals	Up to 35.7 mg TOS/kg cereals
Starch processing for the production of glucose syrups	Starch	Up to 11.9 mg TOS/kg starch

(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.

In brewing processes, the food enzyme is added in the mashing step or in the fermentation step, where it takes part in the degradation of cellulose and cereal beta-D-glucans. It decreases viscosity, thereby improving yield and consistency of the products. The degradation allows the release of molecules such as proteins, pectins, colour or flavours.

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

In distilled alcohol production, the food enzyme is added in the pretreatment, liquefaction, pre-saccharification or the fermentation steps.

In starch processing for glucose syrups production, the food enzyme is added during the saccharification step.

Experimental data have been provided on the removal (> 99%) of protein in the course of distilled alcohol production and starch processing for the production of glucose syrups (Documentation provided to EFSA No°4). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS (including substances other than proteins) are removed by distillation. In addition, taking into account the purification steps applied to the production of glucose syrups, i.e. filtration, ion exchange chromatography, treatment with active carbon, the Panel also considers that the amount of TOS in the final glucose syrup will be removed to a similar degree.

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by distillation and by the purification steps applied during the production of glucose syrups (by > 99%), foods/ingredients derived through these two processes, i.e. distilled alcohols and glucose syrups were excluded from the estimation.

For 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

[25] Technical dossier/Section 3.2.1.4.

Food Consumption Database[26] 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 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 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 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 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

Table 3: 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 (10)	0 (14)	0.000–0.001 (19)	0.000–0.006 (18)	0.002–0.029 (19)	0.001–0.014 (18)
Min–max 95th percentile (number of surveys)	0 (8)	0 (12)	0 (19)	0.000–0.034 (17)	0.016–0.131 (19)	0.004–0.060 (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 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 survey 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.

[26] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

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 (97.6 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates in six human population groups of 0–0.029 mg TOS/kg bw per day at the mean and from 0 to 0.131 mg TOS/kg bw per day at the 95th percentile, resulted in margins of exposure (MOE) above 745 indicating that there is no safety concern.

4. Conclusions

Based on the data provided and, in particular, considering the removal of TOS during distilled alcohol production and starch processing for the production of glucose syrups, and the derived margin of exposure for brewing processes, the Panel concluded that the food enzyme cellulase produced with the genetically modified *T. reesei* strain DP-Nzc36 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 cellulase from a genetically modified strain of *Trichoderma reesei* (DP-Nzc36) in accordance with Regulation (EC) No 1331/2008", July 2015. Submitted by Danisco US Inc.
2) Additional information was received from Danisco US Inc in June 2019.
3) Summary report on GMM part. August 2018. Delivered by contractor (DTU, Kongens Lyngby, Denmark).
4) Additional information on "Food enzyme removal during the production of cereal based distilled alcoholic beverages" and "Food enzyme carry/over in glucose syrups". February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CBS	Westerdijk Fungal Biodiversity Institute
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
CMCU	Carboxymethyl cellulose units
FAO	Food and Agriculture Organization of the United Nations
GMO	Genetically modified organisms
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme TOS in details

Information provided in this appendix is shown in an Excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.5839).

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 of 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[(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).