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Safety evaluation of the food enzyme glucan 1,4- α -maltotetraohydrolase from *Bacillus licheniformis* (strain DP-Dzf24)

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Abstract

The food enzyme glucan 1,4- α -maltotetraohydrolase (EC 3.2.1.8) is produced with the genetically modified *Bacillus licheniformis* strain DP-Dzf24 by Danisco US Inc. The production strain contains multiple copies of a known antimicrobial resistance gene. However, based on the absence of viable cells and DNA in the food enzyme, this is not considered to be a risk. The food enzyme is intended to be used in baking processes and starch processing for the production of glucose syrups. The residual amounts of the Total Organic Solids (TOS) in glucose syrups are removed by filtration and purification during starch processing. Consequently, dietary exposure was not calculated for this use. Based on the maximum use levels recommended for the baking processes and individual data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme–TOS was estimated to be up to 0.271 mg TOS/kg body weight per day in European populations. Toxicological tests with the food enzyme indicated that there was no concern with respect to genotoxicity or systemic toxicity. A no-observed-adverse-effect level (NOAEL) was identified in rats, which, compared with the dietary exposure, results in a margin of exposure of at least 347. The allergenicity was evaluated by searching for similarity of the amino acid sequence to those of known allergens; no match was found. The Panel considers that, under the intended conditions of use, the risk for allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood is considered low. Based on the microbial source, genetic modifications, the manufacturing process, the compositional and biochemical data, the dietary exposure assessment and the findings in the toxicological studies, the Panel concludes that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Keywords: food enzyme, glucan 1, 4- α -maltotetraohydrolase EC 3.2.1.1, *Bacillus licheniformis*, DP-Dzf24, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No. 1332/2008¹ 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. 1331/2008² established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

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

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

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008³ on food enzymes.

Five applications have been introduced by the companies "DSM Food Specialties B.V." for the authorisation of the food enzyme Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG); "Advanced Enzyme Technologies Ltd." for the authorisation of the food enzymes Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC) and Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC); "Danisco US Inc." for the authorisation of the food enzyme Glucan 1,4- α -maltotetrahydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24), and "Amano Enzyme Inc." for the authorisation of the food enzyme Catalase from *Aspergillus niger* (strain AE-CN).

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 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.

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011⁴ implementing Regulation (EC) No 1331/2008⁵, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

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 Carboxypeptidase C from a genetically modified strain of *Aspergillus niger* (strain PEG), Maltogenic amylase from a genetically modified strain of *Escherichia coli* (strain BLASC), Triacylglycerol Lipase from a genetically modified strain of *Aspergillus niger* agg. (strain FL100SC), Glucan 1,4- α -maltotetraohydrolase from a genetically modified strain of *Bacillus licheniformis* (strain DP-Dzf24) and Catalase from *Aspergillus niger* (strain AE-CN) 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 glucan 1,4- α -maltotetraohydrolase from a genetically modified strain of *B. licheniformis* (strain DP-Dzf24).

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- α -maltotetraohydrolase obtained from a genetically modified *B. licheniformis* strain (DP-Dzf24).

Additional information was requested from the applicant during the assessment process on 15 November 2017, 26 June 2018 and 28 November 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, 2009b) as well as in the EFSA Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011) and following the relevant existing guidance'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: glucan 1,4- α -maltotetraohydrolase
Systematic name: 4- α -D-glucan maltotetraohydrolase
Synonyms: exo-maltotetraohydrolase
IUBMB No: EC 3.2.1.60
CAS No: 37288-44-1

The glucan 1,4- α -maltotetraohydrolase catalyses the hydrolysis of (1 \rightarrow 4)- α -D-glucosidic linkages in starch polysaccharides to remove successive maltotetraose units from the non-reducing chain ends. It is intended to be used in starch processing for glucose syrups production and baking processes.

⁴ 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.

⁵ 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.

3.1. Source of the food enzyme

The maltotetrahydrolase amylase is produced with a genetically modified strain of *B. licheniformis* DP-Dzf24 [REDACTED] which is deposited in the CBS International Culture Collection (the Netherlands) with deposition number [REDACTED].⁶

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *B. licheniformis* [REDACTED]. The parental strain [REDACTED] ribotyping of rRNA genes and by partial 16S rRNA gene sequence analysis.⁷ [REDACTED]. The absence of cytotoxicity activity was confirmed on CHO_K1 (Chinese Hamster Ovary cells).⁸

The recipient strain *B. licheniformis* [REDACTED] was developed from the parental strain [REDACTED]

3.1.2. Characteristics of introduced sequences

3.1.3. Description of the genetic modification process¹⁰

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.

Genotypic stability of the *B. licheniformis* DP-Dzf24 production strain was demonstrated [REDACTED]

⁶ Technical dossier/Additional information May 2018/Annex H.

⁷ Technical Dossier/1st submission/Annex BB.

⁸ Technical dossier/Additional information May 2018/Annex K.

⁹ Technical dossier/Additional information May 2018/Annex L.

¹⁰ Technical Dossier/2nd submission/Annex Z

¹¹ Technical dossier/Additional information October 2018/Annexes AA and AB.

¹² Technical Dossier/2nd submission/Annex FF.

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

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No. 852/2004,¹³ with food safety procedures based on hazard analysis and critical control points and in accordance with current good manufacturing practice.¹⁴

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration leaving a supernatant containing the food enzyme. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained while most of the low molecular weight material passes the filtration membrane and is discarded. The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.¹⁵

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 glucan 1,4- α -maltotetrahydrolase is a single polypeptide chain of [REDACTED] amino acids. The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be about [REDACTED] kDa. The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. The gel showed a major band migrating between the 36.5 and 55.4 kDa markers. The protein profile also included bands of lower staining intensity.

The glucan 1,4- α -maltotetrahydrolase activity is quantified based on a colorimetric assay and monitors the rate of hydrolysis of non-reducing-end blocked *p*-nitrophenyl maltoheptoside. The rate of *p*-nitrophenyl release is proportional to glucan 1,4- α -maltotetrahydrolase activity (BMU) and is monitored at 410 nm.

The food enzyme has been characterised with regard to its temperature and pH profiles. The enzyme shows a temperature optimum of 60–65°C and an optimum pH of about 6.0. The thermostability of the glucan 1,4- α -maltotetrahydrolase was tested by incubating a sample to 75–85°C for up to 90 min. At 85°C, the 'half-life' time of maltotetrahydrolase is approx. 18 min, corresponding to a residual activity of ca. 3% after 90 min.

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme have been provided for three batches used for commercialisation (1–3)¹⁶ and one batch (4) used for toxicological testing (Table 1). The mean Total Organic Solids (TOS) of the three commercial batches was 8.2%; the values ranged from 3.7% to 13.8% (Table 1). The mean enzyme activity/TOS ratio of the three commercial food enzyme batches was 3,127 BMU/mg TOS; the values ranged from 2,186 to 4,684 BMU/mg TOS.

¹³ 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.

¹⁴ Technical dossier/1st submission/Annex L

¹⁵ Technical dossier/Additional information May 2018/Annex G.

¹⁶ Technical dossier/Additional information May 2018/Annexes B–D.

Table 1: Compositional data of the food enzyme

Parameter	Units	Batches			
		1	2	3	4 ^(a)
Glucan 1,4- α -maltotetrahydrolase activity	BMU/g batch ^(b)	334,000	302,000	92,919	241,318
Protein	%	6.30	7.77	2.37	7.66
Ash	%	0.28	0.17	0.48	0.51
Water	%	92.6	86.0	95.8	90.40
Total Organic Solids (TOS) ^(c)	%	7.12	13.83	3.72	9.09
Glucan 1,4- α -maltotetrahydrolase activity/mg TOS	BMU/mg TOS	4,684	2,186	2,511	2,655

(a): Batch used for toxicological tests.

(b): BMU: Betamyl Units (for the assay see Section 3.1.2).

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

3.3.3. Purity

The food enzyme 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 *E. coli* and *Salmonella* species are absent in 25 g of sample and total coliforms do not exceed 30 colony forming units (CFU) per gram. No antimicrobial activity as specified in FAO/WHO (2006) was detected in any of these batches.¹⁷

The Panel considered the compositional data provided for the food enzyme as sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the product was demonstrated in 12 production batches¹⁸ analysed in duplicate, by culturing 1 mL of product sample on non-selective agar and incubating at 36°C for 48 h.¹⁹

The absence of recombinant DNA in the food enzyme was demonstrated

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 lower activity/TOS ratio and higher ash concentration compared to the three commercial food enzyme batches, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test²¹

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). Four strains of *Salmonella* Typhimurium (TA98, TA1537, TA100 and TA1535) and *E. coli* WP2uvrA 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 μ g total protein/plate corresponding to 59.3, 178, 593, 1,780 and 5,933 μ g 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,

¹⁷ Technical Dossier/Annex G and Additional Information May 2018/Annex A.

¹⁸ Technical dossier/1st submission/Annex G and Additional information May 2018/Annex I.

¹⁹ Technical Dossier/2nd submission/Annex H.

²⁰ Technical dossier/Additional information March 2019/Annex AC.

²¹ Technical Dossier/1st submission/Annex S.

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 test²²

The *in vitro* mammalian chromosomal aberration test was carried out according to OECD Test Guideline 473 (OECD, 1997) and following GLP. 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 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 $\mu\text{g}/\text{mL}$ and above in the short-term treatments and at 39.06 and above after the continuous treatment. Despite the limited precipitation, 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 rodents²³

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP. Groups of 10 male and 10 female Wistar HanTM:HsdRccHanTM:Wist strain rats received the food enzyme by gavage 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 level in females of all treatment groups and a reduction in chloride level in high-dose females were observed; however, these changes were within relevant 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 correlate were absent, these reductions were considered to be unrelated to treatment.

No other statistically significant differences from controls were observed.

²² Technical Dossier/1st submission/Annex T.

²³ Technical Dossier/1st submission/Annex U.

The Panel identified the no-observed-adverse-effect level (NOAEL) of 94 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The potential allergenicity of glucan 1,4- α -maltotetrahydrolase produced with the genetically modified strain of *B. licheniformis* DP-Dzf24 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 window of 80 amino acids as the criterion, no matches were found.

No information is available on oral- or respiratory sensitisation and elicitation reactions to this glucan 1,4- α -maltotetrahydrolase.

According to the information provided, substances or products that may cause allergies (casein) or intolerances (barley cream, barley meal) (Regulation EU 1169/2011)²⁴ are used as raw materials in the media fed to the microorganisms. However, these proteins will be digested during the fermentation process and consumed by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids will be removed. Therefore, 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. In the starch processing for the production of glucose syrups, experimental data showed a significant removal (> 99%) of protein (Documentation provided to EFSA No 7). However, traces of protein could be present in glucose syrup.

The Panel considers that under the intended conditions of use the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in starch processing for glucose syrup production and baking 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 applicant²⁵

Food manufacturing process ^(a)	Raw material	Recommended use level of the food enzyme
Baking processes	Flour	2.28–22.8 mg TOS/kg flour
Starch processing for glucose syrup production	Starch	2.00–20.0 mg TOS/kg starch

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.

In starch processing, the food enzyme is added to the liquefied starch during the saccharification step in order to reduce viscosity of gelatinised starch, produce soluble dextrans and oligosaccharides and increase the flexibility in production (choice of temperature and pH).

²⁴ 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.

²⁵ Technical dossier: p. 62–65.

Experimental data have been provided on the removal (> 99%) of protein in the course of starch processing for the production of glucose syrups (Documentation provided to EFSA No 7). The Panel considered the evidence as sufficient to conclude that residual amounts of TOS are removed by the purification steps applied during the production of glucose syrups, i.e. filtration, ion exchange chromatography and carbon treatment.

In baking processes, the food enzyme is added to flour during the preparation of dough. The glucan 1,4- α -maltotetrahydrolase catalyses the hydrolysis of 1,4- α -glycosidic linkages in starch glycogen and related polysaccharides and oligosaccharides, resulting in the generation of maltotetraose and other oligosaccharides. This reaction facilitates the handling of the dough, resulting in more uniform products.

The enzyme is specific in its action, not known to catalyse other reactions than this endohydrolysis of starch components, amylopectin and amylose, glycogen and polysaccharides and oligosaccharides into dextrans and other oligosaccharides. These reaction products are naturally present in starch-containing foods. Owing to the substrate specificity of the enzyme, no unintended reaction products in foods are to be expected.

The food enzyme remains in the dough. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the glucan 1,4- α -maltotetrahydrolase may not be fully inactivated during baking processes.

3.5.2. Dietary exposure estimation

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

For the baking 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 Database²⁶ 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 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 mean 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 mean 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/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)	0.004–0.063 (10)	0.048–0.136 (14)	0.055–0.132 (19)	0.030–0.084 (18)	0.022–0.052 (19)	0.022–0.046 (18)
Min–max 95th percentile (number of surveys)	0.025–0.271 (8)	0.120–0.232 (12)	0.107–0.247 (19)	0.067–0.171 (17)	0.049–0.102 (19)	0.044–0.081 (18)

²⁶ <http://www.efsa.europa.eu/en/food-consumption/comprehensive-database>.

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	+/-

+: uncertainty with potential to cause overestimation of exposure; -: uncertainty with potential to cause underestimation of exposure; TOS: total organic solid.

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 rat study with the derived exposure estimates of 0.004–0.136 mg/kg bw per day at the mean and from 0.025–0.271 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposures (MOEs) above 347.

4. Conclusions

Based on the data provided, the removal of the food enzyme during glucose syrup production and the derived MOE from use in baking, the Panel concludes that the food enzyme 1,4- α -maltotetrahydrolase produced with the genetically modified *B. licheniformis* strain DP-Dzf24, does not give rise to safety concerns under the intended conditions of use.

The production strain of the food enzyme contains multiple copies of a known antimicrobial resistance gene. However, based on the absence of viable cells and DNA from the production organism in the food enzyme, this is not considered to be a risk.

Documentation provided to EFSA

- 1) Dossier Application for authorisation of from a genetically modified strain of *B. licheniformis*, March 2015. Submitted by, Dupont
- 2) Additional information. May 2018. Submitted by DuPont
- 3) Additional information. August 2018. Submitted by DuPont
- 4) Additional information. October 2018. Submitted by DuPont
- 5) Additional information. March 2019. Submitted by DuPont
- 6) Summary reports on genetic modifications data were delivered by the Technical University of Denmark (Søborg, Denmark) on 14 July 2017
- 7) Additional information on "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
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MIC	minimum inhibitory concentration
MOE	margin of exposure
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
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 (downloadable <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.5739>).

The file contains two sheets, corresponding to two tables.

Table 1: Mean 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).