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Safety evaluation of the food enzyme α-amylase from the genetically modified *Bacillus licheniformis* strain DP-Dzb45

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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-Dzb45 by Danisco US Inc. 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 was not considered to be a risk. The α-amylase is intended to be used in brewing processes and distilled alcohol production. Since residual amounts of the food enzyme are removed by distillation, no dietary exposure was calculated for this intended use. 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 food enzyme–total organic solids (TOS) was estimated to be up to 0.138 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 was identified in rats, which, compared with the dietary exposure, results in a margin of exposure of at least 484. Similarity of the amino acid sequence to those of known allergens was searched 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 but cannot be excluded when the enzyme is used in 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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
>
> 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

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

- 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 Beta-galactosidase from a genetically modified strain of *Aspergillus oryzae* (DP-Bzg59), Alpha, alpha trehalase from a genetically modified strain of *Trichoderma reesei* (DP-Nzs51), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb45), Glucose oxidase from a genetically modified strain of *Aspergillus niger* (DP-Aze23) and Alpha-amylase from *Geobacillus stearothermophilus* (DP-Gzb47).

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 Beta-galactosidase from a genetically modified strain of *Aspergillus oryzae* (DP-Bzg59), Alpha, alpha trehalase from a genetically modified strain of *Trichoderma reesei* (DP-Nzs51), Alpha-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb45), Glucose oxidase from a genetically modified strain of *Aspergillus niger* (DP-Aze23) and Alpha-amylase from *Geobacillus stearothermophilus* (DP-Gzb47) 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-Dzb45.

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 a genetically modified *Bacillus licheniformis* strain DP-Dzb45.

Additional information was requested from the applicant during the assessment process on 20 December 2018 and 13 September 2019 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) 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.

α-Amylase catalyses the hydrolysis of 1,4-α-glucosidic linkages in starch (amylose and amylopectin), glycogen and related polysaccharides and oligosaccharides, resulting in the generation of soluble dextrins and other malto-oligosaccharides. It is intended to be used in 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-Dzb45 ([redacted]) which is deposited in the Westerdijk Fungal Biodiversity Institute (CBS) with the deposit number [redacted].[4]

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

3.1.1. Characteristics of the parental and recipient microorganisms

The parental microorganism is the bacterium *Bacillus licheniformis* strain [redacted] that was taxonomically identified as *B. licheniformis* by [redacted].[5]

The recipient strain *B. licheniformis* [redacted] was developed from the parental strain [redacted][6] [redacted][7]

3.1.2. Characteristics of the introduced sequences

[redacted][6]

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-Dzb45 was further developed from the recipient strain [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-Dzb45 differs from the parental strain *B. licheniformis* [redacted][9]

Although *B. licheniformis* is included in the list of species considered suitable for qualified presumption of safety (QPS) approach to safety assessment (EFSA BIOHAZ Panel, 2018), [redacted]

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

[5] Technical dossier/1st submission/Annex K.
[6] Technical dossier/2nd submission/Annex V.
[7] Technical dossier/Additional data August 2019/Annex AH_SI.
[8] Technical dossier/2nd submission/Annex V and Additional data August 2019/Annex AH_SI.
[9] Technical dossier/Additional data August 2019/Annex AH_SI.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[10], with food safety procedures based on hazard analysis and critical control points and in accordance with current good manufacturing practice.[11]

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 and treatment with [redacted], 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.[12]

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.[13] The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [redacted] kDa. The food enzyme was analysed by SDS–PAGE analysis.[14] 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. 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, temperature 25°C, reaction time 5 min). The enzymatic activity is determined by measuring the release of *p*-nitrophenyl 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.[15]

The food enzyme has a temperature optimum around 70°C (pH 5.8) and a pH optimum around pH 4.5 (temperature 50°C). Thermostability was tested after a pre-incubation of the food enzyme for 5–40 min at 95°C. Under the conditions (pH 5.8) of the applied temperature stability assay, the α-amylase activity decreased rapidly at 95°C showing less than 2% residual activity after 20 min and no activity after 40 min at this temperature.[16]

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).[17] The average total organic solids (TOS) of the three food enzyme batches for commercialisation was 7.2% and the average enzyme activity/TOS ratio was 446 AAU/mg TOS.

[10] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3–21.
[11] Technical dossier/1st submission/Annex M.
[12] Technical dossier/Additional data August 2019/Annex AC_SI.
[13] Technical dossier/1st submission/Annex I.
[14] Technical dossier/1st submission/p. 39.
[15] Technical dossier/Additional data August 2019; Additional data September 2020.
[16] Technical dossier/1st submission/Annex E.
[17] Technical dossier/1st submission; Additional data September 2020.

Table 1: Compositional data of the food enzyme.

Parameter	Unit	Batch			
		1	2	3	4[(a)]
α-Amylase activity	AAU/g batch[(b)]	30,551	32,093	32,578	10,246
Protein	%	4.9	4.8	5.5	4.9
Ash	%	0.5	0.2	0.8	0.8
Water	%	93.2	92.6	91.2	90.7
Total Organic Solids (TOS)[(c)]	%	6.3	7.2	8.0	8.5
Activity/mg TOS	AAU/mg TOS	485	446	407	121

(a): Batch used for the toxicological studies.
(b): AAU: α-amylase units (see Section 3.1.3).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the three commercial batches and in the batch used for the toxicological studies was below 0.1 and 0.5 mg/kg, respectively[18], which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).[19]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). 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 viable cells of the production strain in the food enzyme was demonstrated in nine independent batches analysed in duplicate. [redacted]. No colonies were produced.[21]

The absence of recombinant DNA in the food enzyme was demonstrated by PCR analysis of three batches in triplicate. No DNA was detected [redacted][22]

3.4. Toxicological data

A battery of toxicological tests including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test, and a repeated dose 90-day oral toxicity study in rats has been provided. Batch 4 (Table 1) used in these studies has lower chemical purity than the batches used for commercialisation and, thus, is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation assay

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a) and following Good Laboratory Practice (GLP).[23] Five strains of Salmonella Typhimurium (TA98, TA100, TA102, TA1535 and TA1537) were used in the presence or absence of metabolic activation, applying the treat and plate method. Two separate experiments were carried out in triplicate. Five to eight different concentrations of the food enzyme from 0.16 to 5,000 μg total proteins/plate (corresponding to 0.28 to 8,673 μg TOS/plate) were tested. The toxicity of the food enzyme, detected by the presence of microcolonies, varied between the bacterial strains, in the presence and absence of S9-mix. The

[18] LoD: Pb = 0.010 mg/kg; Additional data September 2020.
[19] Technical dossier/1st submission/annex G.
[20] Technical dossier/1st submission/annex G ; Additional data September 2020/Annex AN_SI.
[21] Technical dossier/Additional data September 2020/Annex AR_SI.
[22] Technical dossier/Additional data September 2020/Annex AQ_SI.
[23] Technical dossier/Additional data September 2020/Annex AN_SI.

highest non-toxic dose level was in the range 8.67–867 μg/plate. Upon treatment with the food enzyme, there was no biologically relevant 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 in human lymphocytes in primary cultures of whole blood according to OECD Test Guideline 473 (OECD, 1997b) and following GLP.[24]

Two separate experiments were carried out in duplicate. In the first experiment, the cultures were treated with the food enzyme from 78.1 to 5,000 μg/mL (corresponding to 135.5–8,673 μg TOS/mL), for 3 h, followed by 18 h recovery period (short treatment) with and without metabolic activation (S9-mix). The test item caused reductions in mean mitotic index greater than 50% compared to the corresponding negative control value at all concentrations tested, except 78.1 μg/mL, both in the absence and presence of S-9 mix. The test was repeated at 10, 20, 40, 80, 160 and 320 μg/mL (corresponding to 17.34, 34.68, 69.37, 138.75, 277.5 and 555 μg TOS/mL). In a second experiment, the cells were exposed to the same concentrations of food enzyme in a short-term treatment (3 h) with S9-mix and in a continuous treatment (18 h) in the absence of S9-mix. Reductions in mean mitotic index of 54%–58% were observed in cultures treated at 277.5 μg TOS/mL in the absence and presence of S-9. Three concentrations were selected for the metaphase analysis: 40, 80 and 160 μg/mL (corresponding to 69.37, 138.75 and 277 μg TOS/mL) for the short treatment and 20, 40 and 80 μg/mL of food enzyme (corresponding to 34.68, 69.37 and 138.75 μg TOS/mL) for the continuous treatment. The frequency of structural and numerical chromosomal aberrations in treated cultures was comparable to the values detected in the negative controls. The Panel concluded that the food enzyme did not induce chromosome aberrations under the test conditions employed in this study.

3.4.2. 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.[25] Groups of 10 male and 10 female SPF Sprague-Dawley rats of strain Ntac:SD received by gavage the food enzyme in doses of 4.96, 12.4 and 37.2 mg total protein/kg body weight (bw) per day, corresponding to 8.9, 22.27 and 66.81 mg TOS/kg bw per day. Controls received the vehicle (0.9% saline).

No mortality was observed.

Water consumption was statistically significantly increased in mid-dose females in days 84–87. As this was an isolated finding, with no dose-relationship and limited to one sex, it was considered by the Panel as incidental and not treatment-related.

Statistically significant differences to controls in haematological parameters included a lower percentage of reticulocytes in high-dose males, a lower percentage of neutrophils in low- and high-dose females and a higher percentage of lymphocytes in all treated females. As no dose response was observed for these changes, they were considered not to be of toxicological significance.

In urinalysis, statistically significant lower amounts of epithelial cells and of crystals in mid- and high-dose females, and of urates in high-dose females were recorded as compared to controls. As the levels for these parameters were lower than in the control females, these findings were considered to be of no toxicological significance.

No other statistically significant differences to controls were observed.

The Panel identified the no observed adverse effect level (NOAEL) of 66.81 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

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

The allergenicity of α-amylase produced with the genetically modified *B. licheniformis* strain DP-Dzb45 was assessed by comparing its amino acid sequence with those of known allergens

[24] Technical dossier/Additional data September 2020/Annex AO_SI.
[25] Technical dossier/Additional data September 2020/Annex AP_SI.

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. The matching allergen was Asp o 21, an α-amylase from *Aspergillus oryzae*, (Brisman and Belin, 1991; Brisman, 2002).

No information is available on oral and respiratory sensitisation or elicitation reactions of this α-amylase. A-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 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)[26] are used as raw materials ([redacted])[27] 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 residues of these foods employed as protein sources are not expected to be present. The Panel also notes that [redacted] a known allergen, is used during the downstream processing of the food enzyme, and is likely to be present in the final product.

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, but cannot be excluded when the enzyme is used in brewing processes, in particular due to the potential presence of [redacted].

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in two food processes. Intended uses and recommended use levels are summarised in Table 2.[28]

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

Food manufacturing process[(a)]	Raw material	Recommended use level of the food enzyme
Brewing processes	Cereals	2.5–30 mg TOS/kg cereal
Distilled alcohol production	Cereals	2.5–30 mg TOS/kg cereal

(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.

In brewing processes, the food enzyme is added during the mashing step and/or cereal cooking step.[29] The α-amylase is used to convert liquefied starch into a maltose-rich solution, increasing the amounts of fermentable sugars and thus increasing the brewing yield, reducing mash viscosity and removing the beer haze.

[26] 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.

[27] Technical dossier/Additional data August 2019/Annex AC_SI.

[28] Technical dossier/p. 68.

The food enzyme remains in the final brewing product. 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.[29] The α–amylase is intended to increase the starch-based substrate conversion into a maltose-rich solution, resulting in higher alcohol yields.

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

3.5.2. Dietary exposure estimation

As residual amounts of TOS are removed by distillation, foods/ingredients derived through this process, i.e. distilled alcohol, were excluded from the estimation.

For the brewing processes, chronic exposure was calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

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 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 one day per subject were excluded and high-level exposure/intake was calculated only for those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 3 provides an overview of the derived exposure estimates across all surveys. Detailed average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

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

Population group	Estimated exposure (mg TOS/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0 (10)	0 (14)	0–0.001 (19)	0–0.006 (18)	0.002–0.031 (19)	0.001–0.015 (18)
Min–max 95th percentile (number of surveys)	0 (8)	0 (12)	0 (19)	0–0.036 (17)	0.017–0.138 (19)	0.004–0.063 (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.

[29] Technical dossier/2nd submission/pp. 64–67.

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	–

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

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

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

3.6. Margin of exposure

A comparison of the NOAEL (66.81 mg TOS/kg bw per day) from the 90-day oral toxicity study in rats with the derived exposure estimates of 0–0.031 mg TOS/kg bw per day at the mean and from 0 to 0.138 mg TOS/kg bw per day at the 95th percentile, resulted in margin of exposure (MOE) of at least 484.

4. Conclusions

Based on the data provided, the removal of the food enzyme during distilled alcohol production and the derived 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-Dzb45 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.

Documentation provided to EFSA

1) Dossier "Application for authorisation of α-amylase from a genetically modified strain of *Bacillus licheniformis* (DP-Dzb45) in accordance with Regulation (EC) No 1331/2008", February 2016. Submitted by Danisco US Inc.
2) Additional information. August 2019. Submitted by Danisco US Inc.
3) Additional information. September 2020. Submitted by Danisco US Inc.
4) Summary report on GMM part. March 2018. Delivered by contractor (DTU, Kongens Lyngby, Denmark).

References

Armentia A, Dias-Perales A, Castrodeza J, Dueñas-Laita A, Palacin A and Fernándes S, 2009. Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant? Allergologia et Immunopathologia, 37, 203–204. https://doi.org/10.1016/j.aller.2009.05.001

Baur X and Czuppon AB, 1995. Allergic reaction after eating α-amylase (Asp o 2)-containing bred. A case report. Allergy, 50, 85–87. https://doi.org/10.1111/j.1398-9995.1995.tb02487.x

Brisman J, 2002. Baker's asthma. Occupational and Environmental Medicine, 59, 498–502; quiz 502, 426.

Brisman J and Belin L, 1991. Clinical and immunological responses to occupational exposure to α-amylase in the baking industry. British Journal of Industrial Medicine, 48, 604–608.

Cullinan P, Cook A, Jones M, Cannon J, Fitzgerald B and Newman Taylor AJ, 1997. Clinical responses to ingested fungal α-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? Allergy, 52, 346–349. https://doi.org/10.1111/j.1398-9995.1997.tb01003.x

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2006;5(1):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009a. Guidance of EFSA prepared by the Scientific Panel of Food Contact Material, Enzymes, Flavourings and Processing Aids on the Submission of a Dossier on Food Enzymes. EFSA Journal 2009;7(8):1305, 26 pp. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009; 7 (5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Fernández Escámez PS, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli PS, Peixe L, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2018. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 7: suitability of taxonomic units notified to EFSA until September 2017. EFSA Journal 2018;16(1):5131, 43 pp. https://doi.org/10.2903/j.efsa.2018.5131

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Exposure assessment of food enzymes. EFSA Journal 2016;14(11):4581, 9 pp. https://doi.org/10.2903/j.efsa.2016.4581 and Annex B – Process-specific technical data used in exposure assessment of food enzymes (Available online: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2016.4581&file=efs24581-sup-0001-Annex_B.pdf)

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), 2019. Statement on the characterisation of microorganisms used for the production of food enzymes. EFSA Journal 2019;17(6):5741, 13 pp. https://doi.org/10.2903/j.efsa.2019.5741

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal2010;8(7):1700, 168 pp. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United States/World Health Organization), 2006. General specifications and considerations for enzyme preparations used in food processing in Compendium of food additive specifications. 67th meeting. FAO JECFA Monographs 3, pp. 63–67. Available online: http://www.fao.org/3/a-a0675e.pdf.

Gray GL, Mainzer SE, Rey MW, Lamsa MH, Kindle KL, Carmona C and Requadt C, 1986. Structural genes encoding the thermophilic α-amylases of *Bacillus stearothermophilus* and *Bacillus licheniformis*. Journal of Bacteriology, 166, 635–643.

Kanny G and Moneret-Vautrin D-A, 1995. A-amylase contained in bread can induce food allergy. Journal of Allergy and Clinical Immunology, 95, 132–133. https://doi.org/10.1016/S0091-6749(95)70161-3

Losada E, Hinojosa M, Quirce S, Sánchez-Cano M and Moneo I, 1992. Occupational asthma caused by α-amylase inhalation: clinical and immunologic findings and bronchial response patterns. Journal of Allergy and Clinical Immunology, 89, 118–125. https://doi.org/10.1016/S0091-6749(05)80048-X

Moreno-Ancillo A, Domínguez-Noche C, Gil-Adrados AC and Cosmes PM, 2004. Bread eating induced oral angioedema due to α-amylase allergy. Journal of Investigative Allergology and Clinical Immunology, 14, 346–347.

OECD (Organisation for Economic Co-Operation and Development), 1997a. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 471: Bacterial reverse mutation test. 21 July 1997. 11 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en;jsessionid=9zfgzu35paaq.x-oecd-live-01

OECD (Organisation for Economic Co-Operation and Development), 1997b. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 473: *In vitro* mammalian chromosomal aberration test. 21 July 1997. 10 pp. Available online: https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosome-aberration-test_9789264071261-en

OECD (Organisation for Economic Co-Operation and Development), 1998. OECD Guideline for the testing of chemicals, Section 4 Health effects, Test No. 408: Repeated dose 90-day oral toxicity study in rodents. 21 September 1998. 10 pp. Available online: http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Poulsen LK, 2004. Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel food. Molecular Nutrition & Food Research, 48, 413–423. https://doi.org/10.1002/mnfr.200400029

Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González R and Losada E, 1992. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. Journal of Allergy and Clinical Immunology, 90(6 Pt 1), 970–978.

Quirce S, Fernandez-Nieto M, Bartolome B, Bombin C, Cuevas M and Sastre J, 2002. Glucoamylase: another fungal enzyme associated with baker's asthma. AnnAllergy Asthma Immunol, 89, 197–202.

Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE and Baur X, 1998. Allergy to Aspergillus-derived enzymes in the baking industry: identification of beta-xylosidase from Aspergillus niger as a new allergen (Asp n 14). Journal of Allergy and Clinical Immunology, 102, 256–264.

Abbreviations

AAU	alpha-amylase units
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.2020.6311).

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).