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Safety evaluation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain DP-Dzb52

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Abstract

The food enzyme α-amylase (1,4-α-D-glucan glucanohydrolase; EC 3.2.1.1) is produced with the genetically modified *Bacillus licheniformis* strain DP-Dzb52 by Danisco US Inc. The production strain contains multiple copies of an 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. The α-amylase is intended to be used in starch processing for the production of glucose syrups, brewing processes and distilled alcohol production. Since residual amounts of the food enzyme are removed by the purification steps applied during the production of glucose syrups and distillation, no dietary exposure was calculated. Based on the maximum use levels recommended for the brewing processes and individual data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the enzyme–total organic solids (TOS) was estimated to be up to 0.145 TOS/kg body weight per day in European populations. The toxicity studies were carried out with another α-amylase from *B. licheniformis* strain DP-Dzb54, considered by the Panel as a suitable substitute. Toxicological tests indicated that there was no concern with respect to genotoxicity or systemic toxicity. A no observed adverse effect level was identified in rats which, compared with the dietary exposure, results in a margin of exposure of at least 750. A search for similarity of the amino acid sequence to known allergens was made and one match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions can be excluded in distilled alcohol production and is considered low when the enzyme is used in starch processing and brewing. 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, α-amylase, 1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1, glycogenase, *Bacillus licheniformis*, genetically modified microorganism

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1. Introduction

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

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

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

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

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

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

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

1.1.1. Background as provided by the European Commission

Only food enzymes included in the 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 Commission 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, 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, 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 assessment 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 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 α-amylase from the genetically modified *Bacillus licheniformis* strain DP-Dzb52.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain DP-Dzb52.

Additional information was requested from the applicant during the assessment process on 20 December 2018, 10 September 2019 and 8 October 2020 and was consequently provided (see 'Documentation provided to EFSA').

Following the request for additional data sent by EFSA on 20 December 2018, the applicant requested a clarification teleconference, which were held on 10 January 2019.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance of EFSA Scientific Committee.

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

3. Assessment

IUBMB nomenclature:	α-amylase
Systematic name:	1,4-α-D-glucan glucanohydrolase
Synonyms:	glycogenase
IUBMB No:	EC 3.2.1.1
CAS No:	9000-90-2
EINECS No:	232-565-6

The α-amylase catalyses the hydrolysis of 1,4-α-glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides as well as oligosaccharides, resulting in the generation of soluble dextrins and other malto-oligosaccharides. It is intended to be used in starch processing for the production of glucose syrups, brewing processes and distilled alcohol production.

3.1. Source of the food enzyme

The α-amylase is produced with the genetically modified *B. licheniformis* strain DP-Dzb52 ([redacted]), which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [redacted].[4]

[4] Technical dossier/Additional data July 2019/Annex AE_SI.

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *Bacillus licheniformis* strain [redacted], which was identified as *B. licheniformis* by [redacted].

The recipient strain *B. licheniformis* [redacted] was developed from the parental strain [redacted].[5]

3.1.2. Characteristics of introduced sequences

[redacted].[5]

3.1.3. Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise α-amylase [redacted].

The production strain *B. licheniformis* DP-Dzb52 was further developed from the recipient strain [redacted].[7] [redacted].[8]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *B. licheniformis* DP-Dzb52 differs from the parental strain *B. licheniformis* [redacted].[6]

Although *B. licheniformis* is included in the list of species considered suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA BIOHAZ Panel, 2018), [redacted]. [redacted] the presence of multiple copies of [redacted] in the genome of the production strain, which is considered a hazard.

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

[5] Technical dossier/2nd submission/Annex V_updated.
[6] Technical dossier/Additional data July 2019/Annex AF_SI.
[7] Technical dossier/2nd submission/Annex_V_updated.
[8] Technical dossier/2nd submission/Annex V and Additional data July 2019/Annex AF_SI.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[9], with food safety procedures based on hazard analysis and critical control points and in accordance with current good manufacturing practice.[10]

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 mass 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.[11]

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 α-amylase is a single polypeptide of [redacted] amino acids.[12] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa.[13] 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 main protein band corresponding to an apparent molecular mass of about [redacted] kDa. No other enzymatic activities were reported.

The in-house determination of the α-amylase activity is based on the hydrolysis of the substrate *p*-nitrophenyl-maltoheptoside (reaction conditions: pH 5.6, 25°C, 5 min). The enzymatic activity is determined by measuring the release of *p*-nitrophenol spectrophotometrically at 410 nm. One unit of α-amylase activity (AAU) is defined as the amount of enzyme required to hydrolyse 10 mg of starch per minute under the conditions of the assay.[13]

The food enzyme has a temperature optimum around 80°C (pH 5.6) and a pH optimum around 6.0 (temperature 90°C). Thermostability was tested after pre-incubation of the food enzyme for 10–120 min at 75°C (pH 4.6). The α-amylase activity decreased rapidly at 75°C, showing no residual activity after 10 min at this temperature.[14]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch used for the toxicological tests (Table 1).[15] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 8.3 and the mean enzyme activity/TOS ratio was 388 AAU/mg TOS.

[9] 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.
[10] Technical dossier/1st submission/Annex M.
[11] Technical dossier/Additional data July 2019/Annex AC_SI.
[12] Technical dossier/1st submission/Annex I.
[13] Technical dossier/Additional data July 2019.
[14] Technical dossier/1st submission/Annex J.
[15] Technical dossier/1st submission/Annex G; Additional data September 2020/Annex AQ.

Table 1: Compositional data of the food enzyme.

Parameter	Unit	Batch			
		1	2	3	4[(a)]
α-Amylase activity	AAU/g batch[(b)]	27,530	32,110	32,448	46,072
Protein	%	3.4	5.8	6.2	16
Ash	%	0.9	1.1	1.2	2.7
Water	%	93.5	89.6	88.9	75.3
Total Organic Solids (TOS)[(c)]	%	5.6	9.3	9.9	22.0
Activity/mg TOS	AAU/mg TOS	492	345	328	209

(a): Batch used for the toxicological studies.
(b): AAU: α-amylase 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 and the batch used for the toxicological studies was below 5 mg/kg, which complies with the specification for lead (≤ 5 mg/kg),[16] as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[17]

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

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

3.3.4. Viable cells and DNA of the production strain

The absence of the production strain in the food enzyme was demonstrated in nine independent batches analysed in duplicate [redacted]. No colonies were produced.[19]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected [redacted].[20]

3.4. Toxicological data

3.4.1. Choice of test item

No toxicological studies were provided for the α-amylase food enzyme produced with *B. licheniformis* strain DP-Dzb52. Instead, the applicant argued that the assessment could be based on toxicological data from another α-amylase produced with the *B. licheniformis* strain DP-Dzb54, ([redacted]), previously submitted to EFSA (Question No EFSA-Q-2015-00666) following the EFSA guidance (EFSA CEF Panel, 2009).

The production strain of the α-amylase (*B. licheniformis* DP-Dzb54) was developed from the same recipient strain ([redacted]) as that for the α-amylase under assessment (*B. licheniformis* DP-Dzb52), using the same genetic modification system ([redacted]). Thus, the genetic modification in *B. licheniformis* DP-Dzb54 involves the same system, as well as the same gene of interest (α-amylase) introduced at the same locus ([redacted]). No rounds of mutagenesis have been applied in the development of the production strains from the recipient and all the genetic modifications have been described throughout. [redacted]. Therefore, the genetic differences between *B. licheniformis* DP-Dzb52 and *B. licheniformis* DP-Dzb54 are not expected to result in a different toxigenic potential.

[16] LOD: Pb = 0.5 mg/kg.
[17] Technical dossier/2nd submission/annex H.
[18] Technical dossier/1st submission/annex G.
[19] Technical dossier/Additional data July 2019/Annex AG_SI and Additional data September 2020/Annex AS.
[20] Technical dossier/Additional data November 2020/Annex AT.

The batch of α-amylase food enzyme from the *B. licheniformis* strain DP-Dzb54 used for the toxicological studies was produced according to a standard procedure similar to the one described in Section 3.2 of this opinion. According to the data provided by the applicant, the raw materials used and the steps involved in the manufacturing of both α-amylase food enzymes from *B. licheniformis* strains (DP-Dzb52 and DP-Dzb54, respectively) are essentially the same. The temperature and pH conditions used during fermentation are the same in both manufacturing processes.

Taking the microbiological and technical data into account, the Panel considered the α-amylase from *B. licheniformis* DP-Dzb54 as a suitable substitute for the α-amylase produced with *B. lichenformis* strain DP-Dzb52 in the toxicological studies.

3.4.2. Genotoxicity

3.4.2.1. 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 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[21] Four strains of Salmonella Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2uvrA were used in the presence or absence of metabolic activation, applying the plate incorporation method. Two separate experiments were carried out using five concentrations of the food enzyme (50, 150, 500, 1,500 and 5,000 μg total protein/plate, corresponding to 68.8, 206.3, 687.5, 2,062.5 and 6,875.0 μg TOS/plate). A third confirmatory experiment was carried out only in S. Typhimurium strain TA98 in the presence of S9-mix. No cytotoxicity was observed at any concentration of the food enzyme in any experiment. In the first experiment, an increase in revertant colony numbers above the control in S. Typhimurium strain TA98 in the presence of S9-mix was recorded at the concentrations of 150, 500 and 1,500 μg total protein/ plate (1.6-, 1.7- and 2.2-fold increase, respectively). However, this was not reproducible in the second and third confirmatory experiments. Upon treatment with the food enzyme, there was no increase in revertant colony numbers above the control values in other tested strains 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.

3.4.2.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in human peripheral blood lymphocytes according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[22] A preliminary toxicity test was performed at concentrations ranging from 19.53 to 5,000 μg total protein/mL. No cytotoxicity was observed at any concentration level of the test substance. Based on these results, in the first experiment, the cells were exposed to the food enzyme at 1,250, 2,500, 3,750 and 5,000 μg total protein/mL (corresponding to 1,718.75, 3,437.50, 5,156.25 and 6,875,00 μg TOS/mL) in the short-term treatment (4 h followed by 20 h recovery period) with and without metabolic activation (S9-mix). In the second experiment, the cells were exposed to the food enzyme at 625, 1,250, 2,500 and 5,000 μg total protein/mL (corresponding to 859.38, 1,718.75, 3,437.50 and 6,875,00 μg TOS/mL) in the short-term treatment (4 h followed by 20 h recovery period) with S9-mix, and at 39,06, 78.13 and 117.21 μg/mL (corresponding to 53.71, 107.43 and 161.16 μg TOS/mL) in the continuous treatment (24 h) in the absence of S9-mix. 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 control data.

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

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

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[23] Groups of 10 male and 10 female Wistar Han™: HsdRccHan™ rats received by gavage the food enzyme in doses of 8, 40 or 80 mg total protein/kg body weight (bw) per day, corresponding to 11, 55 or 110 mg TOS/kg bw per day. Controls received the vehicle (0.9% saline).

[21] Technical dossier/Additional information, September 2020/Annex AL.
[22] Technical dossier/Additional information, September 2020/Annex AM.
[23] Technical dossier/Additional information, September 2020/Annexes/Annex AK.

No mortality was observed.

Statistically significant differences to the control group in functional performance tests were limited to grip strength, which was decreased in high-dose males and increased in high-dose females. These contradictory findings were considered not to be toxicologically relevant.

In days 57–64, the mean body weight gain of all treated males was statistically significantly lower in comparison to controls. As body weights at the end of the treatment and overall body weight gains of the treated males were not statistically significantly different from those in the control group, it was considered not toxicologically relevant.

Among haematological parameters, statistically significant differences to controls were limited to a lower mean corpuscular volume in mid-dose males and lower absolute lymphocyte count in high-dose males.

Clinical chemistry investigation revealed that the aspartate aminotransferase activity (AST) was statistically significantly higher in high-dose males and blood urea was significantly higher in mid-dose females in comparison to control group.

All the changes in haematology and blood chemistry parameters were considered by the Panel as not toxicologically relevant because the differences were generally small, without an apparent dose dependency (except for the absolute lymphocyte count and AST, seen only at the highest dose) and restricted to one sex.

There was a statistically significant increase in absolute and relative spleen weights in low- and mid-dose females, but not at the high dose. As these changes lacked dose response relationship and histological correlation, they were considered not toxicologically relevant.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 110 mg TOS/kg bw per day, the highest dose tested.

3.4.4. 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 α-amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb52 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, one match was found. [redacted].

No information is available on oral and respiratory sensitisation or elicitation reactions of this α-amylase.

Amylase from *A. oryzae* is known as an occupational respiratory allergen associated with baker's asthma (Brisman and Belin, 1991; Sander et al., 1998; Brisman, 2002; Quirce et al., 2002). However, several studies have shown that adults with occupational asthma to a food enzyme (like α-amylase from *A. oryzae*) may be able to ingest the corresponding enzyme without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Considering the wide use of α-amylase as a food enzyme, only a low number of case reports has been described in the literature that focused on allergic reactions upon oral exposure to α-amylase in individuals respiratory sensitised to α-amylase (Losada et al., 1992; Quirce et al., 1992; Baur and Czuppon, 1995; Kanny and Moneret-Vautrin, 1995; Moreno-Ancillo et al., 2004).

According to the information provided, substances or products that may cause allergies or intolerances (Regulation EU 1169/2011)[24] are used as raw materials ([redacted])[11] in media fed to the microorganisms. However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic

[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.

residues of these materials employed as nitrogen sources are not expected to be present in the food enzyme.

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 removed, (e.g. in distilled alcohol production).

The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions can be excluded in distilled alcohol production and is considered to be low upon dietary exposure to this food enzyme when the enzyme is used in starch processing for the production of glucose syrups or brewing.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in three food processes. Intended uses and recommended use levels are summarised in Table 2.

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

Food manufacturing process(a)	Raw material	Recommended use level of the food enzyme
Brewing processes	Cereals	3.2–31.6 mg TOS/kg cereal
Distilled alcohol production	Cereals	2.9–29.1 mg TOS/kg cereal
Starch processing for the production of glucose syrups	Starch	0.88–8.8 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 adoption of this opinion.
(b): Technical dossier/p. 66.

In brewing processes, the food enzyme is added during the mashing step and/or cereal cooking step.[26] The α-amylase is used to convert liquefied starch into a maltose-rich solution, increasing the amounts of fermentable sugars and thus increasing brewing yield and reducing mash viscosity.

The food enzyme remains in the final brewing product. However, based on data provided on thermostability (see Section 3.3.1), it is anticipated that the α-amylase is inactivated during brewing processes.

In distilled alcohol production, the food enzyme is added during the slurry mixing step, in the liquefaction step and, if needed, in the pre-saccharification step.[26] It is intended to convert liquefied starch into a maltose-rich solution and to increase the amounts of fermentable sugars, which results in higher alcohol yields.

In starch processing for the glucose syrup production, the food enzyme is added during the saccharification step, where it degrades gelatinised starch into dextrins.[26]

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS by distillation and during starch processing were considered by the Panel as sufficient to exclude these processes from the exposure assessment (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated for these two processes.

The food enzyme remains in the final brewing product. Therefore, the dietary exposure to the food enzyme TOS is calculated for the brewing processes.

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (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 (days) and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual average exposure. Based on these distributions, the average and 95th percentile exposures were calculated per survey for

[26] Technical dossier/pp. 61–64.

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 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 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)	0–0.001 (19)	0–0.006 (18)	0.002–0.032 (19)	0.001–0.016 (18)
Min–max 95th percentile (number of surveys)	0 (8)	0 (12)	0 (19)	0–0.038 (17)	0.018–0.145 (19)	0.004–0.066 (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	+/–
Exclusion of other processes from the exposure estimate: – distilled alcohol production – starch processing for the production of glucose syrups	–

+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure.

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

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

3.6. Margin of exposure

The comparison of the NOAEL (110 mg TOS/kg bw per day) from the 90-day rat study with the derived exposure estimates of 0–0.032 mg TOS/kg bw per day at the mean and from 0 to 0.145 mg TOS/kg bw per day at the 95th percentile resulted in a margin of exposure (MoE) of at least 759.

4. Conclusions

Based on the data provided, the removal of the food enzyme during distilled alcohol production and starch processing for the production of glucose syrups, and the margin of exposure from use in brewing processes, the Panel concluded that the food enzyme α-amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb52 does not give rise to safety concerns under the intended conditions of use.

The production strain of the food enzyme contains multiple copies of an 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.

5. Documentation provided to EFSA

1) Dossier "Application for authorisation of α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb52) in accordance with Regulation (EC) No 1331/2008", January 2016. Submitted by Danisco US Inc.
2) Additional information. July 2019. Submitted by Danisco US Inc.
3) Additional information. September 2020. Submitted by Danisco US Inc.
4) Additional information. November 2020. Submitted by Danisco US Inc.

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Abbreviations

AAU	α-amylase units
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
FAO	Food and Agriculture Organization of the United Nations
GMO	genetically modified organisms
IUBMB	International Union of Biochemistry and Molecular Biology

MoE	margin of exposure
NOAEL	no observed adverse effect level
PCR	polymerase chain reaction
QPS	qualified presumption of safety
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequencing
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.2021.6564).

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).