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Safety evaluation of the food enzyme glucan 1,4-α-maltotetraohydrolase from *Bacillus licheniformis* (strain DP-Dzr46)

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Abstract

The food enzyme glucan 1,4-α-maltotetraohydrolase (4-α-D-glucan maltotetraohydrolase, EC 3.2.1.60) is produced with a genetically modified *Bacillus licheniformis* strain DP-Dzr46 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 glucan 1,4-α-maltotetraohydrolase food enzyme is intended to be used in baking processes. Based on the maximum use levels, dietary exposure to the food enzyme–Total Organic Solids (TOS) was estimated to be up to 0.405 mg TOS/kg body weight (bw) per day in European populations. The toxicity studies were carried out with another glucan 1,4-α-maltotetraohydrolase from *B. licheniformis* (strain DP-Dzf24). The Panel considered this food enzyme as a suitable substitute to be used in the toxicological studies, because it derives from the same recipient strain as strain DP-Dzr46, the location of the inserts is comparable, no partial inserts were present and the production methods are comparable. Genotoxicity tests did not raise a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level (NOAEL) at the highest dose of 94 mg TOS/kg bw per day that, compared with the estimated dietary exposure, results in a sufficiently high margin of exposure of at least 232. Similarity of the amino acid sequence to those of known allergens was searched and none 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, glucan 1, 4-α-maltotetraohydrolase, 4-α-D-glucan maltotetraohydrolase, EC 3.2.1.60, exo-maltotetraohydrolase, *Bacillus licheniformis*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No. 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

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

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA 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 European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7(2) of Regulation (EC) No 1332/2008 on food enzymes.

Five applications have been introduced by the company "Danisco US Inc." for the authorisation of the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb52), Glucan 1,4-alpha-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh49), Glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzr46), Glucan 1,4-alpha-maltohydrolase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzr50) and Glucan 1,4-alpha-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh34).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, 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, pp. 7–15.

[2] Regulation (EC) No. 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessments on the food enzymes Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb52), Glucan 1,4-alpha-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh49), Glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzr46), Glucan 1,4-alpha-maltohydrolase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzr50) and Glucan 1,4-alpha-glucosidase from a genetically modified strain of *Trichoderma reesei* (DP-Nzh34) 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 glucan 1,4-alpha-maltotetraohydrolase from a genetically modified *B. licheniformis* (strain DP-Dzr46).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme glucan 1,4-alpha-maltotetraohydrolase from genetically modified *B. licheniformis* (strain DP-Dzr46).

Additional information was sought from the applicant during the assessment process in a request from EFSA sent on 27 June 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, 2011).

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 to the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	Glucan 1,4-α-maltotetraohydrolase
Systematic name:	4-α-D-glucan maltotetraohydrolase
Synonyms:	exo-maltotetraohydrolase; 1,4-α-D-glucan maltotetraohydrolase
IUBMB No.:	EC 3.2.1.60
CAS No.:	37288-44-1
EINECS No.:	686-668-7

The glucan 1,4-α-maltotetraohydrolase catalyses the hydrolysis of 1,4-α-glycosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other oligosaccharides. It is intended to be used in baking processes.

3.1. Source of the food enzyme

The glucan 1,4-α-maltotetraohydrolase is produced with a genetically modified bacterium *B. licheniformis* strain DP-Dzr46, which is deposited at the restricted Westerdijk Fungal Biodiversity Institute Culture collection (CBS-KNAW, The Netherlands) with deposit number [redacted].[4]

[4] Technical dossier/Additional data/Annex AM_SI.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium [redacted] The recipient strain [redacted] as confirmed by whole genome sequencing (WGS) analysis.[7]

3.1.2. Characteristics of introduced sequences

The maltotetraohydrolase encoding gene [redacted]

The vector [redacted]

3.1.3. Description of the genetic modification process

The recipient strain [redacted][9]

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 *B. licheniformis* DP-Dzr46 differs from the parental strain [redacted] Its genotypic stability was demonstrated [redacted].[10]

Although *B. licheniformis* is included in the list of species considered suitable for the QPS (Qualified Presumption of Safety) approach to safety assessment (EFSA BIOHAZ Panel 2017), the production strain does not meet the qualification required. [redacted] its lack of cytotoxicity has not been demonstrated.

No issues of concern arising from the genetic modifications were identified by the Panel except the presence [redacted]

[5] Technical dossier/1st submission/Annex K.
[6] Technical dossier/1st submission/Annexes AA and AB and Additional data/Annex Z.
[7] Technical dossier/Additional data/Annex AS_SI_NGS.
[8] Technical dossier/1st submission/Annex AA and 2nd submission/Annex Z.
[9] Technical dossier/2nd submission/Annex Z.
[10] Technical dossier/2nd submission/Annex AF.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004[11], with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP), and in accordance with current Good Manufacturing Practice (GMP).

The production strain is grown as a pure culture using a typical industrial medium in a submerged, batch 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.[12] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[13]

The Panel considered that sufficient information has been provided on the manufacturing process and on 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 glucan 1,4-α-maltotetraohydrolase is a single polypeptide chain of [redacted] amino acids.[14] 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 gels showed a single major protein band corresponding to an apparent molecular mass of about [redacted] kDa,[15] which is consistent with the expected mass of the enzyme. No other enzymatic side activities were reported.[16]

The in-house determination of glucan 1,4-α-maltotetraohydrolase activity is based on hydrolysis of the substrate non-reducing-end blocked *p*-nitrophenyl maltoheptoside (reaction conditions: pH 5.6, 30°C). The reaction product is further hydrolysed by glucoamylase and α-glucosidase, resulting in the release of glucose and *p*-nitrophenyl. The enzymatic activity is determined by measuring the release of *p*-nitrophenyl spectrophotometrically at 410 nm.[17] The rate of *p*-nitrophenyl release is proportional to glucan 1,4-α-maltotetraohydrolase activity that is finally expressed in betamyl units (BMU)/g.

The food enzyme has been characterised with regard to its temperature and pH profiles. It has a temperature optimum between 60 and 70°C (pH 6.5) and a pH optimum around pH 6 (60°C). Thermostability was tested after a pre-incubation of the food enzyme for up to 10 min at different temperatures. Under the conditions of the applied temperature stability assay (pH 5), the 'half-life' time of glucan 1,4-α-maltotetraohydrolase is less than 1 min, at the highest temperature tested (89°C).[18]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation (Table 1). The average Total Organic Solids (TOS) of the three food enzyme batches for commercialisation was 7.56% (range 7.01–8.18%). The average enzyme activity/TOS ratio of the three food enzyme batches for commercialisation is 3,394 BMU/mg TOS.

[11] Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[12] Technical dossier/Section 3.2.1.2.5.
[13] Additional data/Annex AJ_SI.
[14] Technical dossier/1st submission/Annex H.
[15] Technical dossier/2nd submission/p. 37/Additional data/Annex AI_SI.
[16] Technical dossier/2nd submission/p. 40.
[17] Technical dossier/Annex D.
[18] Technical dossier/1st submission/Annex I and 2nd submission/p. 41-42.

Table 1: Compositional data of the food enzyme[(a)]

Parameter	Unit	Batches		
		1	2	3
Glucan 1,4-α-maltotetraohydrolase activity	BMU/g[(b)]	286,133	232,457	252,520
Protein	%	8.32	6.80	7.66
Ash	%	0.56	0.33	0.40
Water	%	91.26	92.66	92.10
Total Organic Solids (TOS)[(c)]	%	8.18	7.01	7.50
Glucan 1,4-α-maltotetraohydrolase/mg TOS	BMU/mg TOS	3,498	3,316	3,367

(a): Technical dossier/Additional data/Annex AH_SI_CoAs.
(b): BMU: Betamyl Units (see Section 3.3.1).
(c): TOS calculated as 100% - % water - % ash.

3.3.3. Purity

The lead content in the three commercial batches 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).[19,20]

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 unit (CFU) per gram. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[20]

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 production batches.[21] [redacted]

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] in three independent batches in triplicate. No DNA was detected [redacted][23] Therefore, the presence of this gene in the production strain has no consequences for the food enzyme safety.

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. These studies were performed with a glucan-1,4-alpha-maltotetraohydrolase produced by a different strain of *B. licheniformis* (DP-Dzf24).

The focus of the toxicological studies of food enzymes is the assessment of non-protein components of TOS. Only rarely is the enzyme protein itself considered a potential hazard. No potential hazard is expected for glucan-1,4-α-maltotetraohydrolase.

B. licheniformis strain DP-Dzf24 was developed from the same recipient strain [redacted] as strain DP-Dzr46 using the same genetic modification system with a different variant of maltotetraohydrolase gene in each case [redacted].

The genetic modification in strain DP-Dzr46 only differs from that of strain DP-Dzf24 in the [redacted]

[19] LOD: Pb = 0.05 mg/kg.
[20] Technical dossier/Additional data/Annex AH_SI.
[21] Technical dossier/Additional data/Annex AQ.
[22] Technical dossier/2nd submission/Annex G.
[23] Technical dossier/Additional data/Annex AR-SI_rDNA.

The genetic differences between the enzyme 1,4-alpha-maltotetraohydrolase produced by *B. licheniformis* strain DP-Dzf24 and that produced by strain DP-Dzr46 are not expected to result in a different toxigenic potential.

The batch of glucan-1,4-alpha-maltotetraohydrolase food enzyme from the *B. licheniformis* strain DP-Dzf24, used for toxicological studies, was produced according to a standard procedure similar to the one described in Section 3.2 of this opinion.[25] No major differences were encountered in the raw materials used.

Taking the microbiological data, the genetic modifications made and manufacturing process into account, the Panel considered the glucan 1,4-α-maltotetraohydrolase from *B. licheniformis* strain DP-Dzf24 as a suitable substitute for the glucan 1,4-α-maltotetraohydrolase from *B. licheniformis* strain DP-Dzr46 in the toxicological studies. The composition of the batch used to carry out the toxicological studies is shown in Table 2.

Table 2: Compositional data of the food enzyme glucan 1,4-α-maltotetraohydrolase derived from strain DP-Dzf24 used in toxicological studies(a)

Parameter	Unit	Batch 4 (b)
Glucan 1,4-α-maltotetraohydrolase activity	BMU/g batch(c)	234,745
Protein	%	7.66
Ash	%	0.51
Water	%	90.40
Total Organic Solids (TOS) (d)	%	9.09
Glucan 1,4-α-maltotetraohydrolase/mg TOS	BMU/mg TOS	2,582

(a): Technical dossier/Annex V and 2nd Submission/p.71.
(b): Batch used for the toxicological studies.
(c): BMU: Betamyl Units.
(d): TOS calculated as 100% - % water - % ash.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was made according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP).[26] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvrA* were used in the presence or absence of metabolic activation (S9 mix), applying the standard plate incorporation method. Two experiments were carried out using five concentrations of food enzyme (50, 150, 500, 1,500 and 5,000 μg total protein/plate corresponding to 59.3, 178, 593, 1,780 and 5,933 μg TOS/plate), and appropriate positive and negative controls. 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.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 2014) and following GLP.[27] The food enzyme was tested for its ability to induce chromosomal aberrations in human peripheral blood lymphocytes with and without metabolic activation (S9 mix). Three experimental conditions were applied: a short-term treatment followed by a recovery period (4+20 h) in the presence and absence of S9, and a continuous treatment (24+0 h) in

[24] Additional information/Annex Z.
[25] Technical dossier/Additional data/Annex AK.
[26] Technical dossier/1st submission/Annex S.
[27] Technical dossier/1st submission/Annex T.

the absence of S9. In a preliminary cytotoxicity assay, performed in a range of concentrations from 19.53 to 5,000 μg of total protein/mL, precipitate was reported at 156.25 μg/mL and above in the short-term treatments and at 39.06 and above after the continuous treatment. Based on these results, the cells were exposed to the test material at 156.25, 312.5 and 625 μg total protein/mL (corresponding to 185, 371 and 742 μg TOS/mL) in the short-term treatments. No cytotoxicity was observed at any concentration in these test conditions. In the second experiment, the cells were treated with 156.25, 312.5 and 625 μg total protein/mL (corresponding to 185, 371 and 742 μg TOS/mL) for 4 h in the presence of S9 followed by a 20-h recovery period (4+20 treatment), and with 156.25, 234.38 and 312.5 μg total protein/mL (corresponding to 185, 278 and 371 μg TOS/mL) for 24 h in the absence of S9. The highest concentrations induced approximately 70% and 14% reduction in mitotic index in the continuous and short treatment, respectively. In all the tested conditions, the frequency of cells with 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 negative control data.

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

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

The repeated dose 90-days oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998), and following GLP.[28] Groups of 10 male and 10 female Wistar Han™: HsdRccHan™:Wist strain rats received by gavage the food enzyme in doses corresponding to 28, 56 and 94 mg TOS/kg body weight (bw) per day. Controls received the vehicle (saline solution 0.9%).

Mortality was observed on day 90 just prior to terminal kill in one mid-dose female. This animal did not show any clinical signs prior to death. One low-dose female was culled on day 68 following signs of lethargy, piloerection, hunched posture, pallor of the extremities and staining around the snout. These observations were considered as non-treatment related.

Body weight gain increased statistically significantly in mid-dose males during week 8 and was significantly reduced in low-dose males during week 11. These isolated findings were considered to be incidental and of no toxicological importance.

Behavioural observations revealed increased respiratory rate, tiptoe gait and hunched posture in mid-dose females on day 27. One control male displayed decreased respiration and hunched posture on day 20. These effects were considered not to be treatment-related and of no toxicological importance.

In blood chemistry, a statistically significant increase in potassium in females of all treatment groups and a reduction in chloride levels in high-dose females were observed; however, these changes were within historical control ranges.

Statistically significant reductions in absolute and relative ovary weights were detected in females of all treatment groups when compared to controls. As the control values of this parameter were higher than the expected ranges for rats of the age and strain employed, and a convincing dose-related response or any histopathological correlation was absent, these reductions were considered to be unrelated to treatment.

No other statistically significant differences compared to controls were observed. The Panel identified the no observed adverse effect level (NOAEL) of 94 mg TOS/kg bw per day.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of the glucan 1,4-α-maltotetraohydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzr46 was assessed by comparing its amino acid sequence with those of known allergens according to the scientific opinion on the assessment of allergenicity of genetically modified 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.

[28] Technical dossier/1st submission/Annex U.

No information is available on oral sensitisation or elicitation reactions of this glucan 1,4-α-maltotetraohydrolase. Therefore, it can be concluded that an allergic reaction upon oral ingestion of glucan 1,4-α-maltotetraohydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzr46 cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

According to the information provided, substances or products that may cause allergies or intolerances (Regulation (EU) No 1169/2011[29]) are used as raw materials ([redacted]) 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 fungal biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues are not expected.

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 occurring is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes,[30] at a recommended use level of up to 34 mg TOS/kg flour.[31]

During baking, the food enzyme is added to the raw materials during the preparation of the dough in order to reduce staling, and ensure a uniform volume and an improved crumb structure of the bakery product.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is expected that the glucan 1,4-α-maltotetraohydrolase is inactivated during baking processes.

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 (see Section 3.5.1) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. 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 1 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).

[29] 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.

[30] 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 adoption of this opinion.

[31] Technical dossier/Section 3.2.1.4.

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)	0.006–0.095 (10)	0.072–0.204 (14)	0.082–0.196 (19)	0.045–0.125 (18)	0.033–0.078 (19)	0.033–0.069 (18)
Min–max 95th percentile (number of surveys)	0.037–0.405 (8)	0.179–0.346 (12)	0.160–0.369 (19)	0.100–0.255 (17)	0.073–0.153 (19)	0.066–0.121 (18)

3.5.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and are summarised in Table 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	+/–

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.

3.6. Margin of exposure

A comparison of the NOAEL (94 mg TOS/kg bw per day) from the 90-day study with the derived exposure estimates of 0.006–0.204 mg TOS/kg bw per day at the mean and from 0.037 to 0.405 mg TOS/kg bw per day at 95th percentile, resulted in the margin of exposures (MOE) above 232, 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 glucan 1,4-α-maltotetraohydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzr46 does not give rise to safety concerns under the intended conditions of use.

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

Documentation provided to EFSA

1) Technical dossier "Application for authorization of Glucan 1,4-alpha-maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* DP-Dzr46 in accordance with Regulation (EC) No 1331/2008" March 2015. Submitted by Danisco US Inc.
2) Additional information, January 2019. Submitted by Danisco US Inc.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units

EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practices
GMO	EFSA Panel on Genetically Modified Organisms
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kDa	Kilo Daltons
LOD	limit of detection
MOE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
QPS	Qualified Presumption of Safety
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization
WGS	whole genome sequence

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

Information provided in this appendix is shown in an excel file (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5684).

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).